

ENGINEERED TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE VARIANTS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of United States Provisional Patent Application Number 63/499,770, filed May 3, 2023, and United States Provisional Patent Application 63/379,439, filed on October 13, 2022, the entirety of each of which is incorporated herein by reference.

TECHNICAL FIELD

[0002] The present invention provides engineered terminal deoxynucleotidyl transferase (TdT) polypeptides useful in template-independent polynucleotide synthesis, as well as compositions and methods of utilizing these engineered polypeptides.

REFERENCE TO SEQUENCE LISTING, TABLE OR COMPUTER PROGRAM

[0003] The official copy of the Sequence Listing is submitted concurrently with the specification as an XML file, with a file name of "CX10-238WO3_ST26.xml", a creation date of October 11, 2023, and a size of 13,670,249 bytes. The Sequence Listing filed is part of the specification and is incorporated in its entirety by reference herein.

BACKGROUND

[0004] Synthetic biology is becoming established in a diverse range of high value, high growth markets. From food and agriculture to therapeutics, diagnostics, and vaccines; tools such as gene editing, DNA sequencing and gene synthesis are being used to build value-added products with advanced functionality (e.g., cell bioreactors, etc.) and desired end products (e.g., drugs, chemicals, etc.). The barrier to widespread implementation of these technologies is the ability to efficiently synthesize RNA, DNA, and other polynucleotides.

[0005] In particular, silencing RNA (siRNA) therapeutics are a promising class of drugs that have the potential to treat numerous difficult to treat conditions in a highly targeted manner by binding to known mRNA targets (Hu et al. (2020). *Sig Transduct Target Ther* 5, 101; Zhang et al. (2021). *Bioch. Pharmac.*, 189, 114432.) As these therapies become more common and are targeted at larger patient populations, the ability to produce large amounts of the oligonucleotide active pharmaceutical ingredient (API) becomes critical.

[0006] To date, short RNA oligonucleotides have been synthesized almost exclusively by iterative addition of nucleotides in the form of activated phosphoramidites, plus additional processing steps, to a growing immobilized nucleotide chain (Brown, T. *Nucleic Acids Book*. See at: www.atdbio.com/nucleic-acids-book (accessed 2022-10-10).)

[0007] Phosphoramidite chemistry has been developed extensively over the years to synthesize small amounts of DNA and for more complex therapeutic RNA syntheses but suffers from several cost and

sustainability issues that are potentially limiting as API demand grows to triple-quadruple digit kilograms per year (Andrews et al. (2021). *J. Org. Chem.* 86, 49–61). Additionally, RNA synthesis using phosphoramidite synthesis chemistry is limited to producing short oligonucleotides of approximately 200 basepairs (Beaucage & Caruthers. (1981). *Tetrahedron Lett.* 22 (20): 1859.)

[0008] The phosphoramidite iterative methodology is multi-step and based on phosphorous (III) coupling chemistry that requires (i) coupling (ii) capping (iii) oxidation to P(V) forming phosphodiester or phosphorothioate diester (iv) deblocking of 5' O group. After chain synthesis is complete, the final oligo is cleaved from the support where deblocking of phosphate cyanoethyl group and nucleobases can also occur (Brown, T. *Nucleic Acids Book*. See at: www.atdbio.com/nucleic-acids-book (accessed 2022-10-10).) Washes with organic solvents at each step are also required. The phosphate cyanoethyl blocking group and nucleobase protecting groups can be removed in parallel to oligonucleotide cleavage from the solid support to generate the oligonucleotide product, or the cyanoethyl group can be removed under milder condition before chain cleavage, if required.

[0009] Many aspects of the environmental impact of the current phosphoramidite methodology and potential advances have been reviewed (Andrews et al. (2021). *J. Org. Chem.* 86, 49–61). Even at an aspirational high oligonucleotide loading of 20% mass final oligonucleotide to mass solid support, at least five-fold mass of support is required over the final product mass.

[0010] In addition to the high cost of mass support required to immobilize the oligonucleotide, the use of organic solvents and 5'-O-blocking groups entail additional waste and process inefficiencies. Organic solvents such as acetonitrile are required for solubilization of the phosphoramidite coupling partners, or dichloromethane or toluene for deprotection steps. These solvents need to be anhydrous to reduce undesired hydrolysis of the phosphoramidite partners and can come from non-sustainable sources, adding cost, sustainability questions, and potential supply issues to the process.

[0011] The phosphoramidite coupling partners themselves carry a required blocking group at the 5'-O-position, the nucleobase nitrogen atom (in A, C and G), and the nascent phosphate. The most common 5'-O-blocking group, dimethoxytrityl, has a molecular mass of ~ 303 Da that approaches that of the heaviest native ribonucleotide fragment Gp with a mass of ~ 345 Da. This protecting group requires energy, resources, and effort to produce and append, and then requires disposal when separated from the desired materials.

[0012] In conclusion, a paradigm shift in oligonucleotide synthesis is necessary to enable siRNA therapeutics by lowering environmental impact, improving economic efficiency, and increasing scalability. New methods of oligonucleotide synthesis are, therefore, of great interest to the pharmaceutical industry.

[0013] Template-Independent Enzymatic Synthesis

[0014] Enzymatic synthesis may facilitate production of high volumes of complex or long polynucleotides (>200 base pairs) while minimizing toxic waste. A variety of prokaryotic and eukaryotic DNA and RNA polymerases are known to naturally synthesize polynucleotides of thousands of base pairs

or more. Most of these polymerases function during DNA replication associated with cell division or transcription of RNA from DNA associated with gene or protein expression. Both of these processes involve template-dependent polynucleotide synthesis, wherein the polymerase uses an existing template polynucleotide strand to synthesize a complementary polynucleotide strand.

[0015] The potential of template-independent enzymatic polynucleotide synthesis to produce defined sequences has long been recognized. One early report suggested using NTPs with blocked 3' groups to allow stepwise addition of specific nucleotide residues (Bollum. (1962). JBC, 237, 1945–1949).

[0016] However, few polymerases are known to catalyze template-independent polynucleotide synthesis. These include polymerase lambda, polymerase mu, and terminal deoxynucleotidyl transferase (TdT), all members of the X family of DNA polymerases, many of which participate in DNA repair processes (Domínguez et al. (2000). EMBO, 19(7), 1731–1742.) Of these, TdT is known to generate diversity in antigen receptors by indiscriminately adding nucleosides to the 3' end of a single-stranded polynucleotide in a template-independent process (Bentolila et al. (1995). EMBO, 14(17), 4221–4229.)

[0017] Others have published a method of polynucleotide synthesis using a nucleoside 5'-triphosphate with a 3'-OH position protected with a removable blocking moiety and, specifically, a template-independent polynucleotide polymerase, including a terminal deoxynucleotidyl transferase (U.S. Pat. 5,763,594). The blocking group, also known to those skilled in the art as an inhibitor or reversible terminating group, may include a variety of groups that prevent the TdT from adding additional NTPs to the nascent polynucleotide chain. This may include charged molecules, large molecules and moieties, or other blocking groups known to those skilled in the art. Appropriate removable blocking groups may include carbonitriles, phosphates, carbonates, carbamates, esters, ethers, borates, nitrates, sugars, phosphoramidates, phenylsulfonates, and sulfates. Other 3' blocking groups are also known in the art, including 3'-O-amines and methylamines (U.S. Pat. 7,544,794) and 3'-O-azides (U.S. Pat. 10,407,721).

[0018] Although initially promising, use of 3'-blocked NTPs in template-independent synthesis catalyzed by TdT has proven difficult in practice, as TdT struggles to accept 3'-O-blocked NTPs as substrates. Further, wild-type TdTs have low tolerance for oligo acceptor substrates containing one or more modified nucleotides (e.g. 2' modifications).

[0019] Additionally, synthesis of RNA strands present unique challenges due to the additional, reactive 2'-OH on the ribose. While protection of the 2' position facilitates RNA synthesis, this approach reduces efficiency because of steric hindrance by the 2' protecting groups and requires maintenance and removal of the protecting group (CB Reese. (2005). Org Biomol Chem 3, 3851–3868.)

[0020] Recently several reports have described template-independent synthesis methods that use modified NTPs with blocking groups attached to the purine or pyrimidine base, leaving the 3'-OH unmodified and available for additional rounds of synthesis. These base blocking groups may include a cleavable linker that allows removal of the blocking group after each NTP addition step. The cleavable linker may also be attached to a detectable label (U.S. Pat. 7,057,026, among others). A variety of cleavable linkers are known to those skilled in the art. These include linkers attached via reducible

disulfide bonds, photocleavable, electrophilic or nucleophilic, pH sensitive, temperature sensitive, and linkers cleaved by enzymes. One drawback to using cleavable linkers is that, typically, some atoms of the linker moiety remain attached to the NTP following cleavage, leaving a “scar” that may interfere with synthesis of a complementary strand after initial template-independent synthesis of the primary polynucleotide strand.

[0021] Recently, modified NTPs with bases attached to blocking groups with cleavable linkers that are “scarless” and leave the nascent DNA ready for the next round of synthesis have been developed. In one example, the blocking group and cleavable linker are attached to the base via a disulfide bond. Upon addition of a reducing agent, the blocking group is removed and the remaining atoms of the linker self-cyclize to leave the nascent DNA free of any linker atoms (U.S. Pat. 8,808,989, U.S. Pat. 9,695,470, U.S. Pat. 10,041,110). Methods of using NTPs attached to cleavable blocking groups to synthesize polynucleotides are known, including using a microfluidic device or ink jet printing technology (U.S. Pat. 9,279,149). An exonuclease may also be used in a method to synthesize polynucleotides to shorten or completely degrade polynucleotide strands that have not successfully added an NTP after the polynucleotide extension step and prior to removing the blocking group (U.S. Pat. 9,771,613).

[0022] However, NTP bases with bulky blocking groups attached via cleavable linkers are not optimal for efficient synthesis of complex or long oligonucleotides. The large labels may negatively impact enzyme kinetics, and linker scars may lead to an unacceptable rate of misincorporation when synthesizing the oligonucleotide strand. Additionally, larger linkers and necessary deblocking steps may increase the cost, time, and inefficiency of the process as a whole, rendering these methods economically infeasible.

[0023] Recently, several groups have explored modifying the structure or amino acid sequence of TdT or other polymerases to allow template-independent synthesis using 3'-O-blocked groups. Efcavitch et al. describes incorporation of 3' modified dNTPs by TdT in template-independent synthesis using a murine or bacterial TdT with substituted amino acid residues (U.S. Pat. 10,059,929). Other reports describe engineered bovine and gar (*Lepisosteus oculatus*) TdTs that displayed improved activity over wild-type TdT (U.S. Pat. 10,745,727, PCT/GB2020/050247). Similarly, a variety of mutations have been described to improve the activity of Pol X family enzymes (WO 2017216472 A2). Finally, an N-terminal truncation of the BRCT domain (or alternatively mutation of the BRCT domain) of TdT has also been described as enhancing activity in the addition of reversibly blocked NTPs to the 3'-OH of a nucleic acid (US20210164008A1).

[0024] However, no feasible methods of template-independent enzymatic synthesis of complex or long polynucleotides are currently known or commercially available, despite the recognized value of this technology and intensive research efforts devoted to resolving challenges in this field. Improved engineered TdT enzymes are necessary to enable template-independent enzymatic synthesis of complex or long polynucleotides or oligonucleotides of defined sequence using nucleoside triphosphates with 3'-O-removable blocking groups, with 2' modifications, and/or with other modifications.

SUMMARY

[0025] The present invention provides engineered terminal deoxynucleotidyl transferase (TdT) polypeptides useful in template-independent polynucleotide synthesis, as well as compositions and methods of utilizing these engineered polypeptides. The TdTs of the present invention are variants of a predicted splice variant of the wild-type gene from *Monodelphis domestica* (SEQ ID NO: 2). These engineered TdTs are capable of adding nucleoside triphosphates with a 3'-O-removable blocking group and other natural or modified NTPs to the 3'-OH end of a growing oligonucleotide or polynucleotide chain in a template-independent manner. After removal of the blocking group, additional rounds of NTP addition can be used to synthesize a polynucleotide with a defined sequence of bases without using a complementary template strand as a guide for NTP incorporation (template-independent synthesis).

[0026] In some embodiments, the present invention provides an engineered TdT polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to a reference sequence of SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246 comprising at least one substitution or one substitution set at one or more positions, wherein the positions are numbered with reference to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246 and wherein the engineered TdT polypeptide has improved thermostability, increased activity at elevated temperatures, increased soluble expression or isolated protein yield, decreased by-product formation, increased specific activity on NTP-3'-O-RBG and other natural or modified NTP substrates, and/or increased activity on various oligo acceptor substrates as compared to a wild-type TdT or other TdTs or template-independent polymerases known to those of skill in the art. These engineered TdT polypeptides with one or more amino acid substitutions or substitution sets are described, below, in the detailed description of the invention.

[0027] In some additional embodiments, the engineered polypeptide comprises an amino acid sequence with at least 60% sequence identity to any even-numbered sequence set forth in SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476.

[0028] In an additional embodiment, the engineered polypeptide of the present invention further comprises an N-terminal truncation of 1-156 amino acids of the polypeptide sequence relative to any even-numbered sequence set forth in SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476.

[0029] In an additional embodiment, the engineered polypeptide of the present invention is fused with a second polypeptide; optionally, wherein the second polypeptide has inorganic pyrophosphatase (IPP) activity (e.g., an IPP with an amino acid sequence selected from SEQ ID NO: 3942 and 3944). In one embodiment, the engineered polypeptide of the present invention fused with a second polypeptide with IPP activity comprises a sequence selected from SEQ ID NO: 5468, 5470, 5472, and 5474.

[0030] The present invention also provides an engineered polynucleotide encoding at least one engineered polypeptide described in the above paragraphs. In some embodiments, the engineered polynucleotide comprises the odd-numbered sequences set forth in SEQ ID NOs: 3-1959, 2003-3919, 4047-5465, and 5475.

[0031] The present invention further provides vectors comprising at least one engineered polynucleotide described above. In some embodiments, the vectors further comprise at least one control sequence.

[0032] The present invention also provides host cells comprising the vectors provided herein. In some embodiments, the host cell produces at least one engineered polypeptide provided herein.

[0033] The present invention further provides methods of producing an engineered TdT polypeptide, comprising the steps of culturing the host cell provided herein under conditions such that the engineered polynucleotide is expressed and the engineered polypeptide is produced. In some embodiments, the methods further comprise the step of recovering the engineered polypeptide.

[0034] The present invention further provides a method of template-independent synthesis, comprising a TdT or template-independent polymerase with activity on various oligo acceptor substrates and NTP-3'-O-RBG and other natural or modified NTP substrates, wherein the method may comprise an immobilized TdT or an immobilized oligo acceptor substrate or neither an immobilized TdT nor an immobilized oligo acceptor substrate.

DESCRIPTION OF THE INVENTION

[0035] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Generally, the nomenclature used herein and the laboratory procedures of cell culture, molecular genetics, microbiology, organic chemistry, analytical chemistry and nucleic acid chemistry described below are those well-known and commonly employed in the art. Such techniques are well-known and described in numerous texts and reference works well known to those of skill in the art. Standard techniques, or modifications thereof, are used for chemical syntheses and chemical analyses. All patents, patent applications, articles and publications mentioned herein, both *supra* and *infra*, are hereby expressly incorporated herein by reference.

[0036] Although any suitable methods and materials similar or equivalent to those described herein find use in the practice of the present invention, some methods and materials are described herein. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary, depending upon the context they are used by those of skill in the art. Accordingly, the terms defined immediately below are more fully described by reference to the invention as a whole.

[0037] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the present invention. The section headings used herein are for organizational purposes only and not to be construed as limiting the

subject matter described. Numeric ranges are inclusive of the numbers defining the range. Thus, every numerical range disclosed herein is intended to encompass every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein. It is also intended that every maximum (or minimum) numerical limitation disclosed herein includes every lower (or higher) numerical limitation, as if such lower (or higher) numerical limitations were expressly written herein.

[0038] As used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to “a polypeptide” includes more than one polypeptide. Similarly, “comprise,” “comprises,” “comprising” “include,” “includes,” and “including” are interchangeable and not intended to be limiting.

[0039] It is to be understood that where descriptions of various embodiments use the term “comprising,” those skilled in the art would understand that in some specific instances, an embodiment can be alternatively described using language “consisting essentially of” or “consisting of.” It is to be further understood that where descriptions of various embodiments use the term “optional” or “optionally” the subsequently described event or circumstance may or may not occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not. It is to be understood that both the foregoing general description, and the following detailed description are exemplary and explanatory only and are not restrictive of this disclosure. The section headings used herein are for organizational purposes only and not to be construed as limiting the subject matter described.

[0040] Abbreviations

[0041] The abbreviations used for the genetically encoded amino acids are conventional and are as follows:

Amino Acid	Three-Letter	One-Letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartate	Asp	D
Cysteine	Cys	C
Glutamate	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P

Amino Acid	Three-Letter	One-Letter Abbreviation
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[0042] When the three-letter abbreviations are used, unless specifically preceded by an “L” or a “D” or clear from the context in which the abbreviation is used, the amino acid may be in either the L- or D-configuration about α -carbon (C_α). For example, whereas “Ala” designates alanine without specifying the configuration about the α -carbon, “D-Ala” and “L-Ala” designate D-alanine and L-alanine, respectively.

[0043] When the one-letter abbreviations are used, upper case letters designate amino acids in the L-configuration about the α -carbon and lower-case letters designate amino acids in the D-configuration about the α -carbon. For example, “A” designates L-alanine and “a” designates D-alanine. When polypeptide sequences are presented as a string of one-letter or three-letter abbreviations (or mixtures thereof), the sequences are presented in the amino (N) to carboxy (C) direction in accordance with common convention.

[0044] The abbreviations used for the genetically encoding nucleosides are conventional and are as follows: adenosine (A); guanosine (G); cytidine (C); thymidine (T); and uridine (U). These abbreviations are also used interchangeably for nucleosides and nucleotides (nucleosides with one or more phosphate groups). Unless specifically delineated, the abbreviated nucleosides or nucleotides may be either ribonucleosides (or ribonucleotides) or 2'-deoxyribonucleosides (or 2'-deoxyribonucleotides). The nucleosides or nucleotides may also be modified at the 3' position. The nucleosides or nucleotides may be specified as being either ribonucleosides (or ribonucleotides) or 2'-deoxyribonucleosides (or 2'-deoxyribonucleotides) on an individual basis or on an aggregate basis. When nucleic acid sequences are presented as a string of one-letter abbreviations, the sequences are presented in the 5' to 3' direction in accordance with common convention, and the phosphates are not indicated.

[0045] Definitions

[0046] In reference to the present invention, the technical and scientific terms used in the descriptions herein will have the meanings commonly understood by one of ordinary skill in the art, unless specifically defined otherwise. Accordingly, the following terms are intended to have the following meanings.

[0047] “EC” number refers to the Enzyme Nomenclature of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB). The IUBMB biochemical classification is a numerical classification system for enzymes based on the chemical reactions they catalyze.

[0048] “ATCC” refers to the American Type Culture Collection whose biorepository collection includes genes and strains.

[0049] “NCBI” refers to National Center for Biological Information and the sequence databases provided therein.

[0050] “Protein,” “polypeptide,” and “peptide” are used interchangeably herein to denote a polymer of at least two amino acids covalently linked by an amide bond, regardless of length or post-translational modification (e.g., glycosylation, phosphorylation, lipidation, myristilation, ubiquitination, etc.). Included within this definition are D- and L-amino acids, and mixtures of D- and L-amino acids, as well as polymers comprising D- and L-amino acids, and mixtures of D- and L-amino acids.

[0051] “Amino acids” are referred to herein by either their commonly known three-letter symbols or by the one-letter symbols recommended by IUPAC-IUB Biochemical Nomenclature Commission.

Nucleotides, likewise, may be referred to by their commonly accepted single letter codes.

[0052] As used herein, “polynucleotide,” “oligonucleotide,” and “nucleic acid” are used interchangeably herein and refer to two or more nucleosides or nucleotides that are covalently linked together. The polynucleotide may be wholly comprised of ribonucleotides (i.e., RNA), wholly comprised of 2' deoxyribonucleotides (i.e., DNA), wholly comprised of other synthetic nucleotides or comprised of mixtures of synthetic, ribo- and/or 2' deoxyribonucleotides. The polynucleotides may also include modified nucleotides with substitutions, including 2' substitutions (e.g., 2'-fluoro, 2'-O-methyl, 2'-O-methoxyethyl, locked or constrained ethyl modifications, and others known to those skilled in the art). Nucleosides will be linked together via standard phosphodiester linkages or via one or more non-standard linkages, including but not limited to phosphorothioate linkages. The polynucleotide may be single-stranded or double-stranded or may include both single-stranded regions and double-stranded regions. Moreover, while a polynucleotide will typically be composed of the naturally occurring encoding nucleobases (i.e., adenine, guanine, uracil, thymine and cytosine), it may include one or more modified and/or synthetic nucleobases, such as, for example, inosine, xanthine, hypoxanthine, etc. In some embodiments, such modified or synthetic nucleobases are nucleobases encoding amino-acid sequences. Nucleobases that are modified or synthetic may comprise any known or hypothetical or future discovered modification or structure that would be recognized by one of skill in the art as a modified or synthetic nucleobase. Similarly, the terms “polynucleotide,” “oligonucleotide,” and “nucleic acid” are intended to comprise any modified or synthetic structure that is now known or discovered in the future that would be recognized by one of skill in the art as being or having the function of a “polynucleotide,” “oligonucleotide,” or “nucleic acid.” An example of a modified or synthetic structure having the function of a “polynucleotide,” “oligonucleotide,” or “nucleic acid” is PNA or peptide nucleic acid.

[0053] As used herein, “oligo acceptor substrate” and “acceptor substrate” and “growing oligo acceptor substrate strand” and “growing oligonucleotide chain” and “growing polynucleotide strand” are used interchangeably herein and refer to any oligo or nucleotide chain or similar moiety with an exposed 3'-OH or equivalent thereof that may be recognized by a wild-type TdT or polymerase or an engineered TdT or template-independent polymerase of the current disclosure as a substrate for nucleoside addition or synthesis. In some embodiments, the acceptor substrate may be single stranded. In yet other embodiments,

the acceptor substrate may be double stranded or partially doubled stranded. In some embodiments, the acceptor substrate may comprise a nucleotide chain consisting of 1-10 nucleotides, 5-20 nucleotides, 15-50 nucleotides, 30-100 nucleotides, or greater than 100 nucleotides. In some embodiments, the acceptor substrate may comprise a chemical moiety that is not a nucleotide chain but contains a free -OH capable of being recognized as a substrate by a wild-type or engineered TdT, referred to herein as a "3'-OH equivalent". Exemplary oligo acceptor substrates are provided in the Examples.

[0054] As used herein, "nucleoside triphosphate-3'-O-removable blocking group" and "nucleotide triphosphate-3'-O-removable blocking group" and "reversible terminator" and "NTP-3'-O-RBG" are used interchangeably herein and refer to a ribonucleoside triphosphate or a deoxyribonucleoside triphosphate or a synthetic or nucleoside triphosphate composed of an alternate or modified sugar with a removable blocking group attached at the 3' position of the sugar moiety. An NTP-3'-O-RBG may also include other modifications as described herein, including but not limited to modifications at the 2' position, modifications to the nucleobase, and modifications to the phosphates. A nucleotide may also have a 3'-O-RBG, as is expected after reaction of an NTP-3'-O-RBG with an engineered TdT of the present disclosure and an oligo acceptor substrate.

[0055] As used herein, "oligo acceptor product" and "growing oligonucleotide chain" and "oligo acceptor extension product" are used interchangeably herein and refer to the product of a NTP-3'-O-RBG or other natural or modified NTP substrate and an oligo acceptor substrate, wherein a TdT or related polymerase has catalyzed the extension or addition of a nucleotide-3'-O-RBG or other natural or modified nucleotide substrate to an oligo acceptor substrate via reaction with one or more NTP-3'-O-RBGs or other natural or modified NTP substrates.

[0056] As used herein, "removable blocking group" and "blocking group" and "terminator group" and "reversible terminating group" and "inhibitor group" and related variations of these terms are used interchangeably herein and refer to a chemical group that would hinder addition of a second NTP-3'-O-RBG or other natural or modified NTP substrate to the 3' end of the growing oligo acceptor substrate strand prior to removal of the removable blocking from the first round of addition. In some embodiments, the NTP-3'-O-RBG or other natural or modified NTP substrate may comprise a removable blocking group selected from the group consisting of NTP-3'-O-NH₂, or NTP-3'-O-PO₃. In some embodiments, the NTP-3'-O-RBG or other natural or modified NTP substrate may have a natural purine or pyrimidine base, such as adenine, guanine, cytosine, thymine, or uridine. In some embodiments, NTP-3'-O-RBG or other natural or modified NTP substrates may have an unnatural base analog such as inosine, xanthine, hypoxanthine or another base analog, as is known in the art. In some embodiments the blocking group may comprise or may additionally comprise a modification at the 2' position.

[0057] As used herein, "template-independent synthesis" refers to synthesis of an oligonucleotide or a polynucleotide without the use of template strand as a guide for synthesis of a complementary oligo or polynucleotide strand. Thus, template-independent synthesis refers to an iterative process, whereby, successive nucleotides are added to a growing oligo or nucleotide chain or acceptor substrate. Template-

independent synthesis may be in a sequence defined manner or may be random, as is the case with the wild-type TdT in creating antigen receptor diversity. Processes for template-independent synthesis are further described herein.

[0058] “Coding sequence” refers to that portion of a nucleic acid (e.g., a gene) that encodes an amino acid sequence of a protein.

[0059] “Naturally-occurring” or “wild-type” refers to the form found in nature. For example, a naturally occurring or wild-type polypeptide or polynucleotide sequence is a sequence present in an organism that can be isolated from a source in nature and which has not been intentionally modified by human manipulation.

[0060] As used herein, “recombinant,” “engineered,” and “non-naturally occurring” when used with reference to a cell, nucleic acid, or polypeptide, refer to a material, or a material corresponding to the natural or native form of the material, that has been modified in a manner that would not otherwise exist in nature. In some embodiments, the cell, nucleic acid or polypeptide is identical to a naturally occurring cell, nucleic acid or polypeptide, but is produced or derived from synthetic materials and/or by manipulation using recombinant techniques. Non-limiting examples include, among others, recombinant cells expressing genes that are not found within the native (non-recombinant) form of the cell or expressed native genes that are otherwise expressed at a different level.

[0061] “Percentage of sequence identity” and “percentage homology” are used interchangeably herein to refer to comparisons among polynucleotides or polypeptides, and are determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence for optimal alignment of the two sequences. The percentage may be calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Alternatively, the percentage may be calculated by determining the number of positions at which either the identical nucleic acid base or amino acid residue occurs in both sequences or a nucleic acid base or amino acid residue is aligned with a gap to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Those of skill in the art appreciate that there are many established algorithms available to align two sequences. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman (Smith and Waterman, Adv. Appl. Math., 2:482 [1981]), by the homology alignment algorithm of Needleman and Wunsch (Needleman and Wunsch, J. Mol. Biol., 48:443 [1970]), by the search for similarity method of Pearson and Lipman (Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444 [1988]), by computerized implementations of these algorithms (e.g., GAP, BESTFIT, FASTA, and TFASTA in the GCG Wisconsin Software Package), or by

visual inspection, as known in the art. Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity include, but are not limited to the BLAST and BLAST 2.0 algorithms, which are described by Altschul et al. (See, Altschul et al., J. Mol. Biol., 215: 403-410 [1990]; and Altschul et al., Nucl. Acids Res., 3389-3402 [1977], respectively). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information website. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as, the neighborhood word score threshold (Altschul et al., *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSum62 scoring matrix (See, Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 [1989]). Exemplary determination of sequence alignment and % sequence identity can employ the BESTFIT or GAP programs in the GCG Wisconsin Software package (Accelrys, Madison WI), using default parameters provided.

[0062] “Reference sequence” refers to a defined sequence used as a basis for a sequence comparison. A reference sequence may be a subset of a larger sequence, for example, a segment of a full-length gene or polypeptide sequence. Generally, a reference sequence is at least 20 nucleotide or amino acid residues in length, at least 25 residues in length, at least 50 residues in length, or the full length of the nucleic acid or polypeptide. Since two polynucleotides or polypeptides may each (1) comprise a sequence (*i.e.*, a portion of the complete sequence) that is similar between the two sequences, and (2) may further comprise a sequence that is divergent between the two sequences, sequence comparisons between two (or more) polynucleotides or polypeptide are typically performed by comparing sequences of the two polynucleotides or polypeptides over a “comparison window” to identify and compare local regions of sequence similarity. In some embodiments, a “reference sequence” can be based on a primary amino acid sequence, where the reference sequence is a sequence that can have one or more changes in the primary sequence. For instance, a “reference sequence based on SEQ ID NO:4 having at the residue corresponding

to X14 a valine” or X14V refers to a reference sequence in which the corresponding residue at X14 in SEQ ID NO:4, which is a tyrosine, has been changed to valine.

[0063] “Comparison window” refers to a conceptual segment of at least about 20 contiguous nucleotide positions or amino acids residues wherein a sequence may be compared to a reference sequence of at least 20 contiguous nucleotides or amino acids and wherein the portion of the sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The comparison window can be longer than 20 contiguous residues, and includes, optionally 30, 40, 50, 100, or longer windows.

[0064] As used herein, “substantial identity” refers to a polynucleotide or polypeptide sequence that has at least 80 percent sequence identity, at least 85 percent identity, at least between 89 to 95 percent sequence identity, or more usually, at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 20 residue positions, frequently over a window of at least 30-50 residues, wherein the percentage of sequence identity is calculated by comparing the reference sequence to a sequence that includes deletions or additions which total 20 percent or less of the reference sequence over the window of comparison. In some specific embodiments applied to polypeptides, the term “substantial identity” means that two polypeptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 89 percent sequence identity, at least 95 percent sequence identity or more (e.g., 99 percent sequence identity). In some embodiments, residue positions that are not identical in sequences being compared differ by conservative amino acid substitutions.

[0065] “Corresponding to,” “reference to,” and “relative to” when used in the context of the numbering of a given amino acid or polynucleotide sequence refer to the numbering of the residues of a specified reference sequence when the given amino acid or polynucleotide sequence is compared to the reference sequence. In other words, the residue number or residue position of a given polymer is designated with respect to the reference sequence rather than by the actual numerical position of the residue within the given amino acid or polynucleotide sequence. For example, a given amino acid sequence, such as that of an engineered TdT, can be aligned to a reference sequence by introducing gaps to optimize residue matches between the two sequences. In these cases, although the gaps are present, the numbering of the residue in the given amino acid or polynucleotide sequence is made with respect to the reference sequence to which it has been aligned.

[0066] “Amino acid difference” or “residue difference” refers to a change in the amino acid residue at a position of a polypeptide sequence relative to the amino acid residue at a corresponding position in a reference sequence. The positions of amino acid differences generally are referred to herein as “X_n,” where n refers to the corresponding position in the reference sequence upon which the residue difference is based. For example, a “residue difference at position X25 as compared to SEQ ID NO: 2” refers to a change of the amino acid residue at the polypeptide position corresponding to position 25 of SEQ ID

NO:2. Thus, if the reference polypeptide of SEQ ID NO: 2 has a valine at position 25, then a “residue difference at position X25 as compared to SEQ ID NO:2” an amino acid substitution of any residue other than valine at the position of the polypeptide corresponding to position 25 of SEQ ID NO: 2. In most instances herein, the specific amino acid residue difference at a position is indicated as “XnY” where “Xn” specified the corresponding position as described above, and “Y” is the single letter identifier of the amino acid found in the engineered polypeptide (i.e., the different residue than in the reference polypeptide). In some embodiments, more than one amino acid can appear in a specified residue position (i.e., the alternative amino acids can be listed in the form XnY/Z, where Y and Z represent alternate amino acid residues). In some instances (e.g., in Tables 5.1, 6.2, 7.2, 8.2, 9.2, 10.2, 11.2, 12.2, 13.2, 14.2, 15.2, 16.2, 17.2, 18.2, 19.2, 20.2, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 26.3, 26.4, 27.2, 27.3, 27.4, 27.5, 28.1, 28.2, 28.3, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35.2, 36.2, 37.2, 38.2, 39.2, 40.2, 41.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 49.2, 50.2, 51.2, 52.2, 53.2, 54.2, 55.2, 56.2, 56.3, 56.4, 61.2, 63.2, 64.2, 65.2, 66.2, 67.2, 68.2, 69.2, 70.2, 71.2, 72.2, 73.2, 74.2, 75.2, 76.2, 77.2, 78.2, 79.2, and 80.1) the present invention also provides specific amino acid differences denoted by the conventional notation “AnB”, where A is the single letter identifier of the residue in the reference sequence, “n” is the number of the residue position in the reference sequence, and B is the single letter identifier of the residue substitution in the sequence of the engineered polypeptide. Furthermore, in some instances, a polypeptide of the present invention can include one or more amino acid residue differences relative to a reference sequence, which is indicated by a list of the specified positions where changes are made relative to the reference sequence. In some additional embodiments, the present invention provides engineered polypeptide sequences comprising both conservative and non-conservative amino acid substitutions.

[0067] As used herein, “conservative amino acid substitution” refers to a substitution of a residue with a different residue having a similar side chain, and thus typically involves substitution of the amino acid in the polypeptide with amino acids within the same or similar defined class of amino acids. By way of example and not limitation, an amino acid with an aliphatic side chain is substituted with another aliphatic amino acid (e.g., alanine, valine, leucine, and isoleucine); an amino acid with a hydroxyl side chain is substituted with another amino acid with a hydroxyl side chain (e.g., serine and threonine); an amino acid having an aromatic side chain is substituted with another amino acid having an aromatic side chain (e.g., phenylalanine, tyrosine, tryptophan, and histidine); an amino acid with a basic side chain is substituted with another amino acid with a basic side chain (e.g., lysine and arginine); an amino acid with an acidic side chain is substituted with another amino acid with an acidic side chain (e.g., aspartic acid or glutamic acid); and/or a hydrophobic or hydrophilic amino acid is replaced with another hydrophobic or hydrophilic amino acid, respectively. Exemplary conservative substitutions are provided in Table 1 below.

Table 1. Conservative Amino Acid Substitution Examples	
Residue	Possible Conservative Substitutions
A, L, V, I	Other aliphatic (A, L, V, I) Other non-polar (A, L, V, I, G, M)

G, M	Other non-polar (A, L, V, I, G, M)
D, E	Other acidic (D, E)
K, R	Other basic (K, R)
N, Q, S, T	Other polar
H, Y, W, F	Other aromatic (H, Y, W, F)
C, P	None

[0068] “Non-conservative substitution” refers to substitution of an amino acid in the polypeptide with an amino acid with significantly differing side chain properties. Non-conservative substitutions may use amino acids between, rather than within, the defined groups and affects (a) the structure of the peptide backbone in the area of the substitution (*e.g.*, proline for glycine), (b) the charge or hydrophobicity, or (c) the bulk of the side chain. By way of example and not limitation, an exemplary non-conservative substitution can be an acidic amino acid substituted with a basic or aliphatic amino acid; an aromatic amino acid substituted with a small amino acid; and a hydrophilic amino acid substituted with a hydrophobic amino acid.

[0069] “Deletion” refers to modification to the polypeptide by removal of one or more amino acids from the reference polypeptide. Deletions can comprise removal of 1 or more amino acids, 2 or more amino acids, 5 or more amino acids, 10 or more amino acids, 15 or more amino acids, or 20 or more amino acids, up to 10% of the total number of amino acids, or up to 20% of the total number of amino acids making up the reference enzyme while retaining enzymatic activity and/or retaining the improved properties of an engineered TdT enzyme. Deletions can be directed to the internal portions and/or terminal portions of the polypeptide. In various embodiments, the deletion can comprise a continuous segment or can be discontinuous.

[0070] “Insertion” refers to modification to the polypeptide by addition of one or more amino acids from the reference polypeptide. In some embodiments, the improved engineered TdT enzymes comprise insertions of one or more amino acids to the naturally occurring polypeptide as well as insertions of one or more amino acids to other improved TdT polypeptides. Insertions can be in the internal portions of the polypeptide, or to the carboxy or amino terminus. Insertions as used herein include fusion proteins as is known in the art. The insertion can be a contiguous segment of amino acids or separated by one or more of the amino acids in the naturally occurring polypeptide.

[0071] “Fragment” as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the sequence. Fragments can be at least 14 amino acids long, at least 20 amino acids long, at least 50 amino acids long or longer, and up to 70%, 80%, 90%, 95%, 98%, and 99% of the full-length TdT polypeptide, for example the polypeptide of SEQ ID NO: 2 or an TdT provided in the even-numbered sequences of SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476.

[0072] “Isolated polypeptide” refers to a polypeptide which is substantially separated from other contaminants that naturally accompany it, *e.g.*, protein, lipids, and polynucleotides. The term embraces polypeptides which have been removed or purified from their naturally-occurring environment or

expression system (*e.g.*, host cell or *in vitro* synthesis). The engineered TdT enzymes may be present within a cell, present in the cellular medium, or prepared in various forms, such as lysates or isolated preparations. As such, in some embodiments, the engineered TdT enzyme can be an isolated polypeptide. [0073] “Substantially pure polypeptide” refers to a composition in which the polypeptide species is the predominant species present (*i.e.*, on a molar or weight basis it is more abundant than any other individual macromolecular species in the composition), and is generally a substantially purified composition when the object species comprises at least about 50 percent of the macromolecular species present by mole or % weight. Generally, a substantially pure TdT composition will comprise about 60 % or more, about 70% or more, about 80% or more, about 90% or more, about 95% or more, and about 98% or more of all macromolecular species by mole or % weight present in the composition. In some embodiments, the object species is purified to essential homogeneity (*i.e.*, contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species. Solvent species, small molecules (<500 Daltons), and elemental ion species are not considered macromolecular species. In some embodiments, the isolated engineered TdT polypeptide is a substantially pure polypeptide composition.

[0074] As used herein, “improved enzyme property” refers to at least one improved property of an enzyme. In some embodiments, the present invention provides engineered TdT polypeptides that exhibit an improvement in any enzyme property as compared to a reference TdT polypeptide and/or a wild-type TdT polypeptide, and/or another engineered TdT polypeptide. For the engineered TdT polypeptides described herein, the comparison is generally made to the wild-type enzyme from which the TdT is derived, although in some embodiments, the reference enzyme can be another improved engineered TdT. Thus, the level of “improvement” can be determined and compared between various TdT polypeptides, including wild-type, as well as engineered TdTs. Improved properties include, but are not limited, to such properties as enzymatic activity (which can be expressed in terms of percent conversion of the substrate), thermostability, solvent stability, pH activity profile, cofactor requirements, refractoriness to inhibitors (*e.g.*, substrate or product inhibition), activity at elevated temperatures, increased soluble expression, decreased by-product formation, increased specific activity on NTP-3'-O-RBG substrates, increased incorporation efficiency in extension of oligo acceptor substrates, and/or increased activity on various oligo acceptor substrates (including enantioselectivity).

[0075] “Increased enzymatic activity” refers to an improved property of the TdT polypeptides, which can be represented by an increase in specific activity (*e.g.*, product produced/time/weight protein) or an increase in percent conversion of the substrate to the product (*e.g.*, percent conversion of starting amount of substrate to product in a specified time period using a specified amount of TdT) as compared to the reference TdT enzyme. Exemplary methods to determine enzyme activity are provided in the Examples. Any property relating to enzyme activity may be affected, including the classical enzyme properties of K_m , V_{max} or k_{cat} , changes of which can lead to increased enzymatic activity. Improvements in enzyme activity can be from about 1.2 times the enzymatic activity of the corresponding wild-type enzyme, to as much as

2 times, 5 times, 10 times, 20 times, 25 times, 50 times or more enzymatic activity than the naturally occurring or another engineered TdT from which the TdT polypeptides were derived. TdT activity can be measured by any one of standard assays, such as by monitoring changes in properties of substrates, cofactors, or products. In some embodiments, the amount of products generated can be measured by Liquid Chromatography-Mass Spectrometry (LC-MS), HPLC, or other methods, as known in the art. Comparisons of enzyme activities are made using a defined preparation of enzyme, a defined assay under a set condition, and one or more defined substrates, as further described in detail herein. Generally, when lysates are compared, the numbers of cells and the amount of protein assayed are determined as well as use of identical expression systems and identical host cells to minimize variations in amount of enzyme produced by the host cells and present in the lysates.

[0076] “Conversion” refers to the enzymatic conversion of the substrate(s) to the corresponding product(s). “Percent conversion” refers to the percent of the substrate that is converted to the product within a period of time under specified conditions. Thus, the “enzymatic activity” or “activity” of a TdT polypeptide can be expressed as “percent conversion” of the substrate to the product.

[0077] “Thermostable” refers to a polypeptide that maintains similar activity (more than 60% to 80% for example) after exposure to elevated temperatures (*e.g.*, 40-80 °C) for a period of time (*e.g.*, 0.5-24 hrs) compared to the wild-type enzyme exposed to the same elevated temperature.

[0078] “Solvent stable” refers to a polypeptide that maintains similar activity (more than *e.g.*, 60% to 80%) after exposure to varying concentrations (*e.g.*, 5-99%) of solvent (ethanol, isopropyl alcohol, dimethylsulfoxide (DMSO), tetrahydrofuran, 2-methyltetrahydrofuran, acetone, toluene, butyl acetate, methyl tert-butyl ether, etc.) for a period of time (*e.g.*, 0.5-24 hrs) compared to the wild-type enzyme exposed to the same concentration of the same solvent.

[0079] “Thermo- and solvent stable” refers to a polypeptide that is both thermostable and solvent stable.

[0080] The term “stringent hybridization conditions” is used herein to refer to conditions under which nucleic acid hybrids are stable. As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature (T_m) of the hybrids. In general, the stability of a hybrid is a function of ion strength, temperature, G/C content, and the presence of chaotropic agents. The T_m values for polynucleotides can be calculated using known methods for predicting melting temperatures (See *e.g.*, Baldino et al., *Meth. Enzymol.*, 168:761-777 [1989]; Bolton et al., *Proc. Natl. Acad. Sci. USA* 48:1390 [1962]; Bresslauer et al., *Proc. Natl. Acad. Sci. USA* 83:8893-8897 [1986]; Freier et al., *Proc. Natl. Acad. Sci. USA* 83:9373-9377 [1986]; Kierzek et al., *Biochem.*, 25:7840-7846 [1986]; Rychlik et al., 1990, *Nucl. Acids Res.*, 18:6409-6412 [1990] (erratum, *Nucl. Acids Res.*, 19:698 [1991]); Sambrook et al., *supra*; Suggs et al., 1981, in *Developmental Biology Using Purified Genes*, Brown et al. [eds.], pp. 683-693, Academic Press, Cambridge, MA [1981]; and Wetmur, *Crit. Rev. Biochem. Mol. Biol.*, 26:227-259 [1991]). In some embodiments, the polynucleotide encodes the polypeptide disclosed herein and hybridizes under defined conditions, such as moderately stringent or highly stringent conditions, to the complement of a sequence encoding an engineered TdT enzyme of the present invention.

[0081] “Hybridization stringency” relates to hybridization conditions, such as washing conditions, in the hybridization of nucleic acids. Generally, hybridization reactions are performed under conditions of lower stringency, followed by washes of varying but higher stringency. The term “moderately stringent hybridization” refers to conditions that permit target-DNA to bind a complementary nucleic acid that has about 60% identity, preferably about 75% identity, about 85% identity to the target DNA, with greater than about 90% identity to target-polynucleotide. Exemplary moderately stringent conditions are conditions equivalent to hybridization in 50% formamide, 5× Denhart's solution, 5×SSPE, 0.2% SDS at 42 °C, followed by washing in 0.2×SSPE, 0.2% SDS, at 42 °C. “High stringency hybridization” refers generally to conditions that are about 10 °C or less from the thermal melting temperature T_m as determined under the solution condition for a defined polynucleotide sequence. In some embodiments, a high stringency condition refers to conditions that permit hybridization of only those nucleic acid sequences that form stable hybrids in 0.018M NaCl at 65 °C (*i.e.*, if a hybrid is not stable in 0.018M NaCl at 65 °C, it will not be stable under high stringency conditions, as contemplated herein). High stringency conditions can be provided, for example, by hybridization in conditions equivalent to 50% formamide, 5× Denhart's solution, 5×SSPE, 0.2% SDS at 42 °C, followed by washing in 0.1×SSPE, and 0.1% SDS at 65 °C. Another high stringency condition is hybridizing in conditions equivalent to hybridizing in 5X SSC containing 0.1% (w:v) SDS at 65 °C and washing in 0.1x SSC containing 0.1% SDS at 65 °C. Other high stringency hybridization conditions, as well as moderately stringent conditions, are described in the references cited above.

[0082] “Heterologous” polynucleotide refers to any polynucleotide that is introduced into a host cell by laboratory techniques and includes polynucleotides that are removed from a host cell, subjected to laboratory manipulation, and then reintroduced into a host cell.

[0083] “Codon optimized” refers to changes in the codons of the polynucleotide encoding a protein to those preferentially used in a particular organism such that the encoded protein is efficiently expressed in the organism of interest. Although the genetic code is degenerate in that most amino acids are represented by several codons, called “synonyms” or “synonymous” codons, it is well known that codon usage by particular organisms is nonrandom and biased towards particular codon triplets. This codon usage bias may be higher in reference to a given gene, genes of common function or ancestral origin, highly expressed proteins versus low copy number proteins, and the aggregate protein coding regions of an organism's genome. In some embodiments, the polynucleotides encoding the TdT enzymes may be codon optimized for optimal production from the host organism selected for expression.

[0084] As used herein, “preferred, optimal, high codon usage bias codons” refers interchangeably to codons that are used at higher frequency in the protein coding regions than other codons that code for the same amino acid. The preferred codons may be determined in relation to codon usage in a single gene, a set of genes of common function or origin, highly expressed genes, the codon frequency in the aggregate protein coding regions of the whole organism, codon frequency in the aggregate protein coding regions of related organisms, or combinations thereof. Codons whose frequency increases with the level of gene

expression are typically optimal codons for expression. A variety of methods are known for determining the codon frequency (e.g., codon usage, relative synonymous codon usage) and codon preference in specific organisms, including multivariate analysis, for example, using cluster analysis or correspondence analysis, and the effective number of codons used in a gene (See e.g., GCG CodonPreference, Genetics Computer Group Wisconsin Package; CodonW, Peden, University of Nottingham; McInerney, *Bioinform.*, 14:372-73 [1998]; Stenico et al., *Nucl. Acids Res.*, 22:437-46 [1994]; Wright, *Gene* 87:23-29 [1990]). Codon usage tables are available for many different organisms (See e.g., Wada et al., *Nucl. Acids Res.*, 20:2111-2118 [1992]; Nakamura et al., *Nucl. Acids Res.*, 28:292 [2000]; Duret, et al., *supra*; Henaut and Danchin, in *Escherichia coli and Salmonella*, Neidhardt, et al. (eds.), ASM Press, Washington D.C., p. 2047-2066 [1996]). The data source for obtaining codon usage may rely on any available nucleotide sequence capable of coding for a protein. These data sets include nucleic acid sequences actually known to encode expressed proteins (e.g., complete protein coding sequences-CDS), expressed sequence tags (ESTs), or predicted coding regions of genomic sequences (See e.g., Mount, Bioinformatics: Sequence and Genome Analysis, Chapter 8, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. [2001]; Uberbacher, *Meth. Enzymol.*, 266:259-281 [1996]; and Tiwari et al., *Comput. Appl. Biosci.*, 13:263-270 [1997]).

[0085] “Control sequence” is defined herein to include all components, which are necessary or advantageous for the expression of a polynucleotide and/or polypeptide of the present invention. Each control sequence may be native or foreign to the nucleic acid sequence encoding the polypeptide. Such control sequences include, but are not limited to, a leader, polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop signals. The control sequences may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the nucleic acid sequence encoding a polypeptide.

[0086] “Operably linked” is defined herein as a configuration in which a control sequence is appropriately placed (*i.e.*, in a functional relationship) at a position relative to a polynucleotide of interest such that the control sequence directs or regulates the expression of the polynucleotide and/or polypeptide of interest.

[0087] “Promoter sequence” refers to a nucleic acid sequence that is recognized by a host cell for expression of a polynucleotide of interest, such as a coding sequence. The promoter sequence contains transcriptional control sequences, which mediate the expression of a polynucleotide of interest. The promoter may be any nucleic acid sequence which shows transcriptional activity in the host cell of choice including mutant, truncated, and hybrid promoters, and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

[0088] “Suitable reaction conditions” refer to those conditions in the biocatalytic reaction solution (*e.g.*, ranges of enzyme loading, substrate loading, cofactor loading, temperature, pH, buffers, co-solvents, etc.) under which a TdT polypeptide of the present invention is capable of converting one or more substrate

compounds to a product compound (e.g., addition of a nucleotide-3'-O-RBG or other natural or modified nucleotide substrate to an oligo acceptor substrate via reaction with NTP-3'-O-RBG or other natural or modified NTP substrate). Exemplary "suitable reaction conditions" are provided in the present invention and illustrated by the Examples.

[0089] "Composition" refers to a mixture or combination of one or more substances, wherein each substance or component of the composition retains its individual properties. As used herein, a biocatalytic composition refers to a combination of one or more substances useful for biocatalysis.

[0090] "Loading", such as in "compound loading" or "enzyme loading" or "cofactor loading" refers to the concentration or amount of a component in a reaction mixture at the start of the reaction.

[0091] "Substrate" in the context of a biocatalyst mediated process refers to the compound or molecule acted on by the biocatalyst. For example, a TdT biocatalyst used in the synthesis processes disclosed herein acts on an NTP-3'-O-RBG substrate or other natural or modified NTP substrate and an oligo acceptor substrate.

[0092] "Product" in the context of a biocatalyst mediated process refers to the compound or molecule resulting from the action of the biocatalyst. For example, an exemplary product for a TdT biocatalyst used in a process disclosed herein is an oligo acceptor extension product, as depicted in Schemes 1 and 2.

[0093] "Alkyl" refers to saturated hydrocarbon groups of from 1 to 18 carbon atoms inclusively, either straight chained or branched, more preferably from 1 to 8 carbon atoms inclusively, and most preferably 1 to 6 carbon atoms inclusively. An alkyl with a specified number of carbon atoms is denoted in parenthesis (e.g., (C₁-C₆)alkyl refers to an alkyl of 1 to 6 carbon atoms).

[0094] "Alkenyl" refers to hydrocarbon groups of from 2 to 12 carbon atoms inclusively, either straight or branched containing at least one double bond but optionally containing more than one double bond.

[0095] "Alkynyl" refers to hydrocarbon groups of from 2 to 12 carbon atoms inclusively, either straight or branched containing at least one triple bond but optionally containing more than one triple bond, and additionally optionally containing one or more double bonded moieties.

[0096] "Heteroalkyl," "heteroalkenyl," and "heteroalkynyl," refer respectively, to alkyl, alkenyl and alkynyl as defined herein in which one or more of the carbon atoms are each independently replaced with the same or different heteroatoms or heteroatomic groups. Heteroatoms and/or heteroatomic groups which can replace the carbon atoms include, but are not limited to -O-, -S-, -S-O-, -NR^γ-, -PH-, -S(O)-, -S(O)₂-, -S(O)NR^γ-, -S(O)₂NR^γ-, and the like, including combinations thereof, where each R^γ is independently selected from hydrogen, alkyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl.

[0097] "Amino" refers to the group -NH₂. Substituted amino refers to the group -NHR^η, NR^ηR^η, and NR^ηR^ηR^η, where each R^η is independently selected from substituted or unsubstituted alkyl, cycloalkyl, cycloheteroalkyl, alkoxy, aryl, heteroaryl, heteroarylalkyl, acyl, alkoxycarbonyl, sulfanyl, sulfinyl, sulfonyl, and the like. Typical amino groups include, but are limited to, dimethylamino, diethylamino, trimethylammonium, triethylammonium, methylsulfonylamino, furanyl-oxy-sulfamino, and the like.

[0098] "Aminoalkyl" refers to an alkyl group in which one or more of the hydrogen atoms are replaced with one or more amino groups, including substituted amino groups.

[0099] "Aminocarbonyl" refers to $-C(O)NH_2$. Substituted aminocarbonyl refers to $-C(O)NR^nR^q$, where the amino group NR^nR^q is as defined herein.

[0100] "Oxy" refers to a divalent group $-O-$, which may have various substituents to form different oxy groups, including ethers and esters.

[0101] "Alkoxy" or "alkyloxy" are used interchangeably herein to refer to the group $-OR^z$, wherein R^z is an alkyl group, including optionally substituted alkyl groups.

[0102] "Carboxy" refers to $-COOH$.

[0103] "Carbonyl" refers to $-C(O)-$, which may have a variety of substituents to form different carbonyl groups including acids, acid halides, aldehydes, amides, esters, and ketones.

[0104] "Carboxyalkyl" refers to an alkyl in which one or more of the hydrogen atoms are replaced with one or more carboxy groups.

[0105] "Aminocarbonylalkyl" refers to an alkyl substituted with an aminocarbonyl group, as defined herein.

[0106] "Halogen" or "halo" refers to fluoro, chloro, bromo and iodo.

[0107] "Haloalkyl" refers to an alkyl group in which one or more of the hydrogen atoms are replaced with a halogen. Thus, the term "haloalkyl" is meant to include monohaloalkyls, dihaloalkyls, trihaloalkyls, etc. up to perhaloalkyls. For example, the expression " $(C_1 - C_2)$ haloalkyl" includes 1-fluoromethyl, difluoromethyl, trifluoromethyl, 1-fluoroethyl, 1,1-difluoroethyl, 1,2-difluoroethyl, 1,1,1 trifluoroethyl, perfluoroethyl, etc.

[0108] "Hydroxy" refers to $-OH$.

[0109] "Hydroxyalkyl" refers to an alkyl group in which one or more of the hydrogen atoms are replaced with one or more hydroxy groups.

[0110] "Thiol" or "sulfanyl" refers to $-SH$. Substituted thiol or sulfanyl refers to $-S-R^n$, where R^n is an alkyl, aryl or other suitable substituent.

[0111] "Sulfonyl" refers to $-SO_2-$. Substituted sulfonyl refers to $-SO_2-R^n$, where R^n is an alkyl, aryl or other suitable substituent.

[0112] "Alkylsulfonyl" refers to $-SO_2-R^z$, where R^z is an alkyl, which can be optionally substituted. Typical alkylsulfonyl groups include, but are not limited to, methylsulfonyl, ethylsulfonyl, n-propylsulfonyl, and the like.

[0113] "Phosphate" as used herein refers to a functional group comprised of an orthophosphate ion (phosphorous atom covalently linked to four oxygen atoms). The orthophosphate ion is commonly found with one or more hydrogen atoms or organic groups.

[0114] "Phosphorylated" as used herein refers to the addition or presence of one or more phosphoryl groups (phosphorous atom covalently linked to the three oxygen atoms).

[0115] “Optionally substituted” as used herein with respect to the foregoing chemical groups means that positions of the chemical group occupied by hydrogen can be substituted with another atom (unless otherwise specified) exemplified by, but not limited to carbon, oxygen, nitrogen, or sulfur, or a chemical group, exemplified by, but not limited to, hydroxy, oxo, nitro, methoxy, ethoxy, alkoxy, substituted alkoxy, trifluoromethoxy, haloalkoxy, fluoro, chloro, bromo, iodo, halo, methyl, ethyl, propyl, butyl, alkyl, alkenyl, alkynyl, substituted alkyl, trifluoromethyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, thio, alkylthio, acyl, carboxy, alkoxycarbonyl, carboxamido, substituted carboxamido, alkylsulfonyl, alkylsulfinyl, alkylsulfonylamino, sulfonamido, substituted sulfonamido, cyano, amino, substituted amino, alkylamino, dialkylamino, aminoalkyl, acylamino, amidino, amidoximo, hydroxamoyl, phenyl, aryl, substituted aryl, aryloxy, arylalkyl, arylalkenyl, arylalkynyl, pyridyl, imidazolyl, heteroaryl, substituted heteroaryl, heteroaryloxy, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, substituted cycloalkyl, cycloalkyloxy, pyrrolidinyl, piperidinyl, morpholino, heterocycle, (heterocycle)oxy, and (heterocycle)alkyl; where preferred heteroatoms are oxygen, nitrogen, and sulfur. Additionally, where open valences exist on these substitute chemical groups they can be further substituted with alkyl, cycloalkyl, aryl, heteroaryl, and/or heterocycle groups, that where these open valences exist on carbon they can be further substituted by halogen and by oxygen-, nitrogen-, or sulfur-bonded substituents, and where multiple such open valences exist, these groups can be joined to form a ring, either by direct formation of a bond or by formation of bonds to a new heteroatom, preferably oxygen, nitrogen, or sulfur. It is further contemplated that the above substitutions can be made provided that replacing the hydrogen with the substituent does not introduce unacceptable instability to the molecules of the present invention and is otherwise chemically reasonable. One of ordinary skill in the art would understand that with respect to any chemical group described as optionally substituted, only sterically practical and/or synthetically feasible chemical groups are meant to be included. “Optionally substituted” as used herein refers to all subsequent modifiers in a term or series of chemical groups. For example, in the term “optionally substituted arylalkyl,” the “alkyl” portion and the “aryl” portion of the molecule may or may not be substituted, and for the series “optionally substituted alkyl, cycloalkyl, aryl and heteroaryl,” the alkyl, cycloalkyl, aryl, and heteroaryl groups, independently of the others, may or may not be substituted.

[0116] “Reaction” as used herein refers to a process in which one or more substances or compounds or substrates is converted into one or more different substances, compounds, or processes.

[0117] Template-Independent Synthesis by Engineered TdTs

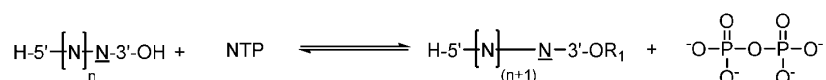
[0118] New methods of efficiently synthesizing high purity strands of DNA, RNA, and other polynucleotides are necessary to overcome the limitations of existing phosphoramidite chemical synthesis methods in order to enable a range of emerging and existing synthetic biology applications.

[0119] The present invention provides novel terminal deoxynucleotidyl transferases that have improved activity in the template-independent synthesis of polynucleotides using 5'-nucleoside triphosphates (“NTPs”) modified with a 3'-O-removable blocking group (NTP-3'-O-RBG) or other natural or modified

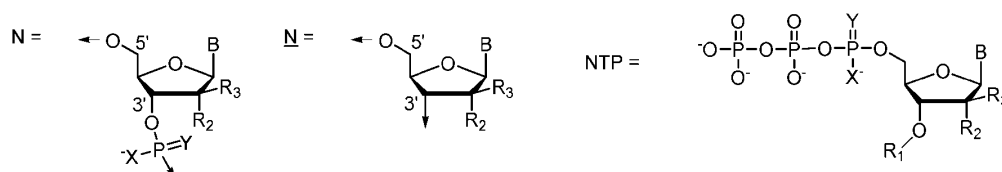
NTP substrates. The TdTs of the present disclosure have improved thermostability, activity at elevated temperatures, increased soluble expression or isolated protein yield, decreased by-product formation, increased affinity for NTP-3'-O-RBG and other natural or modified NTP substrates, increased affinity for oligo acceptor substrates, increased activity or specific activity on NTP-3'-O-RBG and other natural or modified NTP substrates, and/or increased activity or specific activity on various oligo acceptor substrates as compared to a wild-type TdT or other TdTs or template-independent polymerases known to those of skill in the art. The engineered polypeptides of the present disclosure are variants of SEQ ID NO: 2, a predicted splice variant encoded by the genome of species *Monodelphis domestica*. These engineered TdTs are capable of template-independent synthesis of oligonucleotides and polynucleotides.

[0120] Template-independent synthesis of a defined polynucleotide sequence using an engineered TdT is a multistep process. In one embodiment, an oligo acceptor substrate with a 3'-OH allows addition of a defined modified NTP substrate (in this example, an NTP-3'-O-RBG) by an engineered TdT, as depicted in Scheme 1, below.

Scheme 1



where



and

B = adenine, guanine, cytosine, thymine, uracil, pseudouridine, 1-methylpseudouridine

R₁ = benzyl, O-nitrobenzyl, benzoyl, acetyl, cyanoethyl, NO₂, PO₃²⁻, SO₃⁻

R₂ = H, OH, OMe, F, Me, methoxyethyl (MOE)

R₃ = H, OH, OMe, F, Me

X = O, S

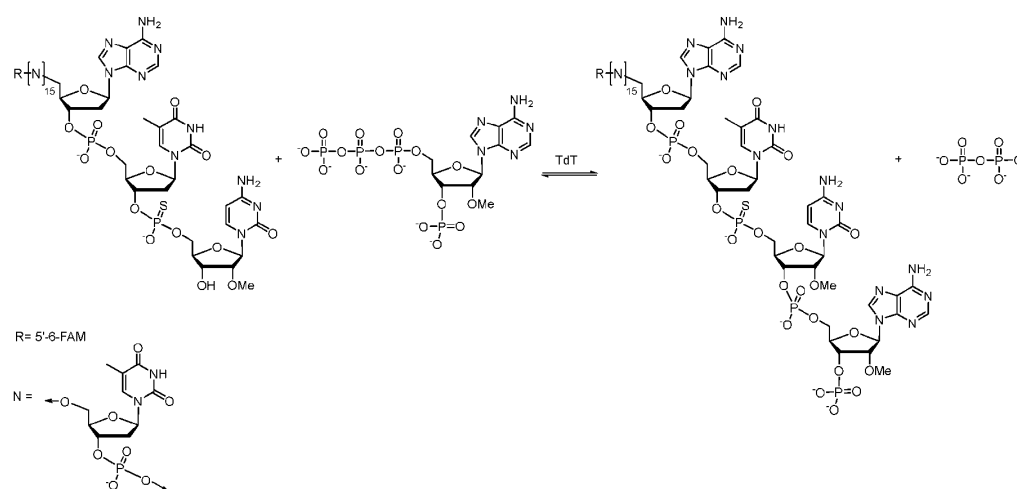
Y = O, S

[0121] After reaction of the NTP-3'-O-RBG with the 3'-OH of oligo acceptor substrate or the growing polynucleotide chain, the TdT is blocked from further reaction by the 3'-O-RBG. The RBG is then removed, exposing the 3'-OH and allowing another round of addition. After each round of addition, the blocking group of the nucleotide-3'-O-RBG or natural or modified nucleotide from the previous round is removed and a new NTP-3'-O-RBG or natural or modified NTP substrate is added to sequentially and efficiently create a defined polynucleotide sequence by addition at the 3'-OH end of the polynucleotide or oligo acceptor substrate without a complementary strand or templating primer sequence. After synthesis of

the defined polynucleotide is complete, the oligonucleotide chain may be cleaved or released from the oligo acceptor substrate.

[0122] A variety of oligo acceptor substrates and NTP-3'-O-RBG or natural or modified NTP substrates may be used in this process, as may be envisioned by one of skill in the art. An example of one reaction is detailed in Scheme 2, below. Scheme 2 depicts the TdT catalyzed reaction of 5'-6-FAM-[N]₁₅AT*mC and 3'-phos-mATP as described in Example 27, while other examples of suitable oligo acceptor substrate and NTP-3'-O-RBG or natural or modified NTP pairs are described in other Examples. These examples are non-limiting.

Scheme 2



[0123] Occasionally, undesired synthesis products are created by the TdT during the addition step. This includes incorporation of NTPs that have lost their blocking group, addition of more than one NTP, or the excision or pyrophosphorolysis of the TdT on the growing polynucleotide chain.

[0124] In some embodiments, one or more additional quality control steps are used, such as adding an exonuclease prior to removing the blocking group and initiating a new round of synthesis. In some embodiments, a phosphatase, such as a pyrophosphatase, is used to breakdown inorganic phosphate and push the reversible TdT reaction toward synthesis.

[0125] As described further herein, the engineered TdT polypeptides of the current disclosure exhibit one of more improved properties in the template-independent polynucleotide synthesis process depicted in Schemes 1 and 2.

[0126] In some embodiments, the present invention provides an engineered TdT polypeptide comprising an amino acid sequence having at least 60% sequence identity to an amino acid reference sequence of SEQ ID NO: 2 and further comprising one or more amino acid residue differences as compared to the reference amino acid sequence, wherein the engineered TdT polypeptide has improved thermostability, increased activity at elevated temperatures, increased soluble expression or isolated protein yield,

decreased by-product formation, increased specific activity on NTP-3'-O-RBG or natural or modified NTP substrates, and/or increased activity on various oligo acceptor substrates as compared to a wild-type TdT or other TdTs or template-independent polymerases known to those of skill in the art.

[0127] In particular, the engineered TdTs polypeptides of the present disclosure have been engineered for efficient synthesis of polynucleotides having a defined sequence using NTP-3'-O-RBG or natural or modified NTP substrates in the process described above.

[0128] A variety of suitable reaction conditions are known to those skilled in the art, as detailed below and in the Examples.

[0129] Engineered Terminal Deoxynucleotidyl Transferase (TdT) Polypeptides

[0130] The present invention provides engineered terminal deoxynucleotidyl transferase (TdT) polypeptides useful in template-independent polynucleotide synthesis using an NTP-3'-O-RBG or natural or modified NTP substrate, as well as compositions and methods of utilizing these engineered polypeptides in template-independent oligonucleotide synthesis.

[0131] The present invention provides TdT polypeptides, polynucleotides encoding the polypeptides, methods of preparing the polypeptides, and methods for using the polypeptides. Where the description relates to polypeptides, it is to be understood that it can describe the polynucleotides encoding the polypeptides.

[0132] Suitable reaction conditions under which the above-described improved properties of the engineered polypeptides carry out the desired reaction can be determined with respect to concentrations or amounts of polypeptide, substrate, co-substrate, buffer, solvent, pH, conditions including temperature and reaction time, and/or conditions with the polypeptide immobilized on a solid support, as further described below and in the Examples.

[0133] In some embodiments, exemplary engineered TdTs comprise an amino acid sequence that has one or more residue differences as compared to SEQ ID NO: 2 at the residue positions indicated in Tables 5.1, 6.2, 7.2, 8.2, 9.2, 10.2, 11.2, 12.2, 13.2, 14.2, 15.2, 16.2, 17.2, 18.2, 19.2, 20.2, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 26.3, 26.4, 27.2, 27.3, 27.4, 27.5, 28.1, 28.2, 28.3, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35.2, 36.2, 37.2, 38.2, 39.2, 40.2, 41.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 49.2, 50.2, 51.2, 52.2, 53.2, 54.2, 55.2, 56.2, 56.3, 56.4, 61.2, 63.2, 64.2, 65.2, 66.2, 67.2, 68.2, 69.2, 70.2, 71.2, 72.2, 73.2, 74.2, 75.2, 76.2, 77.2, 78.2, 79.2, and 80.1.

[0134] The structure and function information for the exemplary engineered polypeptides of the present invention are based on the conversion of an oligo acceptor substrate and a NTP -3'-O-RBG or a dideoxy NTP (e.g. a 2',3'-dideoxy NTP), the results of which are shown below in Tables 5.1, 6.2, 7.2, 8.2, 9.2, 10.2, 11.2, 12.2, 13.2, 14.2, 15.2, 16.2, 17.2, 18.2, 19.2, 20.2, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 26.3, 26.4, 27.2, 27.3, 27.4, 27.5, 28.1, 28.2, 28.3, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35.2, 36.2, 37.2, 38.2, 39.2, 40.2, 41.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 49.2, 50.2, 51.2, 52.2, 53.2, 54.2, 55.2, 56.2, 56.3, 56.4, 61.2, 63.2, 64.2, 65.2, 66.2, 67.2, 68.2, 69.2, 70.2, 71.2, 72.2, 73.2, 74.2, 75.2, 76.2, 77.2, 78.2, 79.2, and 80.1, as further described in the Examples. The odd numbered sequence identifiers (i.e., SEQ ID NOs) in these

Tables refer to the nucleotide sequence encoding the amino acid sequence provided by the even numbered SEQ ID NOs in these Tables. Exemplary sequences are provided in the electronic sequence listing file accompanying this invention, which is hereby incorporated by reference herein. The amino acid residue differences are based on comparison to the reference sequence of SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, as indicated.

[0135] Terminal deoxynucleotidyl transferase, a member of the Pol X family, has been identified in many species. Members of the diverse Pol X family are known to share certain residues, which are conserved across family members. TdT also has a high level of conservation across species for residues thought to be involved in binding divalent metal ions, ternary complex formation, and binding dNTP and DNA ligands (Domínguez et al. (2000). EMBO, 19(7), 1731–1742.) Additionally, TdTs are known to have splice variants which are N-terminal truncations, lacking a BRCT domain. Other template-independent polymerases (including, but not limited to polyA polymerases, polyU polymerases and terminal uridylyltransferases) are also known in the art and may be used to practice the invention. Similarly, other polymerases are known to be capable of template-independent synthesis (including but not limited to reverse transcriptases) and may be used to practice the invention.

[0136] The wild-type TdT from *Monodelphis domestica* (SEQ ID NO: 2) was selected for evolution. The TdT polypeptides of the present disclosure are engineered variants of SEQ ID NO: 2 with a N-terminal 6-histidine tag.

[0137] The polypeptides of the present disclosure have residue differences that result in improved properties necessary to develop an efficient TdT enzyme, capable of template-independent synthesis of polynucleotides having a defined sequence. Various residue differences, at both conserved and non-conserved positions, have been discovered to be related to improvements in various enzymes properties, including improved thermostability, increased activity at elevated temperatures, increased soluble expression or isolated protein yield, decreased by-product formation, increased specific activity on NTP-3'-O-RBG or natural or modified NTP substrates, increased incorporation efficiency in extension of oligo acceptor substrates, and/or increased activity on various oligo acceptor substrates as compared to a wild-type TdT or other TdTs or template-independent polymerases known to those of skill in the art. In some embodiments, the engineered TdT polypeptides exhibit increased incorporation efficiency in extension of an oligo acceptor substrate by addition of an NTP or NQP of greater than 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. Exemplary incorporation efficiency of engineered TdTs are provided in the Examples, e.g., Example 92.

[0138] The activity of each engineered TdT relative to the reference polypeptide of SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246 was determined as conversion of the substrates described in the Examples herein.

In some embodiments, a shake flask purified enzyme (SFP) is used to assess the properties of the engineered TdTs, the results of which are provided in the Examples.

[0139] In some embodiments, the specific enzyme properties are associated with the residues differences as compared to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246 at the residue positions indicated herein. In some embodiments, residue differences affecting polypeptide expression can be used to increase expression of the engineered TdTs.

[0140] In light of the guidance provided herein, it is further contemplated that any of the exemplary engineered polypeptides comprising the sequences of SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246 find use as the starting amino acid sequence for synthesizing other TdT polypeptides, for example by subsequent rounds of evolution that incorporate new combinations of various amino acid differences from other polypeptides in Tables 5.1, 6.2, 7.2, 8.2, 9.2, 10.2, 11.2, 12.2, 13.2, 14.2, 15.2, 16.2, 17.2, 18.2, 19.2, 20.2, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 26.3, 26.4, 27.2, 27.3, 27.4, 27.5, 28.1, 28.2, 28.3, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35.2, 36.2, 37.2, 38.2, 39.2, 40.2, 41.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 49.2, 50.2, 51.2, 52.2, 53.2, 54.2, 55.2, 56.2, 56.3, 56.4, 61.2, 63.2, 64.2, 65.2, 66.2, 67.2, 68.2, 69.2, 70.2, 71.2, 72.2, 73.2, 74.2, 75.2, 76.2, 77.2, 78.2, 79.2, and 80.1, and other residue positions described herein. Further improvements may be generated by including amino acid differences at residue positions that had been maintained as unchanged throughout earlier rounds of evolution.

[0141] In some embodiments, the engineered TdT polypeptide has increased soluble protein expression and comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2 and one or more residue differences as compared to SEQ ID NO: 2, selected from 80/106/121/185/190/205/289/290/293/313/315/336/342/359/391/470/474/499/522/523, 80/106/121/185/190/205/289/290/293/313/342/470/474/499/523, 80/106/121/185/190/244/289/290/293/307/342/359/470/474/499, 80/121/131/185/190/205/244/289/290/293/313/315/336/342/359/391/414/470/474/499/522/523, 80/121/131/185/190/205/244/289/290/293/313/336/342/359/391/414/470/474/499/522/523, 80/121/131/185/190/289/290/293/313/342/470/474/499/522/523, 80/121/174/179/185/190/236/244/288/289/290/293/313/315/317/336/342/359/363/391/394/408/426/462/470/474/499/522/523, 80/121/174/185/186/190/236/244/273/284/288/289/290/293/313/315/317/336/342/352/359/391/394/395/419/428/431/462/470/474/499/522/523, 80/121/174/185/190/193/196/244/273/284/288/289/290/293/297/313/315/317/324/336/342/352/359/376/380/391/394/401/415/419/428/431/435/441/462/470/474/499/522/523,

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80/121/185/190/289/290/293/313/342/499, 80/121/185/315, 80/121/190/289/290, 80/185/236/289/293, 121/185/190/213/289/290/293, and 185/289/290/293. In some embodiments, the engineered TdT polypeptide has increased soluble protein expression and comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2 and one or more residue differences as compared to SEQ ID NO: 2, selected from
80D/106D/121S/185L/190E/205R/289D/290V/293S/313A/315G/336D/342E/359L/391G/470T/474M/499L/522L/523E, 80D/106D/121S/185L/190E/205R/289D/290V/293S/313A/342E/470T/474M/499L/523E, 80D/106D/121S/185L/190E/244V/289D/290V/293S/307K/342E/359L/470T/474M/499L, 80D/121S/131E/185L/190E/205R/244V/289D/290V/293S/313A/315G/336D/342E/359L/391G/414H/470T/474M/499L/522L/523E,

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80D/121S/174L/185L/190E/196R/244V/266K/273V/284L/288E/289D/290V/293S/313A/315G/317T/324I/336D/342E/352P/359L/391G/394R/397R/401G/419A/428V/431S/462F/470T/474M/499L/522L/523E,
80D/121S/174L/185L/190E/236P/244V/273V/282R/284L/288E/289D/290V/293S/313A/315G/317T/336D/342E/352P/359L/391G/394R/395R/419A/428V/431S/462F/470T/474M/499L/522L/523E,
80D/121S/174L/185L/190E/244V/273V/284L/288E/289D/290V/293S/313A/315G/317T/336D/342E/352P/359L/391G/394R/419A/428V/431S/462F/470T/474M/499L/522L/523E,
80D/121S/174L/185L/190E/244V/273V/284L/288E/289D/290V/293S/313A/315G/317T/336D/342E/359L/391G/394R/428V/431S/462F/470T/474M/499L/522L/523E,
80D/121S/174L/185L/190E/244V/273V/284L/288E/289D/290V/293S/313A/315G/317T/336D/342E/359L/391G/394R/419A/428V/431S/462F/470T/474M/499L/522L/523E,
80D/121S/174L/185L/190E/244V/273V/284L/288E/289D/290V/293S/313A/315G/317T/336D/342E/359L/391G/394R/428V/431S/462F/470T/474M/499L/522L/523E,
80D/121S/174L/185L/190E/244V/284L/288E/289D/290V/293S/313A/315G/317T/336D/342E/352P/359L/391G/394R/419A/428V/431S/462F/470T/474M/499L/522L/523E,
80D/121S/174L/185L/190E/244V/284L/288E/289D/290V/293S/313A/315G/317T/336D/342E/359L/391G/394R/428V/431S/462F/470T/474M/499L/522L/523E,
80D/121S/185L/190E/196R/244V/289D/290V/293S/313A/315G/317T/336D/342E/359L/391G/470T/474M/499L/522L/523E, 80D/121S/185L/190E/201R/289D/290V/293S/313A/342E/470T/474M/499L/522L,
80D/121S/185L/190E/244V/273V/289D/290V/293S/313A/315G/317T/336D/342E/352P/359L/391G/419A/435T/470T/474M/499L/522L/523E,
80D/121S/185L/190E/244V/289D/290V/293S/300R/313A/315G/317T/336D/342E/359L/391G/470T/474M/499L/522L/523E,
80D/121S/185L/190E/244V/289D/290V/293S/313A/315G/317T/336D/342E/359L/380D/391G/401G/419A/470T/474M/499L/522L/523E,
80D/121S/185L/190E/244V/289D/290V/293S/313A/315G/317T/336D/342E/359L/391G/392R/470T/474M/499L/522L/523E,
80D/121S/185L/190E/244V/289D/290V/293S/313A/315G/317T/336D/342E/359L/391G/395W/470T/47

4M/499L/522L/523E,
80D/121S/185L/190E/244V/289D/290V/293S/313A/315G/317T/336D/342E/359L/391G/470T/474M/499L/522L/523E,
80D/121S/185L/190E/244V/289D/290V/293S/313A/336D/342E/359L/391G/414H/470T/474M/499L/522L/523E,
80D/121S/185L/190E/289D/290V/293S/313A/315G/336D/342E/359L/391G/414H/470T/474M/499L/522L/523E,
80D/121S/185L/190E/289D/290V/293S/313A/336D/342E/359L/391G/470T/474M/499L/522L/523E,
80D/121S/185L/190E/289D/290V/293S/313A/342E/499L, 80D/121S/185L/315G,
80D/121S/190E/289D/290V, 80D/185L/236P/289D/293S, 121S/185L/190E/213S/289D/290V/293S, and 185L/289D/290V/293S. In some embodiments, the engineered TdT polypeptide has increased soluble protein expression and comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2 and one or more residue differences as compared to SEQ ID NO: 2, selected from
N80D/K106D/C121S/F185L/K190E/M205R/K289D/I290V/D293S/I313A/C315G/L336D/T342E/F359L/C391G/S470T/Q474M/K499L/I522L/Q523E,
N80D/K106D/C121S/F185L/K190E/M205R/K289D/I290V/D293S/I313A/T342E/S470T/Q474M/K499L/Q523E,
N80D/K106D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/C307K/T342E/F359L/S470T/Q474M/K499L,
N80D/C121S/K131E/F185L/K190E/M205R/D244V/K289D/I290V/D293S/I313A/C315G/L336D/T342E/F359L/C391G/F414H/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/K131E/F185L/K190E/M205R/D244V/K289D/I290V/D293S/I313A/L336D/T342E/F359L/C391G/F414H/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/K131E/F185L/K190E/K289D/I290V/D293S/I313A/T342E/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/I174L/Q179K/F185L/K190E/G236P/D244V/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/S363I/C391G/I394R/I408P/H426P/Y462F/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/I174L/F185L/E186G/K190E/G236P/D244V/L273V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/E395R/L419A/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/I174L/F185L/K190E/E193G/E196Y/D244V/L273V/F284L/N288E/K289D/I290V/D293S/K297R/I313A/C315G/S317T/V324I/L336D/T342E/E352P/F359L/Q376H/N380D/C391G/I394R/L401G/Q415S/L419A/E428V/R431S/M435T/E441M/Y462F/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/I174L/F185L/K190E/E193G/D244V/L273V/F284L/N288E/K289D/I290V/D293S/K297R/I

313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/Q415S/L419A/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/I174L/F185L/K190E/E196R/D244V/T266K/L273V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/V324I/L336D/T342E/E352P/F359L/C391G/I394R/T397R/L401G/L419A/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/I174L/F185L/K190E/G236P/D244V/L273V/M282R/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/E395R/L419A/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/I174L/F185L/K190E/D244V/L273V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/L419A/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/I174L/F185L/K190E/D244V/L273V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/L419A/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/I174L/F185L/K190E/D244V/L273V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/L419A/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/I174L/F185L/K190E/D244V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/I174L/F185L/K190E/D244V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/I174L/F185L/K190E/D244V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/F185L/K190E/E196R/D244V/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/C391G/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/F185L/K190E/C201R/K289D/I290V/D293S/I313A/T342E/S470T/Q474M/K499L/I522L,
N80D/C121S/F185L/K190E/D244V/L273V/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/L419A/M435T/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/K300R/I313A/C315G/S317T/L336D/T342E/F359L/C391G/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/N380D/C391G/L401G/L419A/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/C391G/D392R/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/C391G/E395W/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/C391G/S470T/Q474M/K499L/I522L/Q523E,
N80D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/I313A/L336D/T342E/F359L/C391G/F414H/S470T/Q474M/K499L/I522L/Q523E,

N80D/C121S/F185L/K190E/K289D/I290V/D293S/I313A/C315G/L336D/T342E/F359L/C391G/F414H/S470T/Q474M/K499L/I522L/Q523E,

N80D/C121S/F185L/K190E/K289D/I290V/D293S/I313A/L336D/T342E/F359L/C391G/S470T/Q474M/K499L/I522L/Q523E, N80D/C121S/F185L/K190E/K289D/I290V/D293S/I313A/T342E/K499L, N80D/C121S/F185L/C315G, N80D/C121S/K190E/K289D/I290V, N80D/F185L/G236P/K289D/D293S, C121S/F185L/K190E/C213S/K289D/I290V/D293S, and F185L/K289D/I290V/D293S.

[0142] In some embodiments, the engineered TdT polypeptide has increased thermal stability and comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 36 and one or more residue differences as compared to SEQ ID NO: 36, selected from 174/244/273/284/288/315/317/336/352/359/391/394/419/428/431/462/470/474/522/523, 174/244/284/288/315/317/336/359/391/394/428/431/462/470/474/522/523, 244/315/317/336/359/391/470/474/522/523, and 336/359/391/470/474/522/523. In some embodiments, the engineered TdT polypeptide has increased thermal stability and comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 36 and one or more residue differences as compared to SEQ ID NO: 36, selected from

174L/244V/273V/284L/288E/315G/317T/336D/352P/359L/391G/394R/419A/428V/431S/462F/470T/474M/522L/523E,

174L/244V/284L/288E/315G/317T/336D/359L/391G/394R/428V/431S/462F/470T/474M/522L/523E, 244V/315G/317T/336D/359L/391G/470T/474M/522L/523E, and

336D/359L/391G/470T/474M/522L/523E. In some embodiments, the engineered TdT polypeptide has increased thermal stability and comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 36 and one or more residue differences as compared to SEQ ID NO: 36, selected from

I174L/D244V/L273V/F284L/N288E/C315G/S317T/L336D/E352P/F359L/C391G/I394R/L419A/E428V/R431S/Y462F/S470T/Q474M/I522L/Q523E,

I174L/D244V/F284L/N288E/C315G/S317T/L336D/F359L/C391G/I394R/E428V/R431S/Y462F/S470T/Q474M/I522L/Q523E, D244V/C315G/S317T/L336D/F359L/C391G/S470T/Q474M/I522L/Q523E, and L336D/F359L/C391G/S470T/Q474M/I522L/Q523E.

[0143] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 8 and one or more residue differences as compared to SEQ ID NO: 8, selected from 129/196, 173, 183, 186, 193, 195, 196, 263, 266, 268, 281, 282, 297, 300, 303, 316, 318, 320, 324, 343, 360, 392, 395, 397, 411, 415, 417, 421, 454, 456, 477, 481, and 492. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence

having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 8 and one or more residue differences as compared to SEQ ID NO: 8, selected from 129G/196G, 173L, 183R, 186A, 186G, 186L, 186T, 193C, 193G, 193N, 193V, 195R, 195W, 196A, 196G, 196R, 196W, 196Y, 263R, 266K, 268C, 281A, 282Q, 282R, 297F, 297Q, 297R, 297T, 300P, 300R, 303A, 303E, 303M, 316C, 316I, 316T, 318E, 318S, 318T, 318V, 320N, 324I, 343V, 360C, 360V, 392A, 392C, 392R, 395A, 395L, 395R, 395S, 395T, 395W, 395Y, 397R, 411A, 411G, 411R, 415A, 415S, 417G, 417V, 421I, 421M, 454V, 456K, 456R, 477T, 481E, 481V, and 492T. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 8 and one or more residue differences as compared to SEQ ID NO: 8, selected from D129G/E196G, T173L, D183R, E186A, E186G, E186L, E186T, E193C, E193G, E193N, E193V, K195R, K195W, E196A, E196G, E196R, E196W, E196Y, K263R, T266K, V268C, R281A, M282Q, M282R, K297F, K297Q, K297R, K297T, K300P, K300R, K303A, K303E, K303M, V316C, V316I, V316T, K318E, K318S, K318T, K318V, E320N, V324I, I343V, L360C, L360V, D392A, D392C, D392R, E395A, E395L, E395R, E395S, E395T, E395W, E395Y, T397R, L411A, L411G, L411R, Q415A, Q415S, C417G, C417V, L421I, L421M, L454V, V456K, V456R, R477T, R481E, R481V, and D492T.

[0144] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 16 and one or more residue differences as compared to SEQ ID NO: 16, selected from 186, 186/236/318, 186/236/395, 186/282/318, 193/196, 193/196/266/324/376/380, 193/196/297, 193/196/297/324/376/380/401/415/435/441, 193/196/324, 193/196/324/397/401/441, 193/196/376/380, 193/297/324/376/435, 193/297/324/380, 193/297/415, 193/435, 196, 196/266, 196/266/324/397/401, 196/297/324/435, 236/282, 236/282/395, 236/318/481, 266/297/380/397/401, 282, 282/318, 282/481, 297/380/401/441, 297/435, 318/395, 376/401/441, 415, and 435/441. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 16 and one or more residue differences as compared to SEQ ID NO: 16, selected from 186G, 186G/236P/318S, 186G/236P/395R, 186G/282R/318S, 193G/196R/297R, 193G/196Y, 193G/196Y/266K/324I/376H/380D, 193G/196Y/297R/324I/376H/380D/401G/415S/435T/441M, 193G/196Y/324I/397R/401G/441M, 193G/196Y/376H/380D, 193G/297R/324I/376H/435T, 193G/297R/415S, 193G/435T, 193N/196Y/324I, 193N/297R/324I/380D, 196R, 196R/266K, 196R/266K/324I/397R/401G, 196Y/297R/324I/435T, 236P/282R, 236P/282R/395R, 236P/318S/481E, 266K/297R/380D/397R/401G, 282R, 282R/318S, 282R/481E, 297R/380D/401G/441M, 297R/435T, 318S/395R, 376H/401G/441M, 415S, and 435T/441M. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or

more sequence identity to reference sequence SEQ ID NO: 16 and one or more residue differences as compared to SEQ ID NO: 16, selected from E186G, E186G/G236P/K318S, E186G/G236P/E395R, E186G/M282R/K318S, E193G/E196R/K297R, E193G/E196Y, E193G/E196Y/T266K/V324I/Q376H/N380D, E193G/E196Y/K297R/V324I/Q376H/N380D/L401G/Q415S/M435T/E441M, E193G/E196Y/V324I/T397R/L401G/E441M, E193G/E196Y/Q376H/N380D, E193G/K297R/V324I/Q376H/M435T, E193G/K297R/Q415S, E193G/M435T, E193N/E196Y/V324I, E193N/K297R/V324I/N380D, E196R, E196R/T266K, E196R/T266K/V324I/T397R/L401G, E196Y/K297R/V324I/M435T, G236P/M282R, G236P/M282R/E395R, G236P/K318S/R481E, T266K/K297R/N380D/T397R/L401G, M282R, M282R/K318S, M282R/R481E, K297R/N380D/L401G/E441M, K297R/M435T, K318S/E395R, Q376H/L401G/E441M, Q415S, and M435T/E441M.

[0145] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 24 and one or more residue differences as compared to SEQ ID NO: 24, selected from 12, 13, 14, 17, 18, 20, 21, 22, 23, 24, 26, 27, 29, 30, 31, 33, 34, 35, 37, 41, 53, 57, 58, 61, 92, 94, 97, 101, 102, 103, 104, 105, 106, 107, 108, 124, 126, 133, 135, 137, 138, 139, 140, 141, 142, 144, 145, 147, 149, 150, 152, 153, 154, 155, 156, 156/294, 159, 160, 161, 162, and 163. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 24 and one or more residue differences as compared to SEQ ID NO: 24, selected from 12S, 13G, 13K, 13R, 13S, 14G, 14Q, 17A, 17G, 18D, 18R, 20H, 20T, 21E, 22G, 22L, 23E, 23P, 24G, 26G, 27D, 29P, 29R, 30E, 30G, 30V, 31S, 33G, 33K, 33P, 34D, 34K, 34R, 34S, 35E, 35G, 37A, 37F, 37G, 37S, 37T, 37V, 41V, 53E, 57H, 58A, 58S, 61H, 61L, 92M, 92R, 92S, 92V, 92Y, 94E, 94R, 97D, 101E, 102L, 103M, 104G, 104I, 104P, 105N, 106G, 106H, 106S, 107R, 107W, 108D, 108K, 124E, 124I, 126V, 133G, 135R, 137A, 138Q, 139A, 140E, 140G, 141E, 141M, 141R, 142M, 142S, 144C, 145E, 147L, 149R, 150E, 150G, 152G, 152R, 153E, 153G, 153K, 153M, 153P, 153Q, 153V, 154G, 155E, 155T, 156D, 156E/294T, 156M, 156Q, 159D, 160G, 161D, 161E, 161G, 161R, 161S, 162E, 163L, and 163V. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 24 and one or more residue differences as compared to SEQ ID NO: 24, selected from M12S, H13G, H13K, H13R, H13S, R14G, R14Q, T17A, T17G, I18D, I18R, S20H, S20T, D21E, F22G, F22L, G23E, G23P, K24G, R26G, Q27D, K29P, K29R, M30E, M30G, M30V, D31S, H33G, H33K, H33P, I34D, I34K, I34R, I34S, S35E, S35G, M37A, M37F, M37G, M37S, M37T, M37V, I41V, K53E, A57H, T58A, T58S, T61H, T61L, G92M, G92R, G92S, G92V, G92Y, D94E, D94R, A97D, T101E, H102L, K103M, M104G, M104I, M104P, E105N, K106G, K106H, K106S, T107R, T107W,

T108D, T108K, V124E, V124I, K126V, K133G, Q135R, M137A, E138Q, S139A, R140E, R140G, V141E, V141M, V141R, D142M, D142S, A144C, N145E, D147L, T149R, A150E, A150G, T152G, T152R, L153E, L153G, L153K, L153M, L153P, L153Q, L153V, N154G, I155E, I155T, L156D, L156E/K294T, L156M, L156Q, T159D, T160G, K161D, K161E, K161G, K161R, K161S, T162E, I163L, and I163V.

[0146] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 24 and one or more residue differences as compared to SEQ ID NO: 24, selected from 7/135, 12, 13, 14, 15, 16, 17/131, 18, 20, 23, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 44, 45, 46, 57, 65, 77, 85, 89, 93, 94, 97, 101, 102, 103, 105, 106, 108, 109, 110, 119, 123, 124, 126, 130, 131, 132, 133, 134, 135, 137, 138, 139, 149, 150, 153, and 156. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 24 and one or more residue differences as compared to SEQ ID NO: 24, selected from 7Y/135C, 12F, 13E, 14G, 14N, 14Y, 15L, 16V, 17A/131R, 18Y, 20P, 23C, 23E, 23T, 24A, 24M, 24P, 25N, 26G, 27D, 27F, 28R, 29G, 29I, 31V, 32E, 33A, 33C, 34S, 35H, 35W, 44S, 45R, 46M, 57T, 65S, 77C, 77S, 85V, 89A, 93V, 94N, 97T, 101E, 101G, 101V, 102L, 103M, 105N, 105W, 106V, 108G, 108M, 109M, 109N, 109T, 110M, 110V, 119F, 119Q, 123M, 123Q, 124E, 124G, 124I, 124M, 124S, 126C, 130A, 130M, 130Q, 130S, 131G, 131W, 132S, 133G, 133M, 133Q, 134M, 134W, 135E, 135H, 135K, 137A, 137E, 138A, 139G, 149R, 150E, 153E, 153G, 153P, 153Q, and 156D. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 24 and one or more residue differences as compared to SEQ ID NO: 24, selected from H7Y/Q135C, M12F, H13E, R14G, R14N, R14Y, I15L, R16V, T17A/K131R, I18Y, S20P, G23C, G23E, G23T, K24A, K24M, K24P, K25N, R26G, Q27D, Q27F, K28R, K29G, K29I, D31V, N32E, H33A, H33C, I34S, S35H, S35W, H44S, E45R, F46M, A57T, D65S, E77C, E77S, I85V, N89A, S93V, D94N, A97T, T101E, T101G, T101V, H102L, K103M, E105N, E105W, K106V, T108G, T108M, Q109M, Q109N, Q109T, F110M, F110V, I119F, I119Q, K123M, K123Q, V124E, V124G, V124I, V124M, V124S, K126C, T130A, T130M, T130Q, T130S, K131G, K131W, G132S, K133G, K133M, K133Q, Y134M, Y134W, Q135E, Q135H, Q135K, M137A, M137E, E138A, S139G, T149R, A150E, L153E, L153G, L153P, L153Q, and L156D.

[0147] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 268 and one or more residue differences as compared to SEQ ID NO: 268, selected from 14/53/300, 14/53/419, 106/300/415/419/456, 140, and 300/395/419. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 268 and one or more residue differences as compared to SEQ ID NO: 268, selected from 14G/53K/300P, 14G/53K/419L, 106V/300P/415A/419L/456R, 140G, and 300P/395Y/419L. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 268 and one or more residue differences as compared to SEQ ID NO: 268, selected from R14G/E53K/K300P, R14G/E53K/A419L, K106V/K300P/Q415A/A419L/V456R, R140G, and K300P/E395Y/A419L.

[0148] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 648 and one or more residue differences as compared to SEQ ID NO: 648, selected from 12/14/34, 12/14/34/37/94/140/141/145, 12/14/34/37/106/140/142/150/152/153, 12/14/34/37/141/142, 12/14/34/37/142/145, 12/14/34/37/142/161, 12/14/34/37/150, 12/14/34/37/150/153, 12/14/34/140/142, 12/14/34/140/150, 12/14/34/142/150/153, 12/14/92/94, 12/14/94/150/152, 12/14/106/107/141/142, 12/14/106/108/140/141/145/150, 12/14/106/108/152, 12/14/141/142, 12/14/150/152/153, 12/14/153, 12/34/92/140, 12/34/150/152, 12/37/94/141/150/152/153, 12/37/140/141/150/162, 12/161, 14, 14/31/34/37/140/141/145/161/162, 14/34/37, 14/34/37/145, 14/34/37/152, 14/34/94/106/108/141, 14/34/150/153, 14/106, 14/140, 14/141/161, 14/142, 14/142/161/162, 14/153, 14/161, 20/21/24/33/58/104/106/124/155/156, 20/21/33/58/101/104/106/124/155, 20/21/58/104/106/155/156, 20/33/104/106/124/156, 20/58/101/104/106/156, 20/58/101/106, 20/101/106/156, 21/33/58/101/106, 21/33/58/106/155/156, 21/33/101/104/106, 21/33/106, 21/58/101/104/106/155, 21/58/106/155/156, 21/101/104/106/156, 21/104/106, 21/104/106/124, 21/104/106/156, 30/33/58/104/106/155/156, 30/33/101/106/156, 30/33/104/106/155/156, 30/101/104/106/155/156, 30/104/106/155, 30/104/106/155/156, 30/106/155, 33/58/104/106, 33/101/104/106/155, 34/37, 34/37/92, 34/37/140/141/142/145, 34/37/141/142, 34/37/150/153, 34/92/94/141/142, 34/141/142/145, 34/150/152/153, 37/92/142, 37/141/142, 37/153, 58/101/104/106/156, 58/101/106/155, 58/104/106/155/156, 92/94/106/142/145, 101/104/106, 101/104/106/155/156, 101/104/106/156, 101/106, 101/106/124/155, 101/106/155/156, 104/106, 104/106/124, 104/106/155, 104/106/155/156, 104/106/156, 106/107/108/142/220, 106/108/140/141/152/153, 106/108/140/142/150/153, 106/156, 140/141/142, 140/145, 140/145/150/152, 141, 141/152, and 161. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 648 and one or more residue differences as compared to SEQ ID NO: 648, selected from 12S/14G/34D, 12S/14G/34D/37A/106K/140G/142M/150E/152R/153E, 12S/14G/34D/37A/141E/142S, 12S/14G/34D/37A/150E, 12S/14G/34D/37F/94E/140G/141E/145E, 12S/14G/34D/37F/142S/145E, 12S/14G/34D/37F/150E/153E, 12S/14G/34D/140G/142S, 12S/14G/34D/140G/150E,

12S/14G/34S/37A/142M/161E, 12S/14G/34S/142S/150E/153E, 12S/14G/92R/94E, 12S/14G/94E/150E/152R, 12S/14G/106K/107R/141E/142M, 12S/14G/106K/108K/140G/141E/145E/150E, 12S/14G/106K/108K/152R, 12S/14G/141E/142S, 12S/14G/150E/152R/153E, 12S/14G/153E, 12S/34D/92R/140G, 12S/34D/150E/152R, 12S/37A/94E/141E/150E/152R/153E, 12S/37F/140G/141E/150E/162E, 12S/161E, 14G, 14G/31G/34D/37A/140G/141E/145E/161E/162E, 14G/34D/94E/106K/108K/141E, 14G/34D/150E/153E, 14G/34S/37A, 14G/34S/37A/152R, 14G/34S/37F/145E, 14G/34S/150E/153E, 14G/106K, 14G/140G, 14G/141E/161E, 14G/142M, 14G/142S/161E/162E, 14G/153E, 14G/161E, 20T/21E/24G/33K/58A/104P/106S/124E/155E/156E, 20T/21E/33K/58A/101E/104P/106K/124E/155E, 20T/21E/58A/104I/106S/155E/156E, 20T/33K/104P/106K/124E/156E, 20T/58A/101E/106K, 20T/58S/101E/104P/106S/156E, 20T/101E/106K/156E, 21E/33K/58A/101E/106K, 21E/33K/58S/106S/155E/156E, 21E/33K/101E/104P/106S, 21E/33K/106S, 21E/58A/106K/155E/156E, 21E/58S/101E/104I/106S/155E, 21E/101E/104P/106S/156E, 21E/104I/106S/124E, 21E/104P/106S, 21E/104P/106S/156E, 30G/33K/58S/104P/106S/155E/156E, 30G/33K/101E/106S/156E, 30G/33K/104I/106S/155E/156E, 30G/101E/104P/106S/155E/156E, 30G/104P/106S/155E, 30G/104P/106S/155E/156E, 30G/106K/155E, 33K/58A/104I/106S, 33K/101E/104P/106K/155E, 34D/37A, 34D/37A/92R, 34D/37F/141E/142S, 34D/92R/94E/141E/142M, 34D/141E/142S/145E, 34D/150E/152R/153E, 34S/37A/150E/153E, 34S/37F/140G/141E/142M/145E, 37A/141E/142S, 37F/92R/142M, 37F/153E, 58A/101E/106K/155E, 58S/101E/104P/106S/156E, 58S/104I/106K/155E/156E, 92R/94E/106K/142S/145E, 101E/104I/106K, 101E/104I/106K/156E, 101E/104I/106S/156E, 101E/104P/106K/155E/156E, 101E/106K, 101E/106K/155E/156E, 101E/106S/124E/155E, 104I/106K, 104I/106K/124E, 104I/106S, 104I/106S/155E/156E, 104P/106K, 104P/106K/155E, 104P/106K/156E, 104P/106S, 104P/106S/155E, 106K/107R/108K/142S/220R, 106K/108K/140G/141E/152R/153E, 106K/108K/140G/142S/150E/153E, 106K/156E, 140G/141E/142M, 140G/145E, 140G/145E/150E/152R, 141E, 141E/152R, and 161E. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 648 and one or more residue differences as compared to SEQ ID NO: 648, selected from M12S/R14G/I34D, M12S/R14G/I34D/M37A/V106K/R140G/D142M/A150E/T152R/L153E, M12S/R14G/I34D/M37A/V141E/D142S, M12S/R14G/I34D/M37A/A150E, M12S/R14G/I34D/M37F/D94E/R140G/V141E/N145E, M12S/R14G/I34D/M37F/D142S/N145E, M12S/R14G/I34D/M37F/A150E/L153E, M12S/R14G/I34D/R140G/D142S, M12S/R14G/I34D/R140G/A150E, M12S/R14G/I34S/M37A/D142M/K161E, M12S/R14G/I34S/D142S/A150E/L153E, M12S/R14G/G92R/D94E, M12S/R14G/D94E/A150E/T152R, M12S/R14G/V106K/T107R/V141E/D142M, M12S/R14G/V106K/T108K/R140G/V141E/N145E/A150E, M12S/R14G/V106K/T108K/T152R, M12S/R14G/V141E/D142S, M12S/R14G/A150E/T152R/L153E,

M12S/R14G/L153E, M12S/I34D/G92R/R140G, M12S/I34D/A150E/T152R,
M12S/M37A/D94E/V141E/A150E/T152R/L153E, M12S/M37F/R140G/V141E/A150E/T162E,
M12S/K161E, R14G, R14G/D31G/I34D/M37A/R140G/V141E/N145E/K161E/T162E,
R14G/I34D/D94E/V106K/T108K/V141E, R14G/I34D/A150E/L153E, R14G/I34S/M37A,
R14G/I34S/M37A/T152R, R14G/I34S/M37F/N145E, R14G/I34S/A150E/L153E, R14G/V106K,
R14G/R140G, R14G/V141E/K161E, R14G/D142M, R14G/D142S/K161E/T162E, R14G/L153E,
R14G/K161E, S20T/D21E/K24G/H33K/T58A/M104P/V106S/V124E/I155E/L156E,
S20T/D21E/H33K/T58A/T101E/M104P/V106K/V124E/I155E,
S20T/D21E/T58A/M104I/V106S/I155E/L156E, S20T/H33K/M104P/V106K/V124E/L156E,
S20T/T58A/T101E/V106K, S20T/T58S/T101E/M104P/V106S/L156E, S20T/T101E/V106K/L156E,
D21E/H33K/T58A/T101E/V106K, D21E/H33K/T58S/V106S/I155E/L156E,
D21E/H33K/T101E/M104P/V106S, D21E/H33K/V106S, D21E/T58A/V106K/I155E/L156E,
D21E/T58S/T101E/M104I/V106S/I155E, D21E/T101E/M104P/V106S/L156E,
D21E/M104I/V106S/V124E, D21E/M104P/V106S, D21E/M104P/V106S/L156E,
M30G/H33K/T58S/M104P/V106S/I155E/L156E, M30G/H33K/T101E/V106S/L156E,
M30G/H33K/M104I/V106S/I155E/L156E, M30G/T101E/M104P/V106S/I155E/L156E,
M30G/M104P/V106S/I155E, M30G/M104P/V106S/I155E/L156E, M30G/V106K/I155E,
H33K/T58A/M104I/V106S, H33K/T101E/M104P/V106K/I155E, I34D/M37A, I34D/M37A/G92R,
I34D/M37F/V141E/D142S, I34D/G92R/D94E/V141E/D142M, I34D/V141E/D142S/N145E,
I34D/A150E/T152R/L153E, I34S/M37A/A150E/L153E, I34S/M37F/R140G/V141E/D142M/N145E,
M37A/V141E/D142S, M37F/G92R/D142M, M37F/L153E, T58A/T101E/V106K/I155E,
T58S/T101E/M104P/V106S/L156E, T58S/M104I/V106K/I155E/L156E,
G92R/D94E/V106K/D142S/N145E, T101E/M104I/V106K, T101E/M104I/V106K/L156E,
T101E/M104I/V106S/L156E, T101E/M104P/V106K/I155E/L156E, T101E/V106K,
T101E/V106K/I155E/L156E, T101E/V106S/V124E/I155E, M104I/V106K, M104I/V106K/V124E,
M104I/V106S, M104I/V106S/I155E/L156E, M104P/V106K, M104P/V106K/I155E,
M104P/V106K/L156E, M104P/V106S, M104P/V106S/I155E, V106K/T107R/T108K/D142S/S220R,
V106K/T108K/R140G/V141E/T152R/L153E, V106K/T108K/R140G/D142S/A150E/L153E,
V106K/L156E, R140G/V141E/D142M, R140G/N145E, R140G/N145E/A150E/T152R, V141E,
V141E/T152R, and K161E.

[0149] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 660 and one or more residue differences as compared to SEQ ID NO: 660, selected from 16/29/30, 16/29/30/33/153, 16/29/30/101/104, 16/30/104, 16/33, 29/30, 58, 92/94/108/141/155/392, 92/101/137/155/476, 94/101/156/476, 101, 101/104, 101/137/155, 101/141/155/156, and 108. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 660 and one or more residue differences as compared to SEQ ID NO: 660, selected from 16V/29I/30E, 16V/29I/30E/33K/153P, 16V/29I/30E/101E/104V, 16V/30L/104V, 16V/33K, 29I/30E, 58A, 92R/94E/108K/141E/155E/392R, 92R/101E/137A/155E/476R, 94E/101E/156E/476R, 101E, 101E/104V, 101E/137E/155E, 101E/141E/155E/156E, and 108K. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 660 and one or more residue differences as compared to SEQ ID NO: 660, selected from R16V/K29I/M30E, R16V/K29I/M30E/H33K/E153P, R16V/K29I/M30E/T101E/M104V, R16V/M30L/M104V, R16V/H33K, K29I/M30E, T58A, G92R/D94E/T108K/V141E/I155E/D392R, G92R/T101E/M137A/I155E/E476R, D94E/T101E/L156E/E476R, T101E, T101E/M104V, T101E/M137E/I155E, T101E/V141E/I155E/L156E, and T108K.

[0150] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 660 and one or more residue differences as compared to SEQ ID NO: 660, selected from 195, 197, 204/342, 205, 236/297, 258, 261, 262, 264, 268, 269, 276, 278, 280, 281, 282, 290, 291, 297, 300, 303, 306, 308, 309, 310, 312, 315, 316, 342, 344, 353, 360, 385, 391, 410, 413, 419, 421, 448, 454, 456, 473, 476, 515, and 525. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 660 and one or more residue differences as compared to SEQ ID NO: 660, selected from 195E, 197M, 204L/342W, 205E, 205L, 236E/297L, 258A, 258C, 258G, 258L, 258M, 258S, 258W, 261G, 261R, 261V, 262I, 264A, 264E, 264R, 264S, 268L, 269W, 276S, 278C, 278E, 278I, 278R, 278T, 278V, 280F, 281A, 281C, 281G, 281L, 281S, 281T, 281V, 282C, 282G, 282H, 282W, 290A, 290L, 291S, 297C, 297D, 297S, 297V, 300R, 303A, 303N, 303Q, 303S, 303V, 306F, 308F, 308W, 309F, 310A, 310G, 310H, 310R, 310S, 312V, 315A, 315S, 316A, 316L, 342R, 342V, 344V, 353A, 353K, 353M, 353R, 353S, 360I, 385R, 391L, 391R, 410E, 413C, 413F, 413V, 419G, 419H, 421F, 448R, 454M, 456S, 473V, 476V, 515V, 525F, and 525H. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 660 and one or more residue differences as compared to SEQ ID NO: 660, selected from K195E, N197M, F204L/E342W, M205E, M205L, G236E/K297L, R258A, R258C, R258G, R258L, R258M, R258S, R258W, S261G, S261R, S261V, F262I, L264A, L264E, L264R, L264S, V268L, F269W, A276S, K278C, K278E, K278I, K278R, K278T, K278V, Y280F, R281A, R281C, R281G, R281L, R281S, R281T, R281V, M282C, M282G, M282H, M282W, V290A, V290L, R291S, K297C, K297D, K297S, K297V, P300R, K303A, K303N, K303Q, K303S, K303V, L306F,

Y308F, Y308W, Y309F, E310A, E310G, E310H, E310R, E310S, L312V, G315A, G315S, V316A, V316L, E342R, E342V, T344V, F353A, F353K, F353M, F353R, F353S, L360I, Q385R, G391L, G391R, A410E, H413C, H413F, H413V, L419G, L419H, L421F, K448R, L454M, R456S, R473V, E476V, A515V, S525F, and S525H.

[0151] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 882 and one or more residue differences as compared to SEQ ID NO: 882, selected from 175, 196, 199, 203, 208, 275, 313, 314, 317, 321, 322, 325, 329/462, 379, 394, 397, 403/462, 406, 408, 457, 461, 462, 469, 477, 481, 484, and 495. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 882 and one or more residue differences as compared to SEQ ID NO: 882, selected from 175L, 196G, 196Y, 199G, 199M, 199Q, 199R, 199S, 199V, 203A, 203G, 203L, 203R, 203S, 208V, 275V, 313I, 314G, 314K, 314L, 314R, 314V, 314Y, 317G, 321C, 321K, 321S, 322A, 325F, 325T, 325V, 325W, 329R/462E, 379C, 394E, 394T, 397D, 397T, 403F/462H, 406G, 406V, 408A, 408T, 457S, 457V, 461G, 461V, 462I, 462R, 462W, 469Q, 477T, 481D, 481M, 481T, 484M, 484R, and 495S. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 882 and one or more residue differences as compared to SEQ ID NO: 882, selected from N175L, R196G, R196Y, D199G, D199M, D199Q, D199R, D199S, D199V, T203A, T203G, T203L, T203R, T203S, I208V, T275V, A313I, D314G, D314K, D314L, D314R, D314V, D314Y, T317G, A321C, A321K, A321S, D322A, S325F, S325T, S325V, S325W, Q329R/F462E, T379C, R394E, R394T, R397D, R397T, L403F/F462H, R406G, R406V, I408A, I408T, C457S, C457V, R461G, R461V, F462I, F462R, F462W, W469Q, R477T, R481D, R481M, R481T, T484M, T484R, and A495S.

[0152] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 882 and one or more residue differences as compared to SEQ ID NO: 882, selected from 175, 179, 196, 199, 201, 203, 272, 273, 275, 307, 313, 314, 319, 321, 322, 324, 325, 350, 376, 394, 404, 406, 408, 461, 462, 477, 481, 484, 491, 492, 495, and 523. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 882 and one or more residue differences as compared to SEQ ID NO: 882, selected from 175D, 175I, 175V, 179R, 196G, 199A, 199E, 199G, 199H, 199I, 199Q, 199R, 199V, 201A, 201W, 203M, 203R, 272T, 273M, 273W, 275D, 307M, 307V, 313M, 313Q, 313R, 313S, 314G, 314I, 319G, 319R, 321K, 322K, 322Q, 324V, 325A, 325V, 350S, 376V, 394L, 394M, 394S, 394T, 404F, 404W, 406V, 408G, 408L, 408R, 461A,

461G, 461Q, 461S, 462L, 462Q, 477Q, 481W, 484M, 484R, 491I, 492S, 492T, 495G, 495S, and 523H. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 882 and one or more residue differences as compared to SEQ ID NO: 882, selected from N175D, N175I, N175V, Q179R, R196G, D199A, D199E, D199G, D199H, D199I, D199Q, D199R, D199V, C201A, C201W, T203M, T203R, G272T, V273M, V273W, T275D, C307M, C307V, A313M, A313Q, A313R, A313S, D314G, D314I, A319G, A319R, A321K, D322K, D322Q, I324V, S325A, S325V, G350S, Q376V, R394L, R394M, R394S, R394T, P404F, P404W, R406V, I408G, I408L, I408R, R461A, R461G, R461Q, R461S, F462L, F462Q, R477Q, R481W, T484M, T484R, L491I, D492S, D492T, A495G, A495S, and E523H.

[0153] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1336 and one or more residue differences as compared to SEQ ID NO: 1336, selected from 15/199/203/394, 15/199/394, 28/344/353/395, 195/199/203/278/297/394, 195/199/203/278/314/353/394, 195/203/278/394/395, 195/203/297/314/394, 195/203/297/394/419, 195/203/394, 195/278/297/394, 195/278/297/394/395, 195/314/344, 195/394/395, 199/203/297/394/395, 203/278/297/394, 203/297/314/394/395, 203/310/314/394/395/419, 203/344/394, 203/353, 203/394, 203/394/395, 297/394, 314/394/395, 344/394/395, 353, and 394. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1336 and one or more residue differences as compared to SEQ ID NO: 1336, selected from 15F/199R/203A/394E, 15F/199R/394E, 28N/344V/353K/395W, 195E/199R/203A/278E/297D/394E, 195E/199R/203A/278E/314R/353K/394E, 195E/203A/278E/394E/395W, 195E/203A/297D/314R/394E, 195E/203A/297D/394E/419M, 195E/203A/394E, 195E/278E/297D/394E, 195E/278E/297D/394E/395W, 195E/314R/344V, 195E/394E/395W, 199R/203A/297D/394E/395W, 203A/278E/297D/394E, 203A/297D/314R/394E/395W, 203A/310R/314R/394E/395W/419M, 203A/344V/394E, 203A/353K, 203A/394E, 203A/394E/395W, 297D/394E, 314R/394E/395W, 344V/394E/395W, 353K, and 394E. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1336 and one or more residue differences as compared to SEQ ID NO: 1336, selected from I15F/D199R/T203A/R394E, I15F/D199R/R394E, K28N/T344V/F353K/E395W, K195E/D199R/T203A/K278E/K297D/R394E, K195E/D199R/T203A/K278E/D314R/F353K/R394E, K195E/T203A/K278E/R394E/E395W, K195E/T203A/K297D/D314R/R394E, K195E/T203A/K297D/R394E/L419M, K195E/T203A/R394E, K195E/K278E/K297D/R394E, K195E/K278E/K297D/R394E/E395W, K195E/D314R/T344V, K195E/R394E/E395W, D199R/T203A/K297D/R394E/E395W, T203A/K278E/K297D/R394E,

T203A/K297D/D314R/R394E/E395W, T203A/E310R/D314R/R394E/E395W/L419M, T203A/T344V/R394E, T203A/F353K, T203A/R394E, T203A/R394E/E395W, K297D/R394E, D314R/R394E/E395W, T344V/R394E/E395W, F353K, and R394E.

[0154] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1336 and one or more residue differences as compared to SEQ ID NO: 1336, selected from 61, 152/503, 160, 162, 165, 177, 200, 200/425, 213, 217, 219, 223, 236, 246, 248, 292, 292/411, 295, 326, 329, 330, 333, 334, 338, 340, 340/438, 363, 369, 370, 372, 373, 383, 400/401/402, 425, 427, 435, 435/503, 437, 440, 441, 442, 443, 444, 446, 459, 460, 488, 490, 501, 502, 503, 504, and 506. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1336 and one or more residue differences as compared to SEQ ID NO: 1336, selected from 61A, 152H/503S, 160M, 160N, 160S, 160V, 162A, 165K, 177L, 200C, 200C/425K, 200K, 200V, 213S, 217Q, 219L, 219R, 223Q, 236I, 236P, 236V, 246K, 248C, 248S, 292L/411P, 292T, 295S, 326C, 326M, 326N, 326S, 326T, 329K, 329R, 330E, 333A, 333D, 333G, 333H, 333R, 334E, 334R, 334S, 338T, 340A, 340G, 340M, 340M/438V, 340R, 340S, 363C, 369G, 369M, 369N, 370G, 372G, 373H, 373N, 383R, 400A/401E/402F, 425D, 425R, 425T, 427E, 427Q, 435A, 435C, 435E, 435G, 435K, 435Q, 435S, 435S/503M, 435T, 437Q, 437S, 440E, 440V, 441N, 442G, 443T, 444R, 446E, 446P, 459Q, 460G, 488S, 490E, 490H, 490R, 490V, 490W, 501A, 502G, 502R, 503E, 503Q, 503R, 503V, 504N, 504R, 504W, and 506E. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1336 and one or more residue differences as compared to SEQ ID NO: 1336, selected from T61A, R152H/I503S, T160M, T160N, T160S, T160V, T162A, Q165K, H177L, T200C, T200C/H425K, T200K, T200V, C213S, E217Q, V219L, V219R, D223Q, G236I, G236P, G236V, E246K, L248C, L248S, S292L/L411P, S292T, T295S, L326C, L326M, L326N, L326S, L326T, Q329K, Q329R, D330E, W333A, W333D, W333G, W333H, W333R, T334E, T334R, T334S, D338T, L340A, L340G, L340M, L340M/G438V, L340R, L340S, S363C, E369G, E369M, E369N, Q370G, D372G, Q373H, Q373N, K383R, D400A/G401E/K402F, H425D, H425R, H425T, K427E, K427Q, M435A, M435C, M435E, M435G, M435K, M435Q, M435S, M435S/I503M, M435T, T437Q, T437S, N440E, N440V, E441N, S442G, E443T, A444R, S446E, S446P, Y459Q, D460G, K488S, M490E, M490H, M490R, M490V, M490W, K501A, K502G, K502R, I503E, I503Q, I503R, I503V, F504N, F504R, F504W, and K506E.

[0155] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1348 and one or more residue differences as compared to SEQ ID NO: 1348, selected from 3/175/213/313/325/340/457/481/485,

3/307/321/340/353/406/408/445, 148/175/201/457/485, 175/201/333/412/425/457/485, 175/325/397, 175/333, 175/333/369/481, 175/333/485, 175/485, 199/307/321/340/406/408/445/484, 201/213/333/344/397/425/481/485, 201/333/344/457/481, 201/333/481, 201/406/408/462/484/502, 213, 213/333/397, 307/333/340/408/445/462/502, 307/373/406/408/484, 321/333/340, 325/333/369/425, 325/425/457/481, 333, 333/344/369/397, 333/344/369/485, 340/484, 344/485, 353/406, 373, and 406/408. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1348 and one or more residue differences as compared to SEQ ID NO: 1348, selected from 3Q/175D/213S/313R/325T/340R/457V/481W/485D, 3Q/307M/321K/340R/353K/406G/408A/445N, 148T/175D/201A/457V/485D, 175D/201A/333A/412N/425D/457V/485D, 175D/325T/397Q, 175D/333A, 175D/333A/369N/481W, 175D/333A/485D, 175D/485D, 199H/307M/321K/340R/406G/408A/445N/484M, 201A/213S/333A/344V/397Q/425D/481W/485D, 201A/333A/344V/457V/481W, 201A/333A/481W, 201R/406G/408A/462L/484M/502G, 213S, 213S/333A/397Q, 307M/333G/340R/408A/445N/462L/502G, 307M/373H/406G/408A/484M, 321K/333G/340R, 325T/333A/369N/425D, 325T/425D/457V/481W, 333A/344V/369N/397Q, 333A/344V/369N/485D, 333G, 340R/484M, 344V/485D, 353K/406G, 373H, and 406G/408A. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1348 and one or more residue differences as compared to SEQ ID NO: 1348, selected from H3Q/N175D/C213S/A313R/S325T/L340R/C457V/R481W/H485D, H3Q/C307M/A321K/L340R/F353K/R406G/I408A/K445N, P148T/N175D/C201A/C457V/H485D, N175D/C201A/W333A/D412N/H425D/C457V/H485D, N175D/S325T/R397Q, N175D/W333A, N175D/W333A/E369N/R481W, N175D/W333A/H485D, N175D/H485D, D199H/C307M/A321K/L340R/R406G/I408A/K445N/T484M, C201A/C213S/W333A/T344V/R397Q/H425D/R481W/H485D, C201A/W333A/T344V/C457V/R481W, C201A/W333A/R481W, C201R/R406G/I408A/F462L/T484M/K502G, C213S, C213S/W333A/R397Q, C307M/W333G/L340R/I408A/K445N/F462L/K502G, C307M/Q373H/R406G/I408A/T484M, A321K/W333G/L340R, S325T/W333A/E369N/H425D, S325T/H425D/C457V/R481W, W333A/T344V/E369N/R397Q, W333A/T344V/E369N/H485D, W333G, L340R/T484M, T344V/H485D, F353K/R406G, Q373H, and R406G/I408A.

[0156] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1596 and one or more residue differences as compared to SEQ ID NO: 1596, selected from 160/219/460/503/506, 219/307/326, 252, 252/333, 406/408, 406/408/442/446, 406/408/490, 408, and 446. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%,

88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1596 and one or more residue differences as compared to SEQ ID NO: 1596, selected from 160M/219R/460G/503E/506E, 219R/307M/326T, 252K, 252K/333H, 406G/408A, 406G/408A/442G/446P, 406G/408A/490R, 408A, and 446P. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1596 and one or more residue differences as compared to SEQ ID NO: 1596, selected from T160M/V219R/D460G/I503E/K506E, V219R/C307M/L326T, A252K, A252K/A333H, R406G/I408A, R406G/I408A/S442G/S446P, R406G/I408A/M490R, I408A, and S446P.

[0157] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1596 and one or more residue differences as compared to SEQ ID NO: 1596, selected from 162, 163, 165, 171, 177, 179, 188, 200, 205, 208, 233, 252, 253, 260, 261, 277, 307, 325, 329, 330, 353, 371, 376, 382, 393, 400, 402, 405, 406, 407, 410, 413, 419, 441, 442, 460, 464, 484, 488, 490, 495, 506, 508, and 520. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1596 and one or more residue differences as compared to SEQ ID NO: 1596, selected from 162H, 163V, 165P, 171K, 177Y, 179K, 188M, 200A, 205R, 208A, 233D, 252E, 253I, 260K, 261A, 277E, 307L, 325L, 329K, 330E, 353Q, 371D, 376H, 382L, 393I, 400E, 402Q, 405N, 406P, 407A, 410Q, 413G, 419A, 441M, 442A, 460E, 464Y, 484E, 488N, 490L, 495G, 506S, 508D, and 520D. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1596 and one or more residue differences as compared to SEQ ID NO: 1596, selected from T162H, I163V, Q165P, R171K, H177Y, Q179K, L188M, T200A, M205R, I208A, E233D, A252E, V253I, Q260K, S261A, D277E, C307L, S325L, Q329K, D330E, F353Q, E371D, Q376H, W382L, L393I, D400E, K402Q, S405N, R406P, K407A, A410Q, H413G, L419A, E441M, S442A, D460E, F464Y, T484E, K488N, M490L, A495G, K506S, K508D, and E520D.

[0158] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1596 and one or more residue differences as compared to SEQ ID NO: 1596, selected from 161, 162, 163, 165, 179, 188, 205, 208, 231, 233, 251, 252, 253, 261, 277, 306, 307, 321, 325, 327, 329, 353, 368, 370, 371, 376, 380, 393, 400, 402, 406, 410, 413, 414, 419, 426, 441, 442, 460, 464, 484, 488, 490, 495, 506, 508, and 518. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more

sequence identity to reference sequence SEQ ID NO: 1596 and one or more residue differences as compared to SEQ ID NO: 1596, selected from 161R, 162H, 163V, 165P, 179K, 188M, 205R, 208A, 231T, 233D, 251K, 252E, 253I, 261A, 277E, 306F, 307L, 321K, 325L, 327I, 329K, 353Q, 368R, 370T, 371D, 376H, 380D, 393I, 400E, 402Q, 406P, 410Q, 413G, 414H, 419A, 426P, 441M, 442A, 460E, 464Y, 484E, 488N, 490L, 495G, 506S, 508D, and 518D. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1596 and one or more residue differences as compared to SEQ ID NO: 1596, selected from K161R, T162H, I163V, Q165P, Q179K, L188M, M205R, I208A, G231T, E233D, Q251K, A252E, V253I, S261A, D277E, L306F, C307L, A321K, S325L, L327I, Q329K, F353Q, K368R, Q370T, E371D, Q376H, N380D, L393I, D400E, K402Q, R406P, A410Q, H413G, F414H, L419A, H426P, E441M, S442A, D460E, F464Y, T484E, K488N, M490L, A495G, K506S, K508D, and G518D.

[0159] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1654 and one or more residue differences as compared to SEQ ID NO: 1654, selected from 160/165/203/205/219/353/460/488, 160/165/205/219/441, 160/203/205/441, 160/219/330/484, 179/353, 205/307/441/460/488, and 441. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1654 and one or more residue differences as compared to SEQ ID NO: 1654, selected from 160M/165P/203E/205R/219R/353Q/460G/488N, 160M/165P/205R/219R/441M, 160M/203E/205R/441M, 160M/219R/330K/484E, 179K/353Q, 205R/307L/441M/460G/488N, and 441M. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1654 and one or more residue differences as compared to SEQ ID NO: 1654, selected from T160M/Q165P/T203E/M205R/V219R/F353Q/D460G/K488N, T160M/Q165P/M205R/V219R/E441M, T160M/T203E/M205R/E441M, T160M/V219R/D330K/T484E, Q179K/F353Q, M205R/C307L/E441M/D460G/K488N, and E441M.

[0160] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1654 and one or more residue differences as compared to SEQ ID NO: 1654, selected from 3/160/205/208/219/307/353/371/376/413/414/441/488, 160, 160/165/179/203/205/208/219/353/376/413/414/441/488, 160/165/179/203/307/376/413/495, 160/165/203/205/219/353/460/488, 160/165/203/205/307/371/376/413/414, 160/165/203/208/371/376/413/414, 160/165/205, 160/165/205/208, 160/165/205/219/441,

160/165/205/414/441/495, 160/165/307/353/413/414/441/488/495,
160/179/203/205/208/219/371/414/484/506, 160/179/208/219/307/413/414/503/506,
160/179/208/307/371/376/414, 160/179/307/376/441/488/503, 160/203/205/208/219/414/460/506,
160/203/205/208/413/460/484/488, 160/203/205/441, 160/203/208/307/353/495,
160/203/208/371/413/414, 160/203/208/413/414/441/484, 160/203/326/353/413/414/484/495,
160/205/208/219/326/441/484/488/503, 160/208/326/376/414/441/484/488, 160/208/371/441/484/506,
160/208/414/441/452/480/488/495, 160/219/307/371/506, 160/219/330/484,
165/179/203/205/219/414/418/441/488/503, 165/179/203/205/484/503, 165/179/205/413/441,
165/179/208/353/413/414/503, 165/203/205, 165/203/205/307/414/441/484/495/503/506,
165/203/205/484/488, 165/203/208/326/376/503, 165/205, 165/208/326/413/414/484/495/506, 179,
179/203/205, 179/203/208/326/353/376/484, 179/205/208/353/414/441/460/484/488, 179/205/353,
179/208/353/460, 179/353, 203/205/208/307/330/353/441/460/503/506, 203/205/208/307/441,
203/205/208/353, 203/208/219/376/441, 203/208/219/441, 203/208/326/353, 203/413/503/506,
205/208/307/353/376/413, 205/208/414, 205/219/307/353, 205/307, 205/307/376/414/441/495,
205/307/441/460/488, 205/326/488/503/506, 208/488/506, 326/353/371/376/414, and 441. In some
embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%,
70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more
sequence identity to reference sequence SEQ ID NO: 1654 and one or more residue differences as
compared to SEQ ID NO: 1654, selected from
3Q/160M/205R/208A/219R/307L/353Q/371D/376H/413G/414H/441M/488N, 160M,
160M/165P/179K/203E/205R/208A/219R/353Q/376H/413G/414H/441M/488N,
160M/165P/179K/203E/307L/376H/413G/495G, 160M/165P/203E/205R/219R/353Q/460G/488N,
160M/165P/203E/205R/307L/371D/376H/413G/414H, 160M/165P/203E/208A/371D/376H/413G/414H,
160M/165P/205R, 160M/165P/205R/208A, 160M/165P/205R/219R/441M,
160M/165P/205R/414H/441M/495G, 160M/165P/307L/353Q/413G/414H/441M/488N/495G,
160M/179K/203E/205R/208A/219R/371D/414H/484E/506E,
160M/179K/208A/219R/307L/413G/414H/503E/506E, 160M/179K/208A/307L/371D/376H/414H,
160M/179K/307L/376H/441M/488N/503E, 160M/203E/205R/208A/219R/414H/460G/506E,
160M/203E/205R/208A/413G/460G/484E/488N, 160M/203E/205R/441M,
160M/203E/208A/307L/353Q/495G, 160M/203E/208A/371D/413G/414H,
160M/203E/208A/413G/414H/441M/484E, 160M/203E/326T/353Q/413G/414H/484E/495G,
160M/205R/208A/219R/326T/441M/484E/488N/503E,
160M/208A/326T/376H/414H/441M/484E/488N, 160M/208A/371D/441M/484E/506E,
160M/208A/414H/441M/452I/480H/488N/495G, 160M/219R/307L/371D/506E,
160M/219R/330K/484E, 165P/179K/203E/205R/219R/414H/418I/441M/488N/503E,
165P/179K/203E/205R/484E/503E, 165P/179K/205R/413G/441M,
165P/179K/208A/353Q/413G/414H/503E, 165P/203E/205R,

165P/203E/205R/307L/414Y/441M/484E/495G/503E/506E, 165P/203E/205R/484E/488N, 165P/203E/208A/326T/376H/503E, 165P/205R, 165P/208A/326T/413G/414H/484E/495G/506E, 179K, 179K/203E/205R, 179K/203E/208A/326T/353Q/376H/484E, 179K/205R/208A/353Q/414H/441M/460G/484E/488N, 179K/205R/353Q, 179K/208A/353Q/460G, 179K/353Q, 203E/205R/208A/307L/330N/353Q/441M/460G/503E/506E, 203E/205R/208A/307L/441M, 203E/205R/208A/353Q, 203E/208A/219R/376H/441M, 203E/208A/219R/441M, 203E/208A/326T/353Q, 203E/413G/503E/506E, 205R/208A/307L/353Q/376H/413G, 205R/208A/414H, 205R/219R/307L/353Q, 205R/307L, 205R/307L/376H/414H/441M/495G, 205R/307L/441M/460G/488N, 205R/326T/488N/503E/506E, 208A/488N/506E, 326T/353Q/371D/376H/414H, and 441M. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1654 and one or more residue differences as compared to SEQ ID NO: 1654, selected from H3Q/T160M/M205R/I208A/V219R/C307L/F353Q/E371D/Q376H/H413G/F414H/E441M/K488N, T160M, T160M/Q165P/Q179K/T203E/M205R/I208A/V219R/F353Q/Q376H/H413G/F414H/E441M/K488N, T160M/Q165P/Q179K/T203E/C307L/Q376H/H413G/A495G, T160M/Q165P/T203E/M205R/V219R/F353Q/D460G/K488N, T160M/Q165P/T203E/M205R/C307L/E371D/Q376H/H413G/F414H, T160M/Q165P/T203E/I208A/E371D/Q376H/H413G/F414H, T160M/Q165P/M205R, T160M/Q165P/M205R/I208A, T160M/Q165P/M205R/V219R/E441M, T160M/Q165P/M205R/F414H/E441M/A495G, T160M/Q165P/C307L/F353Q/H413G/F414H/E441M/K488N/A495G, T160M/Q179K/T203E/M205R/I208A/V219R/E371D/F414H/T484E/K506E, T160M/Q179K/I208A/V219R/C307L/H413G/F414H/I503E/K506E, T160M/Q179K/I208A/C307L/E371D/Q376H/F414H, T160M/Q179K/C307L/Q376H/E441M/K488N/I503E, T160M/T203E/M205R/I208A/V219R/F414H/D460G/K506E, T160M/T203E/M205R/I208A/H413G/D460G/T484E/K488N, T160M/T203E/M205R/E441M, T160M/T203E/I208A/C307L/F353Q/A495G, T160M/T203E/I208A/E371D/H413G/F414H, T160M/T203E/I208A/H413G/F414H/E441M/T484E, T160M/T203E/L326T/F353Q/H413G/F414H/T484E/A495G, T160M/M205R/I208A/V219R/L326T/E441M/T484E/K488N/I503E, T160M/I208A/L326T/Q376H/F414H/E441M/T484E/K488N, T160M/I208A/E371D/E441M/T484E/K506E, T160M/I208A/F414H/E441M/V452I/R480H/K488N/A495G, T160M/V219R/C307L/E371D/K506E, T160M/V219R/D330K/T484E,

Q165P/Q179K/T203E/M205R/V219R/F414H/F418I/E441M/K488N/I503E,
Q165P/Q179K/T203E/M205R/T484E/I503E, Q165P/Q179K/M205R/H413G/E441M,
Q165P/Q179K/I208A/F353Q/H413G/F414H/I503E, Q165P/T203E/M205R,
Q165P/T203E/M205R/C307L/F414Y/E441M/T484E/A495G/I503E/K506E,
Q165P/T203E/M205R/T484E/K488N, Q165P/T203E/I208A/L326T/Q376H/I503E, Q165P/M205R,
Q165P/I208A/L326T/H413G/F414H/T484E/A495G/K506E, Q179K, Q179K/T203E/M205R,
Q179K/T203E/I208A/L326T/F353Q/Q376H/T484E,
Q179K/M205R/I208A/F353Q/F414H/E441M/D460G/T484E/K488N, Q179K/M205R/F353Q,
Q179K/I208A/F353Q/D460G, Q179K/F353Q,
T203E/M205R/I208A/C307L/D330N/F353Q/E441M/D460G/I503E/K506E,
T203E/M205R/I208A/C307L/E441M, T203E/M205R/I208A/F353Q,
T203E/I208A/V219R/Q376H/E441M, T203E/I208A/V219R/E441M, T203E/I208A/L326T/F353Q,
T203E/H413G/I503E/K506E, M205R/I208A/C307L/F353Q/Q376H/H413G, M205R/I208A/F414H,
M205R/V219R/C307L/F353Q, M205R/C307L, M205R/C307L/Q376H/F414H/E441M/A495G,
M205R/C307L/E441M/D460G/K488N, M205R/L326T/K488N/I503E/K506E, I208A/K488N/K506E,
L326T/F353Q/E371D/Q376H/F414H, and E441M.

[0161] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1830 and one or more residue differences as compared to SEQ ID NO: 1830, selected from 163/179/277/338/340, 163/414/441, 171/200/334/406/490, 177, and 292/406. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1830 and one or more residue differences as compared to SEQ ID NO: 1830, selected from 163V/179K/277E/338T/340A, 163V/414H/441M, 171K/200V/334R/406P/490L, 177L, and 292T/406P. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1830 and one or more residue differences as compared to SEQ ID NO: 1830, selected from I163V/Q179K/D277E/D338T/L340A, I163V/F414H/E441M, R171K/T200V/T334R/G406P/M490L, H177L, and S292T/G406P.

[0162] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1100 and one or more residue differences as compared to SEQ ID NO: 1100, selected from 101/137/264/476/525 and 264. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1100 and one or more residue differences as

compared to SEQ ID NO: 1100, selected from 101E/137A/264R/476R/525F and 264R. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1100 and one or more residue differences as compared to SEQ ID NO: 1100, selected from T101E/M137A/L264R/E476R/S525F and L264R.

[0163] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1654 and one or more residue differences as compared to SEQ ID NO: 1654, selected from

160/163/165/203/205/219/353/414/441/460/488 and 160/165/203/205/219/353/460/488. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1654 and one or more residue differences as compared to SEQ ID NO: 1654, selected from

160M/163V/165P/203E/205R/219R/353Q/414H/441M/460G/488N and

160M/165P/203E/205R/219R/353Q/460G/488N. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1654 and one or more residue differences as compared to SEQ ID NO: 1654, selected from T160M/I163V/Q165P/T203E/M205R/V219R/F353Q/F414H/E441M/D460G/K488N and T160M/Q165P/T203E/M205R/V219R/F353Q/D460G/K488N.

[0164] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1596 and one or more residue differences as compared to SEQ ID NO: 1596, selected from

160/165/203/205/219/353/406/408/442/446/460/488, 160/165/205/219/406/408/441/442/446, 160/203/205/406/408/441/442/446, 160/219/330/406/408/442/446/484,

205/307/406/408/441/442/446/460/488, 406/408/441/442/446, and 406/408/442/446. In some

embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1596 and one or more residue differences as compared to SEQ ID NO: 1596, selected from

160M/165P/203E/205R/219R/353Q/406G/408A/442G/446P/460G/488N,

160M/165P/205R/219R/406G/408A/441M/442G/446P, 160M/203E/205R/406G/408A/441M/442G/446P,

160M/219R/330K/406G/408A/442G/446P/484E, 205R/307L/406G/408A/441M/442G/446P/460G/488N,

406G/408A/441M/442G/446P, and 406G/408A/442G/446P. In some embodiments, the engineered TdT

polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%,

89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1596 and one or more residue differences as compared to SEQ ID NO: 1596, selected from

T160M/Q165P/T203E/M205R/V219R/F353Q/R406G/I408A/S442G/S446P/D460G/K488N,

T160M/Q165P/M205R/V219R/R406G/I408A/E441M/S442G/S446P,

T160M/T203E/M205R/R406G/I408A/E441M/S442G/S446P,

T160M/V219R/D330K/R406G/I408A/S442G/S446P/T484E,

M205R/C307L/R406G/I408A/E441M/S442G/S446P/D460G/K488N,

R406G/I408A/E441M/S442G/S446P, and R406G/I408A/S442G/S446P.

[0165] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1950 and one or more residue differences as compared to SEQ ID NO: 1950, selected from 163/169, 185, 186, 192, 193, 194, 197, 198, 245, 251, 258, 259, 261, 263, 271, 274, 278, 280, 284, 286, 290, 291, 297, 304, 306, 308, 316, 347, 352, 359, 362, 378, 393, 396, 398, 399, 405, 407, 409/414, 410/414, 411/414, 413/414, 414, 414/415, 414/417, 415, 455, 465, 466, 468, 494, and 509. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1950 and one or more residue differences as compared to SEQ ID NO: 1950, selected from 163I/169E, 163I/169R, 185C, 185F, 186C, 192C, 192G, 192I, 192L, 192R, 193R, 193S, 194A, 194C, 194D, 194G, 194M, 194W, 197G, 197L, 197R, 198A, 198L, 245A, 251R, 258E, 258G, 258K, 258L, 258Q, 258W, 259N, 259V, 261A, 261G, 263I, 263S, 271C, 274M, 274N, 274P, 274Q, 274T, 274V, 278C, 278L, 278N, 280L, 280S, 284I, 284M, 286N, 286S, 290A, 291M, 291W, 297L, 297P, 304L, 306I, 306M, 308N, 316A, 316C, 347Q, 352G, 352R, 359V, 362S, 362Y, 378L, 393R, 396T, 398W, 399C, 399D, 399F, 399G, 399T, 405G, 405L, 405Y, 407F, 407N, 407S, 409K/414F, 409Q/414F, 410F/414F, 410I/414F, 410S/414F, 410V/414F, 410Y/414F, 411A/414F, 411F/414F, 411G/414F, 411I/414F, 411Q/414F, 411R/414F, 411T/414F, 413A/414F, 413F/414F, 413G/414F, 413I/414F, 414F/415W, 414F/417W, 414G, 415F, 455I, 455L, 465E, 466M, 468M, 468Q, 468S, 468W, 494A, 494C, 494G, 494L, 494W, 509G, and 509K. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1950 and one or more residue differences as compared to SEQ ID NO: 1950, selected from V163I/Q169E, V163I/Q169R, L185C, L185F, E186C, Y192C, Y192G, Y192I, Y192L, Y192R, E193R, E193S, F194A, F194C, F194D, F194G, F194M, F194W, N197G, N197L, N197R, D198A, D198L, G245A, Q251R, R258E, R258G, R258K, R258L, R258Q, R258W, Y259N, Y259V, S261A, S261G, K263I, K263S, V271C, K274M, K274N, K274P, K274Q, K274T, K274V, K278C, K278L, K278N, Y280L, Y280S, L284I, L284M, T286N, T286S, V290A, R291M, R291W, K297L, K297P, A304L, L306I, L306M, Y308N, V316A, V316C, F347Q,

P352G, P352R, L359V, T362S, T362Y, V378L, L393R, S396T, F398W, E399C, E399D, E399F, E399G, E399T, S405G, S405L, S405Y, K407F, K407N, K407S, D409K/H414F, D409Q/H414F, A410F/H414F, A410I/H414F, A410S/H414F, A410V/H414F, A410Y/H414F, L411A/H414F, L411F/H414F, L411G/H414F, L411I/H414F, L411Q/H414F, L411R/H414F, L411T/H414F, H413A/H414F, H413F/H414F, H413G/H414F, H413I/H414F, H414F/A415W, H414F/C417W, H414G, A415F, V455I, V455L, A465E, L466M, G468M, G468Q, G468S, G468W, H494A, H494C, H494G, H494L, H494W, S509G, and S509K.

[0166] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2008 and one or more residue differences as compared to SEQ ID NO: 2008, selected from 158/274/411/413/414, 185/274/413/414, 185/411/413/414, 263/411/413/414/468, 263/413/414, 274/286/411/413/417/468, 274/411/417/468, 274/411/468, 274/413/414/417/468, 274/468, 278/411/413/468, 411/413/417, 411/413/417/468, 411/413/468, 411/414, 413, 413/414, 413/414/468, 413/417/468, 413/468, 414, and 468. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2008 and one or more residue differences as compared to SEQ ID NO: 2008, selected from 158H/274V/411G/413A/414G, 185F/274V/413A/414G, 185F/411G/413A/414G, 263H/411G/413A/414G/468Q, 263H/413A/414G, 274V/286N/411G/413A/417W/468Q, 274V/411G/417W/468Q, 274V/411G/468Q, 274V/413A/414G/417W/468Q, 274V/468Q, 278N/411G/413A/468Q, 411G/413A/417W, 411G/413A/417W/468Q, 411G/413A/468Q, 411G/414G, 413A, 413A/414G, 413A/414G/468Q, 413A/417W/468Q, 413A/468Q, 414G, and 468Q. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2008 and one or more residue differences as compared to SEQ ID NO: 2008, selected from P158H/K274V/L411G/H413A/H414G, L185F/K274V/H413A/H414G, L185F/L411G/H413A/H414G, K263H/L411G/H413A/H414G/G468Q, K263H/H413A/H414G, K274V/T286N/L411G/H413A/C417W/G468Q, K274V/L411G/C417W/G468Q, K274V/L411G/G468Q, K274V/H413A/H414G/C417W/G468Q, K274V/G468Q, K278N/L411G/H413A/G468Q, L411G/H413A/C417W, L411G/H413A/C417W/G468Q, L411G/H413A/G468Q, L411G/H414G, H413A, H413A/H414G, H413A/H414G/G468Q, H413A/C417W/G468Q, H413A/G468Q, H414G, and G468Q.

[0167] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2008 and one or more residue differences as compared to SEQ ID NO: 2008, selected from 3, 8, 11, 12, 15, 26, 26/27, 36, 39, 50, 58, 62, 76, 90, 116, 147, 151, 157, 246, 248, 249, 253, and 255. In some embodiments, the engineered TdT

polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2008 and one or more residue differences as compared to SEQ ID NO: 2008, selected from 3A, 3G, 8P, 11C, 12V, 15A, 26A/27R, 26G, 26I, 26Q, 26T, 36G, 36K, 39A, 50L, 58M, 58N, 58S, 62W, 76P, 90L, 90S, 116A, 147G, 151S, 157V, 246N, 248R, 248V, 249A, 249G, 249R, 253L, and 255G. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2008 and one or more residue differences as compared to SEQ ID NO: 2008, selected from H3A, H3G, G8P, G11C, S12V, I15A, R26A/Q27R, R26G, R26I, R26Q, R26T, S36G, S36K, Y39A, I50L, T58M, T58N, T58S, F62W, N76P, N90L, N90S, S116A, D147G, G151S, P157V, E246N, L248R, L248V, E249A, E249G, E249R, V253L, and N255G.

[0168] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2008 and one or more residue differences as compared to SEQ ID NO: 2008, selected from 9, 11, 12, 15, 16, 26, 38, 39, 58, 70, 79, 81, 90, 151, 157, 158, and 249. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2008 and one or more residue differences as compared to SEQ ID NO: 2008, selected from 9A, 9T, 11S, 12A, 12N, 12Q, 15L, 15Q, 16S, 26S, 26W, 38L, 38T, 39G, 58A, 70A, 79T, 81E, 90M, 151I, 157L, 158A, and 249T. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2008 and one or more residue differences as compared to SEQ ID NO: 2008, selected from G9A, G9T, G11S, S12A, S12N, S12Q, I15L, I15Q, R16S, R26S, R26W, I38L, I38T, Y39G, T58A, K70A, S79T, S81E, N90M, G151I, P157L, P158A, and E249T.

[0169] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2008 and one or more residue differences as compared to SEQ ID NO: 2008, selected from 189, 196, 205, 206, 262, 307, 314, 318, 324, 353, 397, 408, 410, 413, 469, 473, 480, 481, 491, and 493. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2008 and one or more residue differences as compared to SEQ ID NO: 2008, selected from 189E, 189Q, 189R, 196A, 196S, 196T, 196Y, 205E, 206N, 262V, 307E, 307H, 307L, 307S, 314A, 314M, 314Q, 318R, 324V, 353R, 397A, 408E, 408L, 408W, 410E, 413E, 413G, 413L, 413M, 413S, 469F, 469Y, 473A, 473D, 473G, 473P, 473Q, 473S, 480A, 480E, 480G, 480L, 480S, 480W, 481A,

481L, 481S, 481V, 491M, 493E, and 493Q. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2008 and one or more residue differences as compared to SEQ ID NO: 2008, selected from A189E, A189Q, A189R, R196A, R196S, R196T, R196Y, R205E, R206N, F262V, C307E, C307H, C307L, C307S, R314A, R314M, R314Q, K318R, I324V, Q353R, Q397A, A408E, A408L, A408W, A410E, H413E, H413G, H413L, H413M, H413S, W469F, W469Y, R473A, R473D, R473G, R473P, R473Q, R473S, R480A, R480E, R480G, R480L, R480S, R480W, W481A, W481L, W481S, W481V, L491M, N493E, and N493Q.

[0170] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2254 and one or more residue differences as compared to SEQ ID NO: 2254, selected from 197/407/455, 197/455, 284/398/466, 362/407/455, 396/398/410/466, 398/466, 399/411/416, and 466. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2254 and one or more residue differences as compared to SEQ ID NO: 2254, selected from 197R/407S/455L, 197R/455L, 284M/398W/466M, 362S/407S/455L, 396T/398W/410V/466M, 398W/466M, 399D/411Q/416Q, and 466M. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2254 and one or more residue differences as compared to SEQ ID NO: 2254, selected from N197R/K407S/V455L, N197R/V455L, L284M/F398W/L466M, T362S/K407S/V455L, S396T/F398W/A410V/L466M, F398W/L466M, E399D/G411Q/K416Q, and L466M.

[0171] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2514 and one or more residue differences as compared to SEQ ID NO: 2514, selected from 26/90/94/248/261/266/362/455, 26/90/246, 26/90/246/248, 26/90/246/248/261, 26/90/246/248/362/455, 26/90/246/248/455, 26/90/246/266/362, 26/90/246/362, 26/90/246/455, 26/90/248, 26/90/248/266/455, 26/90/248/455, 26/90/248/455/459, 26/90/266, 26/90/362/455, 26/173/248, 26/246, 26/246/248/362, 26/246/248/362/455, 26/246/248/455, 26/248, 26/248/261/266, 26/248/261/266/362/455, 26/248/266/362, 26/248/362/455, 26/248/455, 26/362, 26/362/455, 58/197/249/407/410, 62/249, 90/246/248, 90/246/248/261/266/362/455, 90/246/248/266/362/455, 246/248, 246/248/362, 246/266/455, 248, 248/266/362, 248/362/455, 248/455, and 362. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2514 and one or more residue differences as compared to SEQ ID NO: 2514, selected from
26G/90L/94K/248R/261A/266V/362S/455L, 26G/90L/246N, 26G/90L/246N/248R,
26G/90L/246N/266V/362S, 26G/90L/248V/455L, 26G/90L/248V/455L/459H, 26G/90L/266V,
26G/90L/362S/455L, 26G/246N, 26G/246N/248R/362S, 26G/246N/248V/455L, 26G/248R,
26G/248V/261A/266V, 26I/90L/246N/248R, 26I/90L/248R, 26I/246N/248R/362S/455L,
26I/248R/266V/362S, 26I/248R/455L, 26Q/90L/246N/248R/261A, 26Q/90L/246N/248R/362S/455L,
26Q/90L/246N/248R/455L, 26Q/90L/246N/248V/362S/455L, 26Q/90L/246N/362S,
26Q/90L/246N/455L, 26Q/90L/248R/266V/455L, 26Q/173I/248R, 26Q/246N/248V/362S, 26Q/248R,
26Q/248R/261A/266V/362S/455L, 26Q/248R/362S/455L, 26Q/248R/455L, 26Q/248V,
26Q/248V/266V/362S, 26Q/248V/362S/455L, 26Q/362S, 26Q/362S/455L, 58N/197L/249R/407N/410S,
62W/249R, 90L/246N/248R, 90L/246N/248R/261A/266V/362S/455L,
90L/246N/248R/266V/362S/455L, 246N/248R, 246N/248R/362S, 246N/266V/455L, 248R, 248R/455L,
248V/266V/362S, 248V/362S/455L, and 362S. In some embodiments, the engineered TdT polypeptide
comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%,
91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ
ID NO: 2514 and one or more residue differences as compared to SEQ ID NO: 2514, selected from
R26G/N90L/E94K/L248R/S261A/K266V/T362S/V455L, R26G/N90L/E246N,
R26G/N90L/E246N/L248R, R26G/N90L/E246N/K266V/T362S, R26G/N90L/L248V/V455L,
R26G/N90L/L248V/V455L/Y459H, R26G/N90L/K266V, R26G/N90L/T362S/V455L, R26G/E246N,
R26G/E246N/L248R/T362S, R26G/E246N/L248V/V455L, R26G/L248R, R26G/L248V/S261A/K266V,
R26I/N90L/E246N/L248R, R26I/N90L/L248R, R26I/E246N/L248R/T362S/V455L,
R26I/L248R/K266V/T362S, R26I/L248R/V455L, R26Q/N90L/E246N/L248R/S261A,
R26Q/N90L/E246N/L248R/T362S/V455L, R26Q/N90L/E246N/L248R/V455L,
R26Q/N90L/E246N/L248V/T362S/V455L, R26Q/N90L/E246N/T362S, R26Q/N90L/E246N/V455L,
R26Q/N90L/L248R/K266V/V455L, R26Q/T173I/L248R, R26Q/E246N/L248V/T362S, R26Q/L248R,
R26Q/L248R/S261A/K266V/T362S/V455L, R26Q/L248R/T362S/V455L, R26Q/L248R/V455L,
R26Q/L248V, R26Q/L248V/K266V/T362S, R26Q/L248V/T362S/V455L, R26Q/T362S,
R26Q/T362S/V455L, T58N/N197L/E249R/K407N/V410S, F62W/E249R, N90L/E246N/L248R,
N90L/E246N/L248R/S261A/K266V/T362S/V455L, N90L/E246N/L248R/K266V/T362S/V455L,
E246N/L248R, E246N/L248R/T362S, E246N/K266V/V455L, L248R, L248R/V455L,
L248V/K266V/T362S, L248V/T362S/V455L, and T362S.

[0172] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having
at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
99% or more sequence identity to reference sequence SEQ ID NO: 2524 and one or more residue
differences as compared to SEQ ID NO: 2524, selected from 9/16/62/157/246/249/362, 11/58/227/246,
12/16/158/246/248/249, 30/189/261/266/353/465/468, 30/189/266, 30/261/266/353/468, 30/266,

30/266/303, 30/266/353, 38, 38/81/318, 38/197, 39, 39/79, 58/157/158/362, 70/353, 79/81, 81, 189, 189/261, 189/353, 246/249, 261/353, 266/307/353/468, 266/353/468, and 266/468. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2524 and one or more residue differences as compared to SEQ ID NO: 2524, selected from 9T/16S/62W/157L/246E/249R/362T, 11S/58N/227M/246E, 12A/16S/158A/246E/248L/249R, 30E/189R/266V, 30G/261A/266V/353R/468S, 30G/266V, 30G/266V/353R, 30T/189R/261A/266V/353R/465E/468S, 30T/266V/303E, 38T, 38T/81E/318R, 38T/197L, 39G, 39G/79T, 58N/157L/158A/362T, 70A/353R, 79T/81E, 81E, 189R, 189R/261A, 189R/353R, 246E/249R, 261A/353R, 266V/307S/353R/468S, 266V/353R/468S, and 266V/468S. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2524 and one or more residue differences as compared to SEQ ID NO: 2524, selected from G9T/R16S/F62W/P157L/N246E/E249R/S362T, G11S/T58N/L227M/N246E, S12A/R16S/P158A/N246E/R248L/E249R, M30E/A189R/K266V, M30G/S261A/K266V/Q353R/Q468S, M30G/K266V, M30G/K266V/Q353R, M30T/A189R/S261A/K266V/Q353R/A465E/Q468S, M30T/K266V/K303E, I38T, I38T/S81E/K318R, I38T/N197L, Y39G, Y39G/S79T, T58N/P157L/P158A/S362T, K70A/Q353R, S79T/S81E, S81E, A189R, A189R/S261A, A189R/Q353R, N246E/E249R, S261A/Q353R, K266V/C307S/Q353R/Q468S, K266V/Q353R/Q468S, and K266V/Q468S.

[0173] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2524 and one or more residue differences as compared to SEQ ID NO: 2524, selected from 47/326, 169, 191/413, 200, 292, 304/329, 327/406, 329, 340, 353/459, 373, 379, 382, 402, 403, 404, 427, 429, 459, 461, 484, 490, 495, 504, and 506. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2524 and one or more residue differences as compared to SEQ ID NO: 2524, selected from 47A/326R, 169M, 191V/413V, 200V, 292N, 304V/329R, 327M/406T, 329R, 340I, 340V, 353H/459V, 373G, 379I, 379L, 379M, 379V, 382F, 382L, 402G, 402S, 402V, 403A, 403E, 403P, 403R, 403S, 404D, 404S, 427L, 427M, 427R, 427W, 429R, 459I, 461S, 484A, 490R, 495C, 495G, 495S, 504K, and 506P. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2524 and one or more residue differences as compared to SEQ ID NO: 2524, selected from V47A/L326R, Q169M, N191V/A413V, T200V, S292N, A304V/Q329R, L327M/G406T,

Q329R, L340I, L340V, Q353H/Y459V, Q373G, T379I, T379L, T379M, T379V, W382F, W382L, K402G, K402S, K402V, L403A, L403E, L403P, L403R, L403S, P404D, P404S, K427L, K427M, K427R, K427W, D429R, Y459I, G461S, T484A, M490R, A495C, A495G, A495S, F504K, and K506P.

[0174] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2524 and one or more residue differences as compared to SEQ ID NO: 2524, selected from 175, 179, 189, 200, 203, 292, 293, 325, 340, 373, 379, 402, 403, 404, 406, 427, 459, 461, 484, 495, 506, and 508. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2524 and one or more residue differences as compared to SEQ ID NO: 2524, selected from 175L, 179L, 189V, 200N, 203D, 203M, 292N, 293Q, 325W, 340V, 373C, 373G, 379L, 379M, 402E, 402G, 402S, 403A, 403E, 403G, 403P, 404D, 404E, 404S, 406N, 427C, 427F, 427L, 427M, 427N, 427W, 427Y, 459I, 461S, 484A, 484H, 484M, 495G, 495S, 506P, 506S, 506T, 508S, and 508T. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2524 and one or more residue differences as compared to SEQ ID NO: 2524, selected from N175L, Q179L, A189V, T200N, E203D, E203M, S292N, S293Q, S325W, L340V, Q373C, Q373G, T379L, T379M, K402E, K402G, K402S, L403A, L403E, L403G, L403P, P404D, P404E, P404S, G406N, K427C, K427F, K427L, K427M, K427N, K427W, K427Y, Y459I, G461S, T484A, T484H, T484M, A495G, A495S, K506P, K506S, K506T, K508S, and K508T.

[0175] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2638 and one or more residue differences as compared to SEQ ID NO: 2638, selected from 11/30/79/189/480, 30/58/79/189/307/480, 79/189/307/410, and 79/307. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2638 and one or more residue differences as compared to SEQ ID NO: 2638, selected from 11S/30G/79T/189R/480E, 30G/58N/79T/189R/307L/480E, 79T/189R/307L/410E, and 79T/307L. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2638 and one or more residue differences as compared to SEQ ID NO: 2638, selected from G11S/M30G/S79T/A189R/R480E, M30G/T58N/S79T/A189R/C307L/R480E, S79T/A189R/C307L/V410E, and S79T/C307L.

[0176] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2804 and one or more residue differences as compared to SEQ ID NO: 2804, selected from 169/304/340/402/427, 169/304/340/427/429/504, 169/304/340/429/506, 169/304/340/504, 169/304/402/403/427/506, 169/304/403/427/504, 169/304/427/504/506, 169/304/504, 169/340/402/403/427/429/504, 169/340/402/403/427/504/506, 169/340/402/504, 169/340/427, 169/340/506, 169/402/403/504/506, 169/402/427/429/504, 169/402/504, 169/402/504/506, 169/403/427/506, 266/327/329/404/410, 292/327/329/468, 304, 304/340/402, 304/340/402/403/427/429/504/506, 304/340/402/403/504/506, 304/340/402/403/506, 304/340/402/427/504/506, 304/340/402/506, 304/340/403/427/429/504/506, 304/340/427, 304/402/403, 304/402/403/427/504, 304/402/403/429, 304/402/403/504/506, 304/402/403/506, 304/403/504, 304/504, 304/504/506, 327, 327/329, 327/329/379/404/406/410, 327/329/404/410, 327/329/465/484, 327/382/406/410/484, 327/410/484, 340, 340/402/403, 340/402/427/429/506, 340/402/429/504/506, 340/402/504, 340/403/504, 340/504, 340/506, 379/382/468, 379/404/410, 379/410, 379/465/468/484, 379/468, 402/403/427/504/506, 402/403/429/504, 402/427/504, 402/504, 403/427, 427, 484, 504, 504/506, and 506. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2804 and one or more residue differences as compared to SEQ ID NO: 2804, selected from 169M/304V/340I/429R/506P, 169M/304V/340I/504K, 169M/304V/340V/402G/427L, 169M/304V/340V/427L/429R/504K, 169M/304V/402G/403S/427L/506P, 169M/304V/403S/427L/504K, 169M/304V/427M/504K/506P, 169M/304V/504K, 169M/340I/402G/403A/427M/429R/504K, 169M/340I/427L, 169M/340I/506P, 169M/340V/402G/403P/427M/504K/506P, 169M/340V/402G/504K, 169M/402G/403A/504K/506P, 169M/402G/427L/429R/504K, 169M/402G/504K, 169M/402G/504K/506P, 169M/403A/427L/506P, 266V/327M/329R/404S/410V, 292N/327M/329R/468S, 304V, 304V/340I/402G, 304V/340I/402G/403A/427L/429R/504K/506P, 304V/340I/402G/403S/506P, 304V/340I/402G/506P, 304V/340I/403A/427L/429R/504K/506P, 304V/340I/427M, 304V/340V/402G/403S/504K/506P, 304V/340V/402G/427L/504K/506P, 304V/402G/403A/429R, 304V/402G/403A/504K/506P, 304V/402G/403P/427M/504K, 304V/402G/403P/506P, 304V/402G/403S, 304V/403S/504K, 304V/504K, 304V/504K/506P, 327M, 327M/329R, 327M/329R/379M/404D/406T/410V, 327M/329R/404D/410V, 327M/329R/465E/484A, 327M/382L/406T/410V/484A, 327M/410V/484A, 340I/402G/429R/504K/506P, 340I/402G/504K, 340I/403S/504K, 340I/506P, 340V, 340V/402G/403A, 340V/402G/427L/429R/506P, 340V/504K, 379L/465E/468S/484A, 379L/468S, 379M/382L/468S, 379M/404D/410V, 379M/410V, 379M/468S, 402G/403A/427M/504K/506P, 402G/403S/427M/504K/506P, 402G/403S/429R/504K, 402G/427L/504K, 402G/504K, 403S/427L, 427L, 427M, 484A, 504K, 504K/506P, and 506P. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2804 and one or more residue differences as compared to SEQ ID NO: 2804, selected from Q169M/A304V/L340I/D429R/K506P, Q169M/A304V/L340I/F504K, Q169M/A304V/L340V/K402G/K427L, Q169M/A304V/L340V/K427L/D429R/F504K, Q169M/A304V/K402G/L403S/K427L/K506P, Q169M/A304V/L403S/K427L/F504K, Q169M/A304V/K427M/F504K/K506P, Q169M/A304V/F504K, Q169M/L340I/K402G/L403A/K427M/D429R/F504K, Q169M/L340I/K427L, Q169M/L340I/K506P, Q169M/L340V/K402G/L403P/K427M/F504K/K506P, Q169M/L340V/K402G/F504K, Q169M/K402G/L403A/F504K/K506P, Q169M/K402G/K427L/D429R/F504K, Q169M/K402G/F504K, Q169M/K402G/F504K/K506P, Q169M/L403A/K427L/K506P, K266V/L327M/Q329R/P404S/E410V, S292N/L327M/Q329R/Q468S, A304V, A304V/L340I/K402G, A304V/L340I/K402G/L403A/K427L/D429R/F504K/K506P, A304V/L340I/K402G/L403S/K506P, A304V/L340I/K402G/K506P, A304V/L340I/L403A/K427L/D429R/F504K/K506P, A304V/L340I/K427M, A304V/L340V/K402G/L403S/F504K/K506P, A304V/L340V/K402G/K427L/F504K/K506P, A304V/K402G/L403A/D429R, A304V/K402G/L403A/F504K/K506P, A304V/K402G/L403P/K427M/F504K, A304V/K402G/L403P/K506P, A304V/K402G/L403S, A304V/L403S/F504K, A304V/F504K, A304V/F504K/K506P, L327M, L327M/Q329R, L327M/Q329R/T379M/P404D/G406T/E410V, L327M/Q329R/P404D/E410V, L327M/Q329R/A465E/T484A, L327M/W382L/G406T/E410V/T484A, L327M/E410V/T484A, L340I/K402G/D429R/F504K/K506P, L340I/K402G/F504K, L340I/L403S/F504K, L340I/K506P, L340V, L340V/K402G/L403A, L340V/K402G/K427L/D429R/K506P, L340V/F504K, T379L/A465E/Q468S/T484A, T379L/Q468S, T379M/W382L/Q468S, T379M/P404D/E410V, T379M/E410V, T379M/Q468S, K402G/L403A/K427M/F504K/K506P, K402G/L403S/K427M/F504K/K506P, K402G/L403S/D429R/F504K, K402G/K427L/F504K, K402G/F504K, L403S/K427L, K427L, K427M, T484A, F504K, F504K/K506P, and K506P.

[0177] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2812 and one or more residue differences as compared to SEQ ID NO: 2812, selected from 30, 30/179/200/373/403, 30/179/373/379, 30/184/246/325/379/429/495, 30/184/246/327/329/459, 30/184/246/379/404, 30/184/246/459/461/495/500/504, 30/200/373, 30/200/373/379, 30/246/325/327/329/404/461, 30/246/325/379/404/427/429/461, 30/246/427/459/461, 30/325/327, 30/325/327/379/404/429/495, 30/327/404/504, 30/329/379, 30/373, 30/373/403, 30/379/429/459/461, 30/379/459/461/504, 30/403/441/460, 200/373/379, 246/325/329/379/461, 246/327/404/461/495, 327/329/379/504, 327/459/461/495, 353/403, 373/379, 373/379/403, 379/403/406/468, and 403/441. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2812 and one or more residue differences as compared to SEQ ID NO: 2812, selected from 30G, 30G/179A/200N/373G/403E, 30G/179A/373G/379M, 30G/184S/246E/325W/379M/429R/495S, 30G/184S/246E/327M/329R/459I, 30G/184S/246E/379M/404D, 30G/184S/246E/459I/461S/495S/500N/504Q, 30G/200N/373G, 30G/200N/373G/379M, 30G/246E/325W/327M/329R/404D/461S, 30G/246E/325W/379M/404D/427L/429R/461S, 30G/246E/427L/459I/461S, 30G/325W/327M, 30G/325W/327M/379M/404D/429R/495S, 30G/327M/404D/504S, 30G/329R/379M, 30G/373G, 30G/373G/403L, 30G/379M/429R/459I/461S, 30G/379M/459I/461S/504Q, 30G/403E/441K/460P, 200N/373G/379M, 246E/325W/329R/379M/461S, 246E/327M/404D/461S/495S, 327M/329R/379M/504S, 327M/459I/461S/495S, 353R/403E, 373G/379M, 373G/379M/403L, 379M/403L/406N/468S, and 403L/441K. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2812 and one or more residue differences as compared to SEQ ID NO: 2812, selected from M30G, M30G/Q179A/T200N/Q373G/S403E, M30G/Q179A/Q373G/T379M, M30G/A184S/N246E/S325W/T379M/D429R/A495S, M30G/A184S/N246E/L327M/Q329R/Y459I, M30G/A184S/N246E/T379M/P404D, M30G/A184S/N246E/Y459I/G461S/A495S/T500N/F504Q, M30G/T200N/Q373G, M30G/T200N/Q373G/T379M, M30G/N246E/S325W/L327M/Q329R/P404D/G461S, M30G/N246E/S325W/T379M/P404D/K427L/D429R/G461S, M30G/N246E/K427L/Y459I/G461S, M30G/S325W/L327M, M30G/S325W/L327M/T379M/P404D/D429R/A495S, M30G/L327M/P404D/F504S, M30G/Q329R/T379M, M30G/Q373G, M30G/Q373G/S403L, M30G/T379M/D429R/Y459I/G461S, M30G/T379M/Y459I/G461S/F504Q, M30G/S403E/M441K/G460P, T200N/Q373G/T379M, N246E/S325W/Q329R/T379M/G461S, N246E/L327M/P404D/G461S/A495S, L327M/Q329R/T379M/F504S, L327M/Y459I/G461S/A495S, Q353R/S403E, Q373G/T379M, Q373G/T379M/S403L, T379M/S403L/G406N/Q468S, and S403L/M441K.

[0178] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2812 and one or more residue differences as compared to SEQ ID NO: 2812, selected from 198, 231, 233, 248, 253, 264, 266, 278, 326, 367, 370, 396, 414, 433, 435, 437, 444, 446, 485, 499, 503, 520, and 525. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2812 and one or more residue differences as compared to SEQ ID NO: 2812, selected from 198E, 231S, 233R, 248K, 248T, 253I, 264E, 264L, 264T, 264V, 266R,

278M, 326R, 367D, 370D, 370G, 370M, 370S, 396R, 414E, 433A, 433E, 433G, 433M, 433P, 433R, 433S, 435A, 435E, 435G, 435S, 437G, 437R, 444G, 444R, 446G, 485E, 499M, 499R, 503A, 503E, 503T, 503V, 520P, 525Q, 525R, and 525S. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2812 and one or more residue differences as compared to SEQ ID NO: 2812, selected from D198E, G231S, E233R, R248K, R248T, V253I, R264E, R264L, R264T, R264V, K266R, K278M, L326R, E367D, Q370D, Q370G, Q370M, Q370S, T396R, G414E, W433A, W433E, W433G, W433M, W433P, W433R, W433S, M435A, M435E, M435G, M435S, T437G, T437R, A444G, A444R, P446G, D485E, L499M, L499R, I503A, I503E, I503T, I503V, E520P, F525Q, F525R, and F525S.

[0179] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2812 and one or more residue differences as compared to SEQ ID NO: 2812, selected from 186, 188, 198, 231, 233, 235, 243, 248, 253, 264, 266, 287, 297/440, 366, 367, 368, 370, 414, 433, 435, 437, 439, 442, 444, 485, 501, and 515. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2812 and one or more residue differences as compared to SEQ ID NO: 2812, selected from 186D, 188I, 198E, 231S, 233A, 235R, 243L, 243R, 243S, 243T, 248S, 253I, 264A, 264T, 266R, 266T, 287I, 297S/440K, 366V, 367A, 368R, 370M, 370N, 370S, 414E, 433M, 433P, 433V, 435E, 435I, 435P, 437A, 437G, 437K, 437P, 439G, 439P, 442A, 444G, 444H, 485E, 485S, 501R, and 515E. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2812 and one or more residue differences as compared to SEQ ID NO: 2812, selected from E186D, L188I, D198E, G231S, E233A, K235R, E243L, E243R, E243S, E243T, R248S, V253I, R264A, R264T, K266R, K266T, L287I, K297S/N440K, A366V, E367A, K368R, Q370M, Q370N, Q370S, G414E, W433M, W433P, W433V, M435E, M435I, M435P, T437A, T437G, T437K, T437P, S439G, S439P, G442A, A444G, A444H, D485E, D485S, K501R, and A515E.

[0180] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2956 and one or more residue differences as compared to SEQ ID NO: 2956, selected from 140/142/153/177/427/434/441/444/461/502, 140/142/153/427/484, 140/142/177, 140/142/177/441, 140/142/365/373/404/427/484, 140/142/373/427/484, 140/148/161/177/404, 140/150/153/365/373/427/484, 140/150/177/404/436/441/484/502, 140/161/177/404/427/484, 140/177/404, 140/177/404/484, 142/150/158/177/427/445, 142/150/177/404, 142/153/177/441/444, 142/177/373, 142/177/373/441,

142/461/484/502, 150/177, 153, 153/161/325/404/427/441/484, 153/484, 177, 177/365/427/434, 325/427, 427, and 484. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2956 and one or more residue differences as compared to SEQ ID NO: 2956, selected from

140V/142V/153V/177R/427L/434N/441V/444S/461S/502G, 140V/142V/153V/427L/484L, 140V/142V/177R, 140V/142V/177R/441V, 140V/142V/365A/373R/404D/427L/484L, 140V/142V/373R/427L/484L, 140V/148T/161H/177R/404D, 140V/150P/153V/365A/373R/427L/484L, 140V/150P/177R/404D/436S/441V/484L/502G, 140V/161H/177R/404D/427L/484L, 140V/177R/404D, 140V/177R/404D/484L, 142V/150P/158S/177R/427L/445N, 142V/150P/177R/404D, 142V/153V/177R/441V/444S, 142V/177R/373R, 142V/177R/373R/441V, 142V/461S/484L/502G, 150P/177R, 153V, 153V/161H/325L/404D/427L/441V/484L, 153V/484L, 177R, 177R/365A/427L/434N, 325L/427L, 427L, and 484L. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2956 and one or more residue differences as compared to SEQ ID NO: 2956, selected from G140V/M142V/E153V/H177R/K427L/E434N/M441V/A444S/G461S/K502G, G140V/M142V/E153V/K427L/T484L, G140V/M142V/H177R, G140V/M142V/H177R/M441V, G140V/M142V/G365A/Q373R/P404D/K427L/T484L, G140V/M142V/Q373R/K427L/T484L, G140V/P148T/K161H/H177R/P404D, G140V/E150P/E153V/G365A/Q373R/K427L/T484L, G140V/E150P/H177R/P404D/P436S/M441V/T484L/K502G, G140V/K161H/H177R/P404D/K427L/T484L, G140V/H177R/P404D, G140V/H177R/P404D/T484L, M142V/E150P/P158S/H177R/K427L/K445N, M142V/E150P/H177R/P404D, M142V/E153V/H177R/M441V/A444S, M142V/H177R/Q373R, M142V/H177R/Q373R/M441V, M142V/G461S/T484L/K502G, E150P/H177R, E153V, E153V/K161H/W325L/P404D/K427L/M441V/T484L, E153V/T484L, H177R, H177R/G365A/K427L/E434N, W325L/K427L, K427L, and T484L.

[0181] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3174 and one or more residue differences as compared to SEQ ID NO: 3174, selected from 186/188/248/253/365/366/444/445, 186/231/248/253/484, 186/231/248/365/366/368/484, 186/231/248/366/368/444, 186/231/248/484, 186/231/368/416/441/442/444/484/485, 186/231/441/444/445, 186/484/485, 188/231/248/253/365/441/442/444/445/484, 198/243/264/431/441, 198/243/396/414/431/433/441/499, 198/243/396/414/433/437/441/515, 198/264/266/414, 198/264/396/414/433/441/515, 198/266/396/414/433/441/499/501, 198/266/414/433/441/515, 198/266/437/441/499, 198/414/431/441/499/520, 198/414/433/441/515/520, 231/248/441/484, 243/264/515/520,

243/396/414/433/441/515/520, 243/414/433/437/441, 243/414/437/441/515, 248/442/444/445/484, 264/266/396/414/433/441/515, 264/266/414/441/499/501/515/520, 264/414, 264/414/441, 264/433/441, 266/437/441/499/515/520, 365/366/441/484/485, 396/414/441, 396/414/441/515, and 414/441/520. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3174 and one or more residue differences as compared to SEQ ID NO: 3174, selected from 186D/188I/248K/253I/365A/366V/444H/445N, 186D/231S/248K/253I/484L, 186D/231S/248K/365A/366V/368R/484L, 186D/231S/248K/366V/368R/444H, 186D/231S/248K/484L, 186D/231S/368R/416N/441M/442K/444A/484L/485E, 186D/231S/441M/444H/445N, 186D/484L/485E, 188I/231S/248K/253I/365A/441M/442K/444A/445N/484L, 198E/243S/264A/431R/441M, 198E/243S/396S/414E/431R/433V/441M/499M, 198E/243S/396S/414E/433R/437R/441M/515E, 198E/264A/266T/414E, 198E/264A/396S/414E/433V/441M/515E, 198E/266T/396S/414E/433V/441M/499M/501R, 198E/266T/414E/433S/441M/515E, 198E/266T/437R/441M/499M, 198E/414E/431R/441M/499M/520D, 198E/414E/433V/441M/515E/520D, 231S/248K/441M/484L, 243S/264A/515E/520D, 243S/396S/414E/433V/441M/515E/520D, 243S/414E/433S/437R/441M, 243S/414E/437R/441M/515E, 248K/442K/444A/445N/484L, 264A/266T/396S/414E/433R/441M/515E, 264A/266T/414E/441M/499M/501R/515E/520D, 264A/414E, 264A/414E/441M, 264A/433S/441M, 266T/437R/441M/499M/515E/520D, 365A/366V/441M/484L/485E, 396S/414E/441M, 396S/414E/441M/515E, and 414E/441M/520D. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3174 and one or more residue differences as compared to SEQ ID NO: 3174, selected from E186D/L188I/R248K/V253I/G365A/A366V/S444H/K445N, E186D/G231S/R248K/V253I/T484L, E186D/G231S/R248K/G365A/A366V/K368R/T484L, E186D/G231S/R248K/A366V/K368R/S444H, E186D/G231S/R248K/T484L, E186D/G231S/K368R/K416N/V441M/G442K/S444A/T484L/D485E, E186D/G231S/V441M/S444H/K445N, E186D/T484L/D485E, L188I/G231S/R248K/V253I/G365A/V441M/G442K/S444A/K445N/T484L, D198E/E243S/R264A/S431R/V441M, D198E/E243S/T396S/G414E/S431R/W433V/V441M/L499M, D198E/E243S/T396S/G414E/W433R/T437R/V441M/A515E, D198E/R264A/K266T/G414E, D198E/R264A/T396S/G414E/W433V/V441M/A515E, D198E/K266T/T396S/G414E/W433V/V441M/L499M/K501R, D198E/K266T/G414E/W433S/V441M/A515E, D198E/K266T/T437R/V441M/L499M, D198E/G414E/S431R/V441M/L499M/E520D, D198E/G414E/W433V/V441M/A515E/E520D, G231S/R248K/V441M/T484L, E243S/R264A/A515E/E520D, E243S/T396S/G414E/W433V/V441M/A515E/E520D, E243S/G414E/W433S/T437R/V441M,

E243S/G414E/T437R/V441M/A515E, R248K/G442K/S444A/K445N/T484L,
R264A/K266T/T396S/G414E/W433R/V441M/A515E,
R264A/K266T/G414E/V441M/L499M/K501R/A515E/E520D, R264A/G414E, R264A/G414E/V441M,
R264A/W433S/V441M, K266T/T437R/V441M/L499M/A515E/E520D,
G365A/A366V/V441M/T484L/D485E, T396S/G414E/V441M, T396S/G414E/V441M/A515E, and
G414E/V441M/E520D.

[0182] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3174 and one or more residue differences as compared to SEQ ID NO: 3174, selected from 122/473, 189, 190, 193, 194, 196, 263, 264, 266, 267, 268, 269, 273, 273/501, 274, 278, 279, 281, 297, 298, 299, 300, 301, 302, 304, 309, 312, 315, 347, 350, 352, 353, 359, 390, 392, 394, 407, 408, 410, 411, 413, 414, 416, 436, 454, 468, 472, 473, 477, 479, 480, and 493. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3174 and one or more residue differences as compared to SEQ ID NO: 3174, selected from 122I/473K, 189L, 190L, 190M, 190R, 193S, 193T, 194C, 194L, 194S, 194W, 196G, 196L, 196M, 196N, 196T, 263M, 263R, 264C, 266F, 266I, 266R, 266T, 266V, 266Y, 267A, 267C, 267H, 267V, 267Y, 268C, 268I, 268L, 268T, 269W, 273C, 273D, 273E, 273E/501N, 273F, 273G, 273I, 273L, 273Y, 274A, 274G, 274I, 274V, 278F, 278H, 278M, 278R, 278V, 279Y, 281K, 281L, 297C, 297L, 297M, 297R, 297T, 297V, 298I, 298M, 299A, 299L, 299M, 299N, 299Y, 300H, 301S, 301T, 301V, 302N, 302S, 304D, 304M, 304T, 309F, 309G, 309I, 309L, 309M, 309V, 309W, 312M, 312T, 312V, 315Q, 347I, 350L, 350R, 350W, 352V, 353A, 359G, 390C, 390I, 390L, 390V, 392V, 394A, 394F, 394G, 394M, 394V, 394Y, 407L, 407M, 407N, 407R, 407W, 408G, 408I, 408L, 408M, 408T, 408V, 410F, 410G, 410I, 411E, 411N, 413F, 413L, 413P, 413S, 413V, 414A, 416G, 436S, 454F, 454M, 454V, 468F, 468H, 468M, 468T, 472G, 473G, 477G, 479F, 479V, 480H, 480K, 493V, and 493Y. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3174 and one or more residue differences as compared to SEQ ID NO: 3174, selected from M122I/R473K, R189L, E190L, E190M, E190R, E193S, E193T, F194C, F194L, F194S, F194W, R196G, R196L, R196M, R196N, R196T, H263M, H263R, R264C, K266F, K266I, K266R, K266T, K266V, K266Y, S267A, S267C, S267H, S267V, S267Y, V268C, V268I, V268L, V268T, F269W, V273C, V273D, V273E, V273E/K501N, V273F, V273G, V273I, V273L, V273Y, K274A, K274G, K274I, K274V, K278F, K278H, K278M, K278R, K278V, W279Y, R281K, R281L, K297C, K297L, K297M, K297R, K297T, K297V, L298I, L298M, T299A, T299L, T299M, T299N, T299Y, P300H, M301S, M301T, M301V, Q302N, Q302S, V304D, V304M, V304T, Y309F, Y309G, Y309I, Y309L, Y309M, Y309V, Y309W, L312M, L312T, L312V, G315Q, F347I, G350L, G350R, G350W,

P352V, Q353A, L359G, Y390C, Y390I, Y390L, Y390V, R392V, E394A, E394F, E394G, E394M, E394V, E394Y, K407L, K407M, K407N, K407R, K407W, A408G, A408I, A408L, A408M, A408T, A408V, E410F, E410G, E410I, G411E, G411N, A413F, A413L, A413P, A413S, A413V, G414A, K416G, P436S, L454F, L454M, L454V, Q468F, Q468H, Q468M, Q468T, S472G, R473G, R477G, L479F, L479V, R480H, R480K, N493V, and N493Y.

[0183] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3174 and one or more residue differences as compared to SEQ ID NO: 3174, selected from 196, 263, 266, 268, 273, 281, 394, 416, 454, 468, 473, 477, and 493. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3174 and one or more residue differences as compared to SEQ ID NO: 3174, selected from 196G, 263R, 266I, 266T, 266V, 268I, 273I, 273L, 281K, 394A, 394F, 394G, 394M, 394V, 394Y, 416S, 454M, 468A, 468F, 468H, 468M, 468T, 473G, 477S, and 493V. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3174 and one or more residue differences as compared to SEQ ID NO: 3174, selected from R196G, H263R, K266I, K266T, K266V, V268I, V273I, V273L, R281K, E394A, E394F, E394G, E394M, E394V, E394Y, K416S, L454M, Q468A, Q468F, Q468H, Q468M, Q468T, R473G, R477S, and N493V.

[0184] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1830 and one or more residue differences as compared to SEQ ID NO: 1830, selected from 194, 196, 266, 267, 268, 269, 273, 273/501, 274, 277, 278, 297, 298, 299, 301, 302, 309, 312, 347, 359, 390, 392, 394, 407, 408, 413, 416, 454, 468, 473, 477, 479, and 493. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1830 and one or more residue differences as compared to SEQ ID NO: 1830, selected from 194L, 194W, 196G, 196M, 196N, 266R, 266T, 266V, 267A, 268L, 268T, 269W, 273D, 273E, 273E/501N, 273F, 273G, 273I, 273L, 273Y, 274A, 274G, 274I, 274V, 277E, 278Y, 297R, 298M, 299A, 299N, 299S, 301S, 301T, 301V, 302S, 309F, 309L, 309M, 309W, 312V, 347I, 359G, 390C, 390V, 392V, 394Y, 407D, 407L, 407M, 407N, 407R, 408I, 413S, 416G, 416S, 454M, 454V, 468T, 473G, 477G, 479V, and 493V. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 1830 and one or more residue differences as compared to SEQ ID NO: 1830, selected from F194L, F194W, R196G, R196M, R196N, K266R, K266T,

K266V, S267A, V268L, V268T, F269W, V273D, V273E, V273E/K501N, V273F, V273G, V273I, V273L, V273Y, K274A, K274G, K274I, K274V, D277E, K278Y, K297R, L298M, T299A, T299N, T299S, M301S, M301T, M301V, Q302S, Y309F, Y309L, Y309M, Y309W, L312V, F347I, L359G, Y390C, Y390V, R392V, E394Y, K407D, K407L, K407M, K407N, K407R, A408I, A413S, K416G, K416S, L454M, L454V, Q468T, R473G, R477G, L479V, and N493V.

[0185] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3222 and one or more residue differences as compared to SEQ ID NO: 3222, selected from 186/194/248/396, 186/198/243/248/366/368/394/501, 186/231/243/368/394/485/499, 186/231/248/485, 186/231/366/368/394/485, 186/243, 186/243/248/366/368/394, 186/243/248/394/484/485, 186/243/484/520, 186/248, 186/365, 186/365/366/368/394/499, 186/365/366/394/485, 186/366/368/394/396/484, 186/366/368/394/396/484/485, 186/394/396/485, 194/198/243/366/368/499, 194/515/520, 198/231/243/248/485, 198/243/248/365/394/501, 198/248/394/396/484/485/499, 198/394/396/484/485/499, 198/394/396/499/515/520, 198/394/499/501, 231/365/368/394/499/520, 231/368/394, 231/484/485/499/501, 243/248/394/396/484/485, 243/484, 243/484/485/499, 248/365/366/368/394/484/520, 248/394/484, 365/366/368/394, 365/368/394/396/520, 394/396, and 394/499. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3222 and one or more residue differences as compared to SEQ ID NO: 3222, selected from 186D/194L/248K/396T, 186D/198E/243S/248K/366V/368R/394Y/501R, 186D/231S/243S/368R/394Y/485E/499M, 186D/231S/248K/485E, 186D/231S/366V/368R/394Y/485E, 186D/243S, 186D/243S/248K/366V/368R/394Y, 186D/243S/248K/394Y/484L/485E, 186D/243S/484L/520D, 186D/248K, 186D/365A, 186D/365A/366V/368R/394Y/499M, 186D/365A/366V/394Y/485E, 186D/366V/368R/394Y/396T/484L, 186D/366V/368R/394Y/396T/484L/485E, 186D/394Y/396T/485E, 194L/198E/243S/366V/368R/499M, 194L/515A/520D, 198E/231S/243S/248K/485E, 198E/243S/248K/365A/394Y/501R, 198E/248K/394Y/396T/484L/485E/499M, 198E/394Y/396T/484L/485E/499M, 198E/394Y/396T/499M/515A/520D, 198E/394Y/499M/501R, 231S/365A/368R/394Y/499M/520D, 231S/368R/394Y, 231S/484L/485E/499M/501R, 243S/248K/394Y/396T/484L/485E, 243S/484L, 243S/484L/485E/499M, 248K/365A/366V/368R/394Y/484L/520D, 248K/394Y/484L, 365A/366V/368R/394Y, 365A/368R/394Y/396T/520D, 394Y/396T, and 394Y/499M. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3222 and one or more residue differences as compared to SEQ ID NO: 3222, selected from E186D/F194L/R248K/S396T,

E186D/D198E/E243S/R248K/A366V/K368R/E394Y/K501R,
E186D/G231S/E243S/K368R/E394Y/D485E/L499M, E186D/G231S/R248K/D485E,
E186D/G231S/A366V/K368R/E394Y/D485E, E186D/E243S,
E186D/E243S/R248K/A366V/K368R/E394Y, E186D/E243S/R248K/E394Y/T484L/D485E,
E186D/E243S/T484L/E520D, E186D/R248K, E186D/G365A,
E186D/G365A/A366V/K368R/E394Y/L499M, E186D/G365A/A366V/E394Y/D485E,
E186D/A366V/K368R/E394Y/S396T/T484L, E186D/A366V/K368R/E394Y/S396T/T484L/D485E,
E186D/E394Y/S396T/D485E, F194L/D198E/E243S/A366V/K368R/L499M, F194L/E515A/E520D,
D198E/G231S/E243S/R248K/D485E, D198E/E243S/R248K/G365A/E394Y/K501R,
D198E/R248K/E394Y/S396T/T484L/D485E/L499M, D198E/E394Y/S396T/T484L/D485E/L499M,
D198E/E394Y/S396T/L499M/E515A/E520D, D198E/E394Y/L499M/K501R,
G231S/G365A/K368R/E394Y/L499M/E520D, G231S/K368R/E394Y,
G231S/T484L/D485E/L499M/K501R, E243S/R248K/E394Y/S396T/T484L/D485E, E243S/T484L,
E243S/T484L/D485E/L499M, R248K/G365A/A366V/K368R/E394Y/T484L/E520D,
R248K/E394Y/T484L, G365A/A366V/K368R/E394Y, G365A/K368R/E394Y/S396T/E520D,
E394Y/S396T, and E394Y/L499M.

[0186] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3670 and one or more residue differences as compared to SEQ ID NO: 3670, selected from 273, 273/309/493/499, 273/493/499, 273/499, 274/299/408/416, 274/408/416, 274/416, 288/299/416, 298/299/416, 309, 309/499, 416, and 493/499. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3670 and one or more residue differences as compared to SEQ ID NO: 3670, selected from 273I, 273I/309M/493V/499L, 273I/493V/499L, 273I/499L, 274A/299N/408I/416S, 274A/408I/416S, 274I/416S, 288V/299N/416S, 298M/299N/416S, 309M, 309M/499L, 416S, and 493V/499L. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3670 and one or more residue differences as compared to SEQ ID NO: 3670, selected from V273I, V273I/Y309M/N493V/M499L, V273I/N493V/M499L, V273I/M499L, K274A/T299N/A408I/K416S, K274A/A408I/K416S, K274I/K416S, E288V/T299N/K416S, L298M/T299N/K416S, Y309M, Y309M/M499L, K416S, and N493V/M499L.

[0187] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3670 and one or more residue differences as compared to SEQ ID NO: 3670, selected from 168, 198, 267, 301, 307, 308, 308/361, 313,

372, 392, 397, 415, 419, 451, 452, 456, 472, 473, 475, 493/499, and 528. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3670 and one or more residue differences as compared to SEQ ID NO: 3670, selected from 168S, 198S, 267M, 267R, 301G, 301Q, 301S, 307A, 307G, 307S, 308A, 308A/361T, 308G, 308H, 308K, 308S, 308V, 313M, 372E, 392V, 397F, 397W, 415L, 415W, 419G, 419M, 451K, 452L, 456P, 456T, 472A, 473A, 473S, 475V, 493R/499L, and 528L. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3670 and one or more residue differences as compared to SEQ ID NO: 3670, selected from C168S, D198S, S267M, S267R, M301G, M301Q, M301S, L307A, L307G, L307S, Y308A, Y308A/I361T, Y308G, Y308H, Y308K, Y308S, Y308V, A313M, D372E, R392V, Q397F, Q397W, A415L, A415W, L419G, L419M, R451K, V452L, R456P, R456T, S472A, R473A, R473S, F475V, N493R/M499L, and N528L.

[0188] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3670 and one or more residue differences as compared to SEQ ID NO: 3670, selected from 303/396, 308, 473, and 493/499. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3670 and one or more residue differences as compared to SEQ ID NO: 3670, selected from 303H/396A, 308L, 473A, 473M, 473S, and 493V/499L. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3670 and one or more residue differences as compared to SEQ ID NO: 3670, selected from K303H/S396A, Y308L, R473A, R473M, R473S, and N493V/M499L.

[0189] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3670 and one or more residue differences as compared to SEQ ID NO: 3670, selected from 273, 273/309/413/499, 273/309/493/499, 273/493, 273/493/499, 273/499, 274/299/408/416, 274/408/416, 274/416, 281/413/499, 288/299/416, 298/299/416, 309, 309/413, 413, 413/493/499, 413/499, 416, and 493/499. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3670 and one or more residue differences as compared to SEQ ID NO: 3670, selected from 273E/493V, 273I, 273I/309M/413S/499L, 273I/309M/493V/499L,

273I/493V/499L, 273I/499L, 273S/309M/413S/499L, 274A/299N/408I/416S, 274A/408I/416S, 274I/416S, 281K/413S/499L, 288V/299N/416S, 298M/299N/416S, 309M, 309M/413S, 413S, 413S/493V/499L, 413S/499L, 416S, and 493V/499L. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3670 and one or more residue differences as compared to SEQ ID NO: 3670, selected from V273E/N493V, V273I, V273I/Y309M/A413S/M499L, V273I/Y309M/N493V/M499L, V273I/N493V/M499L, V273I/M499L, V273S/Y309M/A413S/M499L, K274A/T299N/A408I/K416S, K274A/A408I/K416S, K274I/K416S, R281K/A413S/M499L, E288V/T299N/K416S, L298M/T299N/K416S, Y309M, Y309M/A413S, A413S, A413S/N493V/M499L, A413S/M499L, K416S, and N493V/M499L.

[0190] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3674 and one or more residue differences as compared to SEQ ID NO: 3674, selected from 194/196/390, 194/196/390/394/460/480, 194/196/390/394/480, 194/196/390/454/480, 194/196/390/480, 194/196/394/454/480, 194/196/454, 194/390, 194/394, 194/394/454, 194/394/454/480, 196, 196/390, 196/390/394, 196/390/394/454, 196/390/394/454/480, 196/390/394/480, 196/390/454, 196/394, 196/394/454, 196/394/454/480, 196/394/480, 196/454, 297/470/473, 297/473/493, 390, 390/394, 390/394/454, 390/394/454/480, 390/394/480, 390/454, 390/480, 394, 394/480, and 454. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3674 and one or more residue differences as compared to SEQ ID NO: 3674, selected from 194L/196G/390C, 194L/196G/390C/394F/460V/480K, 194L/196G/390C/394F/480K, 194L/196G/390C/454M/480K, 194L/196G/390C/480K, 194L/196G/394F/454M/480K, 194L/196G/454M, 194L/390C, 194L/394F, 194L/394F/454M, 194L/394F/454M/480K, 196G, 196G/390C, 196G/390C/394F, 196G/390C/394F/454M, 196G/390C/394F/454M/480K, 196G/390C/394F/480K, 196G/390C/454M, 196G/394F, 196G/394F/454M, 196G/394F/454M/480K, 196G/394F/480K, 196G/454M, 297R/470S/473G, 297R/473G/493V, 390C, 390C/394F, 390C/394F/454M, 390C/394F/454M/480K, 390C/394F/480K, 390C/454M, 390C/480K, 394F, 394F/480K, and 454M. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3674 and one or more residue differences as compared to SEQ ID NO: 3674, selected from F194L/R196G/Y390C, F194L/R196G/Y390C/E394F/G460V/R480K, F194L/R196G/Y390C/E394F/R480K, F194L/R196G/Y390C/L454M/R480K, F194L/R196G/Y390C/R480K, F194L/R196G/E394F/L454M/R480K, F194L/R196G/L454M, F194L/Y390C, F194L/E394F,

F194L/E394F/L454M, F194L/E394F/L454M/R480K, R196G, R196G/Y390C, R196G/Y390C/E394F, R196G/Y390C/E394F/L454M, R196G/Y390C/E394F/L454M/R480K, R196G/Y390C/E394F/R480K, R196G/Y390C/L454M, R196G/E394F, R196G/E394F/L454M, R196G/E394F/L454M/R480K, R196G/E394F/R480K, R196G/L454M, K297R/T470S/R473G, K297R/R473G/N493V, Y390C, Y390C/E394F, Y390C/E394F/L454M, Y390C/E394F/L454M/R480K, Y390C/E394F/R480K, Y390C/L454M, Y390C/R480K, E394F, E394F/R480K, and L454M.

[0191] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3796 and one or more residue differences as compared to SEQ ID NO: 3796, selected from 198, 198/267/313/451/475/494/499, 198/267/314/451, 198/267/314/475, 198/267/409/451, 198/267/475, 198/451/493, 208/308, 208/308/461, 267, 267/314/328/451/494/499, 267/451, 267/451/494/499, 308, 314/328/451/499, 314/451, 328/409/451, 328/451, 409, 409/475/494, 451, 451/493/494, 451/493/499, 451/494, and 475. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3796 and one or more residue differences as compared to SEQ ID NO: 3796, selected from 198P, 198P/267Q/314A/451K, 198P/267Q/314A/475V, 198P/267Q/409L/451K, 198P/267Q/475V, 198P/267R/313L/451K/475V/494V/499L, 198P/451K/493V, 208V/308A/461N, 208V/308V, 267Q/451K/494V/499L, 267R, 267R/314A/328T/451K/494R/499L, 267R/451K, 308A, 308V, 314A/328T/451K/499L, 314A/451K, 328T/409L/451K, 328T/451K, 409L, 409L/475V/494V, 451K, 451K/493V/494V, 451K/493V/499L, 451K/494V, and 475V. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 3796 and one or more residue differences as compared to SEQ ID NO: 3796, selected from D198P, D198P/S267Q/R314A/R451K, D198P/S267Q/R314A/F475V, D198P/S267Q/D409L/R451K, D198P/S267Q/F475V, D198P/S267R/A313L/R451K/F475V/H494V/M499L, D198P/R451K/N493V, I208V/Y308A/S461N, I208V/Y308V, S267Q/R451K/H494V/M499L, S267R, S267R/R314A/V328T/R451K/H494R/M499L, S267R/R451K, Y308A, Y308V, R314A/V328T/R451K/M499L, R314A/R451K, V328T/D409L/R451K, V328T/R451K, D409L, D409L/F475V/H494V, R451K, R451K/N493V/H494V, R451K/N493V/M499L, R451K/H494V, and F475V.

[0192] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **3870** and one or more residue differences as compared to SEQ ID NO: **3870** at a position or set of positions selected from 165, 169, 171, 173, 175, 176, 179, 183, 187, 191, 192, 195, 197, 199, 200, 203, 204, 210, 257, 259, 267, 291, 293, 295, 301, 319, 325, 340, 341, 342, 374, 387, 398, 399, 403, 404, 406, 429, 480, 481, 483, 484, 490, 491, 493,

494, 495, 521, 507, 508, 509, and 522. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO:

3870 and one or more residue differences as compared to SEQ ID NO: **3870** selected from P165A, P165C, P165R, P165T, Q169M, Q169T, R171G, T173S, N175L, N175M, N175V, N176V, Q179K, N175I, Q179M, Q179P, Q179S, Q179T, Q179V, D183C, D183W, I187R, N191F, N191M, N191Q, N191V, Y192A, Y192M, Y192T, K195R, N197C, N197E, N197F, N197R, N197T, D199G, D199R, D199S, D199V, D199W, T200K, T200M, E203F, E203G, E203M, E203V, F204I, F204V, V210M, E257P, E257S, Y259F, R267S, R291M, S293C, S293T, T295A, T295G, T295N, T295S, T295V, M301G, M301W A319E, W325G, W325L, I340S, V341C, E342Q, L374V, L387I, W398V, E399S, S403K, S403L, S403R, S403T, S403V, S403W, P404G, P404M, G406L, G406R, G406S, G406T, R429F, R429H, R429V, R480A, R480Q, W481Q, A483C, L484S, M490A, M490E, M490L, M490R, M490S, M490V, L491V, N493K, H494A, H494T, S495C, S495M, S495N, S495R, A507G, K508N, S509R, Y521V, and L522I.

[0193] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **3918** and one or more residue differences as compared to SEQ ID NO: **3918** at a position or set of positions selected from 3, 7/400/459/504, 164, 173/367/459/500, 184, 203, 203/367/459, 203/367/459/500/501, 203/400/459/501, 203/459, 203/459/499/504, 203/459/500, 215, 218, 294, 335, 335/402/481/484, 335/402/512, 335/481/484, 335/481/493, 335/481/512, 336, 338, 339, 367, 367/459/500, 370, 373, 376, 380, 384, 390, 395, 395/402, 395/402/481/484, 395/481, 395/481/512, 395/484, 400, 400/459, 400/459/499/500/504, 400/459/500/501, 400/459/501/504, 400/501, 400/504, 402, 402/481, 402/481/484, 402/481/484/512, 402/481/493, 402/484/493, 402/512, 458, 459, 459/501, 460, 481, 481/512, 484, 485, 493, 499, 500, 501, 504, 512, 515, and 516. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **3918** and one or more residue differences as compared to SEQ ID NO: **3918** selected from 3Q, H7-

/D400W/Y459R/F504Q, S164G, T173I/E367S/Y459V/T500S, S184A, E203R, E203R/E367S/Y459R, E203R/E367S/Y459V/T500S/R501P, E203R/D400W/Y459R/R501P, E203R/Y459R/M499R/F504G, E203R/Y459R/T500S, E203R/Y459V, P215Q, V218L, K294D, F335V, F335V/G402K/W481R/L484E, F335V/G402K/W481R/L484R, F335V/G402K/E512R, F335V/G402R/W481R/L484R, F335V/W481D/L484R, F335V/W481R/L484R, F335V/W481R/N493T, F335V/W481R/E512R, D336Q, D338E, D338Q, A339R, E367R, E367S, E367S/Y459V/T500S, E367T, Q370E, Q373G, Q376S, N380G, K384S, C390A, W395A, W395E, W395N, W395N/G402K, W395N/W481R/E512R, W395N/L484R, W395T, W395T/G402K/W481R/L484R, W395T/W481R, D400W, D400W/Y459R/T500S/R501P, D400W/Y459V, D400W/Y459V/M499R/T500S/F504Q, D400W/Y459V/R501P/F504G, D400W/R501P,

D400W/F504Q, G402K, G402K/W481D, G402K/W481D/L484R/E512R, G402K/W481D/N493T, G402K/W481R/L484R, G402K/E512R, G402R, G402R/L484R/N493T, G402S, P458R, Y459R, Y459V, Y459V/R501P, G460M, G460R, W481D, W481R, W481R/E512R, L484E, L484R, E485Q, N493T, M499P, M499R, T500S, R501N, R501P, F504G, F504Q, E512G, E512R, E515P, and H516T.

[0194] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **4266** and one or more residue differences as compared to SEQ ID NO: **4266** at a position or set of positions selected from 95/428/480, 163/190, 163/203/366, 163/328/363/480, 163/363/480/485, 172/174/178/340, 178, 188, 190/202/203/363/366/480/483/485, 190/202/203/480, 190/480/485, 192, 192/498/499/503, 202, 202/203/328/362/363/366/428/480/485/498/499/503, 202/203/485, 203/328/363/428/483, 203/328/428, 203/328/480/485, 203/362/366, 203/498/499/503, 272, 280, 280/498/499/503, 296, 297, 299, 299/498/499/503, 301, 301/503, 308, 308/503, 311, 328/428, 328/480/483, 328/485, 343, 346, 349, 358, 359/498/499/503, 362/363, 362/363/366/428/480/483/498/499/503, 392/498/499/503, 406, 407, 410, 411, 418/498/499/503, 418/503, 419/503, 465, 471, 472/498/499/503, 473, 480/483, 491, and 498/499/503. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **4266** and one or more residue differences as compared to SEQ ID NO: **4266** selected from L95M/R428V/W480D, S163Q/N190M, S163Q/F203I/E366S, S163Q/Q328L/P363G/W480D, S163Q/P363G/W480D/E485Q, T172S/N174M/Q178K/V340C, Q178K, R188K, N190M/E202R/F203I/P363G/E366S/W480D/L483R/E485Q, N190M/E202R/F203I/W480D, N190M/W480D/E485Q, E192G, E192S/M498P/T499S/Q503G, E192T, E192V, E192Y, E192Y/M498P/T499S/Q503G, E202R, E202R/F203I/Q328A/S362T/P363G/E366S/R428V/W480D/E485Q/M498P/T499S/Q503G, E202R/F203I/E485Q, F203I/Q328A/P363G/R428V/L483R, F203I/Q328A/R428V, F203I/Q328A/W480D/E485Q, F203I/S362T/E366S, F203I/M498P/T499S/Q503G, V272P, R280G/M498P/T499S/Q503G, R280H, K296G, K296P, L297F, P299E, P299K/M498P/T499S/Q503G, P299S, P299T, Q301G, Q301S/Q503G, Y308H/Q503G, Y308K, Y308Q, Y308R, Y308T, L311R, Q328A/E485Q, Q328L/R428V, Q328L/W480D/L483R, V343C, V343L, F346Q, F346W, G349Q, L358G, L359I/M498P/T499S/Q503G, L392R/M498P/T499S/Q503G, S362T/P363G, S362T/P363R/E366S/R428V/W480D/L483R/M498P/T499S/Q503G, K406S, A407S, G410A, G410Q, D411E, L418G/M498P/T499S/Q503G, L418V/Q503G, I419V/Q503G, M465E, S471N, R472G/M498P/T499S/Q503G, R472S/M498P/T499S/Q503G, M473Q, W480D/L483R, D491G, D491R, and M498P/T499S/Q503G.

[0195] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,

99% or more sequence identity to reference sequence SEQ ID NO: **4558** and one or more residue differences as compared to SEQ ID NO: **4558** at a position or set of positions selected from 94/365/367, 172/174/178/401/403, 172/174/178/401/403/507, 172/174/178/402/508, 172/174/401/403/507, 172/178, 172/178/401, 174/178, 178/401/403, 178/402/403, 318/375/380, 324/379/405/483, 340, 340/394, 365, 365/367/428, 365/389/394, 367, 375/376, 375/379/483, 375/380, 375/380/400/483, 375/380/483, 376/483, 379/483, 389/394, 394, 401, 401/402/403/507, 402/403, 405/483, and 483. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **4558** and one or more residue differences as compared to SEQ ID NO: **4558** selected from V94I/A365F/K367R, T172S/N174M/Q178K/S402L/S508R, T172S/N174M/Q178R/G401K/P403G, T172S/N174M/Q178R/G401K/P403G/K507R, T172S/N174M/G401K/P403G/K507R, T172S/Q178K, T172S/Q178K/G401K, N174M/Q178R, Q178K/G401K/P403G, Q178K/S402L/P403G, A318E/Q375G/L380V, W324L/N379T/G405L/L483R, V340C, V340C/W394T, A365F, A365F/K367R/R428C, A365H/C389A/W394E, K367R, Q375G/K376H, Q375G/N379T/L483R, Q375G/L380V, Q375G/L380V/G400P/L483R, Q375G/L380V/L483R, K376H/L483R, N379T/L483R, C389A/W394E, W394T, G401K, G401K/S402L/P403G/K507N, S402L/P403G, G405L/L483R, and L483R.

[0196] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **4442** and one or more residue differences as compared to SEQ ID NO: **4442** at a position or set of positions selected from 174/296/299, 182, 185/190, 189/190, 190, 190/193, 192/280, 192/402/507, 257, 259, 260, 281, 289, 296/299, 305, 306, 307, 308, 312, 313, 316, 318, 327, 374, 381, 394, 395, 402, 402/507, 404, 405, 414, 432, 451, 455, 460, 461, 476/480, 480, 480/481, 493, 494, and 522. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **4442** and one or more residue differences as compared to SEQ ID NO: **4442** selected from N174M/K296P/P299E, D182A, D182C, D182N, D182R, E185G/N190M, E189A/N190M, E189H/N190M, E189R/N190M, E189V/N190M, N190M, N190M/L193F, E192T/R280H, E192T/S402L/K507R, W257K, W257R, Q259C, Q259K, Q259R, S260A, M281C, M281L, V289I, K296P/P299T, L306A, L306C, L305F, L306M, L306R, L306T, Y307N, Y308Q, A312H, A312K, A312Q, A312R, A312V, R313A, R313I, R313L, T316F, A318L, A318M, A318N, A318P, A318R, A318T, A318V, A318Y, V327A, L374M, W381F, W381M, W394L, W394M, W394Y, S395G, S402L, S402L/K507R, S404A, G405L, A414C, A414E, A414P, R432H, V451L, R455A, S460A, F461M, R476L/W480D, R476W/W480D, W480D, W480E, W480L, W480M, W480D/Y481W, H493K, S494Q, S494R, S494T, and E522T.

[0197] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **4654** and one or more residue differences as compared to SEQ ID NO: **4654** at a position or set of positions selected from 190, 190/197/308, 190/308/380/405, 190/375, 190/375/380, 190/380/405, 190/405/406, 272/301/393/394/480, 272/318/480/483, 301/394/480, 318, 375, 375/380, 375/405, 375/405/406, 380, 394, 394/480, and 480/483. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **4654** and one or more residue differences as compared to SEQ ID NO: **4654** selected from N190M, N190M/D197N/Y308Q, N190M/Y308Q/L380V/G405L, N190M/Q375G, N190M/Q375G/L380V, N190M/L380V/G405L, N190M/G405L/K406S, V272A/Q301S/E393K/W394T/W480D, V272A/A318E/W480D/L483R, Q301S/W394T/W480D, A318E, Q375G, Q375G/L380V, Q375G/G405L, Q375G/G405L/K406S, L380V, W394T, W394T/W480D, and W480D/L483R.

[0198] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **4850** and one or more residue differences as compared to SEQ ID NO: **4850** at a position or set of positions selected from 189, 189/193/207/307/353, 190/322, 193, 193/307, 261/322/421, 297/298/300/392, 297/300, 297/300/328, 298/300/328, 298/300/328/395, 298/300/360, 298/300/392/395, 298/300/392/395/492, 298/300/395, 298/300/481, 300, 300/392/395, 319, 322, 392, 421, and 492. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **4850** and one or more residue differences as compared to SEQ ID NO: **4850** selected from R189K, R189K/E193V/A207V/L307T/Q353R, E190R/D322N, E193V, E193V/L307T, S261A/D322N/L421V, K297P/L298F/P300T/R392S, K297P/P300E/V328A, K297P/P300S, L298F/P300E/V328A/W395L, L298F/P300E/R392S/W395L, L298F/P300E/W395L, L298F/P300E/D481W, L298F/P300S/V328A, L298F/P300S/R392S/W395L, L298F/P300S/R392S/W395L/D492E, L298F/P300T/L360V, P300E, P300E/R392S/W395L, P300T, A319P, D322N, R392S, L421V, and D492E.

[0199] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **4856** and one or more residue differences as compared to SEQ ID NO: **4856** at a position or set of positions selected from 10/413, 260, 268, 302/307, 317, 353, 354, 362, 364, 392, 393, 394, 395, 397, 402, 404, 412, 413, 419, 436/512, 460, 477, 486, 490, 495, and 518. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%,

95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **4856** and one or more residue differences as compared to SEQ ID NO: **4856** selected from S10N/A413S, Q260R, V268T, Q302A/T307L, Q302S/T307L, T317C, Q353R, Q353S, G354S, S362V, P364Q, R392G, R392H, R392K, L393R, L393V, E394A, E394V, W395Y, Q397G, Q397S, G402K, G402L, G402P, G402T, G402V, P404L, P404Q, D412G, D412M, A413G, L419G, L419V, P436S/E512D, G460A, G460P, G460S, R477Q, R477S, E486V, M490E, M490N, M490Q, M490S, M490T, M490V, S495Q, S495R, and G518S.

[0200] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **4904** and one or more residue differences as compared to SEQ ID NO: **4904** at a position or set of positions selected from 190, 190/287/300/302, 190/300/477/490, 194/300/302/413, 194/300/302/481, 297/298/308/392/395, 298/392/525, 300, 300/317, 300/490, and 395. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **4904** and one or more residue differences as compared to SEQ ID NO: **4904** selected from E190R, E190R/L287V/P300T/Q302A, E190R/P300T/R477Q/M490E, L194F/P300E/Q302S/D481M, L194F/P300T/Q302A/A413G, K297P/L298F/Y308N/R392K/W395Y, L298F/R392K/F525A, P300E, P300E/T317C, P300T/M490E, and W395Y.

[0201] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **5002** and one or more residue differences as compared to SEQ ID NO: **5002** at a position or set of positions selected from 11/523, 190/194, 194, 194/198, 202, 208, 264, 273, 273/347/354, 274, 281, 290, 298/300/302, 298/302/392/393/394/433, 298/302/392/394, 298/392/393/394, 298/392/393/394/477, 298/392/394/490, 298/393/394/395/433/477, 298/393/394/477/495, 298/394/433, 300/302/303, 308/402/460, 309, 313, 314, 324, 352, 359, 360, 361, 392/393/394/433, 392/393/394/477, 392/393/394/477/495, 392/393/394/490, 392/394, 392/394/395, 392/394/433/477, 392/394/433/495, 392/394/477/495, 392/394/495, 393/394, 393/394/433/477/490, 394/477, 394/490, 405, 408/413, 411/413, 413, 460/525, 463, 466, 467, 472, 473, 477, 492, 523, and 526. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **5002** and one or more residue differences as compared to SEQ ID NO: **5002** selected from G11D/E523R, E190L/F194L, E190R/F194L, E190T/F194L, F194L, F194L/D198G, F194L/D198P, F194L/D198Q, F194L/D198V, F194W, F194Y, L202T, I208G, I208S, I208V, A264S, V273M/Q347F/S354G, V273Q, V273S, K274G, K274V, K274W, R281G, R281Q, V290A, L298F/A302S/R392K/L393V/E394A/R433H, L298F/A302S/R392K/E394A, L298F/R392K/L393V/E394A/R477L, L298F/R392K/L393V/E394V,

L298F/R392K/E394V/M490E, L298F/L393V/E394V/W395Y/R433H/R477V,
 L298F/L393V/E394V/R477L/S495Q, L298F/E394V/R433H, L298I/T300P/A302Q,
 T300P/A302Q/K303V, Y308N/G402V/G460A, Y309R, A313S, R314L, I324M, I324T, P352T, L359C,
 L359V, L360V, I361L, R392H/L393V/E394A/R477L, R392H/L393V/E394V/M490E,
 R392H/E394A/R433H/R477L, R392H/E394V/R433H/S495Q, R392K/L393V/E394V/R433H,
 R392K/L393V/E394V/R477V/S495Q, R392K/E394A/W395Y, R392K/E394V,
 R392K/E394V/R477V/S495Q, R392K/E394V/S495Q, L393V/E394A,
 L393V/E394V/R433H/R477V/M490E, E394V/R477V, E394V/M490E, S405T, A408G/G413A,
 A408L/G413A, G411L/G413A, G413A, G460S/F525A, A463P, A463V, M466V, L467A, S472T,
 R473S, R477L, D492R, E523T, E526L, and E526Y.

[0202] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **5028** and one or more residue differences as compared to SEQ ID NO: **5028** at a position or set of positions selected from 82/194/198/313, 194, 194/198, 194/198/208/313, 194/198/309, 194/198/313, 194/198/411, 194/208/411, 194/309, 194/313, 194/411, 198, 198/208, 198/208/309/411, 198/208/313/411, 273/274, 274, 274/281/526, 274/359/526, 274/523, 309, 309/313/411, 324/526, 411, 466, 466/526, 523, and 526. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **5028** and one or more residue differences as compared to SEQ ID NO: **5028** selected from V82I/F194L/D198G/A313S, F194L, F194L/D198Q, F194L/D198Q/I208V/A313S, F194L/D198V, F194L/D198V/Y309R, F194L/D198V/G411L, F194L/I208V/G411L, F194L/Y309R, F194W, F194W/D198Q, F194W/D198V, F194W/D198V/A313S, F194W/A313S, F194W/G411L, D198G, D198Q, D198Q/I208S/A313S/G411L, D198Q/I208V/Y309R/G411L, D198V/I208S, V273Q/K274G, V273S/K274W, K274G/R281Q/E526Y, K274M/E523T, K274V, K274V/L359V/E526Y, K274W, Y309R, Y309R/A313S/G411L, I324M/E526Y, G411L, M466V, M466V/E526Y, E523T, and E526Y.

[0203] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **5192** and one or more residue differences as compared to SEQ ID NO: **5192** at a position or set of positions selected from 96/295, 169, 176, 177, 179, 184, 187, 193, 195, 197, 197/307, 198, 199, 200, 203, 292, 295, 300/394, 304, 325, 326, 326/380, 329, 373, 376, 377, 383, 394, 403, 409, 430, 485, 508, and 520/526. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **5192** and one or more residue differences as compared to SEQ ID NO: **5192** selected from L96S/T295N, Q169E, N176H, R177L, R177T, R177W, Q179G, Q179L,

S184G, S184H, S184P, I187V, V193C, V193F, V193R, V193S, K195L, N197F, N197M/T307A, D198L, D198S, D199G, D199R, D199S, T200A, T200E, T200K, T200Q, T200R, T200S, T200V, T200Y, E203A, E203C, E203G, E203L, S292L, S292M, S292Q, S292R, T295G, T295H, T295K, T295L, T300A/V394F, V304I, W325M, L326Q, L326R, L326T, L326M/N380R, Q329K, Q329L, Q373G, Q376R, K377N, K377R, K383H, K383R, K383S, V394F, V394H, V394I, V394L, V394Q, V394S, V394W, V394Y, L403R, L403V, D409S, K430R, E485L, K508A, K508S, and E520I/Y526E.

[0204] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **5246** and one or more residue differences as compared to SEQ ID NO: **5246** at a position or set of positions selected from 177/198/200, 177/200/203, 177/200/203/295, 177/200/295/326, 180, 184/198/200/203/295, 184/200/295/326, 190/198/200/203, 190/200/203/295/380, 190/200/295, 197, 198, 198/200, 198/200/203, 198/200/203/295, 200/326, 200/380, 203, 203/380, 233, 252, 295, 336, 364, 365, 367, 381, 384, 441, 459, and 485. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: **5246** and one or more residue differences as compared to SEQ ID NO: **5246** selected from R177W/D198L/T200K, R177W/T200K/E203G, R177W/T200K/E203G/T295N, R177W/T200K/T295N/L326M, R180K, S184H/D198L/T200K/E203G/T295N, S184H/T200K/T295N/L326M, E190M/D198L/T200K/E203G, E190M/T200K/E203G/T295N/N380R, E190M/T200K/T295N, N197Q, D198L, D198L/T200K, D198L/T200K/E203G, D198L/T200K/E203G/T295N, T200K/L326M, T200K/N380R, E203G, E203G/N380R, E233L, E233R, A252R, T295N, D336C, P364A, G365H, G365R, E367S, L381H, L381Y, K384A, M441E, R459M, R459W, and E485L.

[0205] In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2 or 660 and one or more residue differences as compared to SEQ ID NO: 2 or 660, selected from 26/30/38/79/81/90/92/94/101/108/137/140/141/142/153/155/160/163/165/177/184/189/194/196/201/203/205/213/219/231/248/258/263/264/266/267/301/304/307/314/318/325/333/340/344/353/362/379/390/392/394/395/397/398/402/403/406/408/410/411/413/414/416/425/427/429/433/434/441/442/444/446/451/455/460/461/466/468/476/481/484/485/488/495/499/501/502/506/515/525, 26/30/38/79/81/90/92/94/101/108/137/140/141/142/153/155/160/163/165/177/184/189/201/203/205/213/219/231/248/258/263/264/266/304/307/314/318/325/333/340/344/353/362/379/392/394/395/397/398/402/403/406/408/410/411/413/414/416/425/427/429/433/434/441/442/444/446/455/460/461/466/468/476/481/484/485/488/495/499/501/502/506/515/525, 26/38/79/81/90/92/94/101/108/137/141/155/160/163/165/189/201/203/205/213/219/246/248/258/263/264/304/307/314/318/333/340/344/353/362/392/394/395/396/397/398/402/403/406/408/410/411/413/414/425

/441/442/446/455/460/461/466/468/476/481/485/488/506/525,
92/94/101/108/137/141/155/160/163/165/201/203/205/213/219/258/263/264/314/333/344/353/392/394/39
5/397/406/408/411/413/414/425/441/442/446/460/461/468/476/481/485/488/525, and
92/94/101/108/137/141/155/201/213/264/314/333/344/392/394/395/397/406/408/425/442/446/461/476/48
1/485/525. In some embodiments, the engineered TdT polypeptide comprises an amino acid sequence
having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 2 or 660 and one or more
residue differences as compared to SEQ ID NO: 2 or 660, selected from
26Q/30G/38T/79T/81E/90L/92R/94E/101E/108K/137A/140V/141E/142V/153V/155E/160M/163V/165P/
177R/184S/189R/194L/196G/201A/203E/205R/213S/219R/231S/248R/258W/263H/264A/266T/267R/30
1W/304V/307L/314R/318R/325W/333A/340I/344V/353Q/362S/379M/390C/392R/394E/395W/397Q/39
8W/402G/403S/406G/408A/410E/411G/413A/414E/416S/425D/427L/429R/433R/434N/441M/442G/444
S/446P/451K/455L/460G/461S/466M/468Q/476R/481W/484L/485E/488N/495S/499M/501R/502G/506P
/515E/525F,
26Q/30G/38T/79T/81E/90L/92R/94E/101E/108K/137A/140V/141E/142V/153V/155E/160M/163V/165P/
177R/184S/189R/201A/203E/205R/213S/219R/231S/248R/258W/263H/264A/266T/304V/307L/314R/31
8R/325W/333A/340I/344V/353Q/362S/379M/392R/394E/395W/397Q/398W/402G/403S/406G/408A/41
0E/411G/413A/414E/416S/425D/427L/429R/433R/434N/441M/442G/444S/446P/455L/460G/461S/466
M/468Q/476R/481W/484L/485E/488N/495S/499M/501R/502G/506P/515E/525F,
26Q/38T/79T/81E/90L/92R/94E/101E/108K/137A/141E/155E/160M/163V/165P/189R/201A/203E/205R
/213S/219R/246N/248R/258W/263H/264R/304V/307L/314R/318R/333A/340I/344V/353Q/362S/392R/3
94E/395W/396T/397Q/398W/402G/403S/406G/408A/410E/411G/413A/414G/425D/441M/442G/446P/4
55L/460G/461G/466M/468Q/476R/481W/485D/488N/506P/525F,
92R/94E/101E/108K/137A/141E/155E/160M/163V/165P/201A/203E/205R/213S/219R/258W/263H/264
R/314R/333A/344V/353Q/392R/394E/395W/397Q/406G/408A/411G/413A/414G/425D/441M/442G/446
P/460G/461G/468Q/476R/481W/485D/488N/525F, and
92R/94E/101E/108K/137A/141E/155E/201A/213S/264R/314R/333A/344V/392R/394E/395W/397Q/406
G/408A/425D/442G/446P/461G/476R/481W/485D/525F. In some embodiments, the engineered TdT
polypeptide comprises an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%,
89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference
sequence SEQ ID NO: 2 or 660 and one or more residue differences as compared to SEQ ID NO: 2 or
660, selected from
R26Q/M30G/I38T/S79T/S81E/N90L/G92R/D94E/T101E/T108K/M137A/G140V/V141E/M142V/E153V
/I155E/T160M/I163V/Q165P/H177R/A184S/A189R/F194L/R196G/C201A/T203E/M205R/C213S/V219
R/G231S/L248R/R258W/K263H/L264A/K266T/S267R/M301W/A304V/C307L/D314R/K318R/S325W/
W333A/L340I/T344V/F353Q/T362S/T379M/Y390C/D392R/R394E/E395W/R397Q/F398W/K402G/L40
3S/R406G/I408A/A410E/L411G/H413A/F414E/K416S/H425D/K427L/D429R/W433R/E434N/E441M/S

442G/A444S/S446P/R451K/V455L/D460G/R461S/L466M/G468Q/E476R/R481W/T484L/H485E/K488N/A495S/L499M/K501R/K502G/K506P/A515E/S525F,
 R26Q/M30G/I38T/S79T/S81E/N90L/G92R/D94E/T101E/T108K/M137A/G140V/V141E/M142V/E153V/I155E/T160M/I163V/Q165P/H177R/A184S/A189R/C201A/T203E/M205R/C213S/V219R/G231S/L248R/R258W/K263H/L264A/K266T/A304V/C307L/D314R/K318R/S325W/W333A/L340I/T344V/F353Q/T362S/T379M/D392R/R394E/E395W/R397Q/F398W/K402G/L403S/R406G/I408A/A410E/L411G/H413A/F414E/K416S/H425D/K427L/D429R/W433R/E434N/E441M/S442G/A444S/S446P/V455L/D460G/R461S/L466M/G468Q/E476R/R481W/T484L/H485E/K488N/A495S/L499M/K501R/K502G/K506P/A515E/S525F,
 R26Q/I38T/S79T/S81E/N90L/G92R/D94E/T101E/T108K/M137A/V141E/I155E/T160M/I163V/Q165P/A189R/C201A/T203E/M205R/C213S/V219R/E246N/L248R/R258W/K263H/L264R/A304V/C307L/D314R/K318R/W333A/L340I/T344V/F353Q/T362S/D392R/R394E/E395W/S396T/R397Q/F398W/K402G/L403S/R406G/I408A/A410E/L411G/H413A/F414G/H425D/E441M/S442G/S446P/V455L/D460G/R461G/L466M/G468Q/E476R/R481W/H485D/K488N/K506P/S525F,
 G92R/D94E/T101E/T108K/M137A/V141E/I155E/T160M/I163V/Q165P/C201A/T203E/M205R/C213S/V219R/R258W/K263H/L264R/D314R/W333A/T344V/F353Q/D392R/R394E/E395W/R397Q/R406G/I408A/L411G/H413A/F414G/H425D/E441M/S442G/S446P/D460G/R461G/G468Q/E476R/R481W/H485D/K488N/S525F, and
 G92R/D94E/T101E/T108K/M137A/V141E/I155E/C201A/C213S/L264R/D314R/W333A/T344V/D392R/R394E/E395W/R397Q/R406G/I408A/H425D/S442G/S446P/R461G/E476R/R481W/H485D/S525F.

[0206] As will be appreciated by the skilled artisan, in some embodiments, one or a combination of residue differences above that is selected can be kept constant (i.e., maintained) in the engineered TdT as a core feature, and additional residue differences at other residue positions incorporated into the sequence to generate additional engineered TdT polypeptides with improved properties. Accordingly, it is to be understood for any engineered TdT containing one or a subset of the residue differences above, the present invention contemplates other engineered TdTs that comprise the one or subset of the residue differences, and additionally one or more residue differences at the other residue positions disclosed herein.

[0207] As noted above, the engineered TdT polypeptides are also capable of converting substrates (e.g., NTP-3'-O-RBG or a natural or modified NTP and an oligo acceptor substrate) to products (e.g., an oligo acceptor substrate with an added nucleotide-3'-O-RBG). In some embodiments, the engineered TdT polypeptide is capable of converting the substrate compounds to the product compound with at least 1.2 fold, 1.5 fold, 2 fold, 3 fold, 4 fold, 5 fold, 10 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, 100 fold, or more activity relative to the activity of the reference polypeptide of SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192, and/or 5246.

[0208] In some embodiments, the engineered TdT capable of converting the substrate compounds to the product compounds with at least 2 fold the activity relative to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192, and/or 5246, comprises an amino acid sequence selected from the even-numbered sequences in SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476.

[0209] In some embodiments, the engineered TdT has an amino acid sequence comprising one or more residue differences as compared to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192, and/or 5246, that increases soluble expression or isolated protein yield of the engineered TdT in a bacterial host cell, particularly in *E. coli*, as compared to a wild-type or engineered reference TdT, comprises an amino acid sequence selected from the even-numbered sequences in SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476.

[0210] In some embodiments, the engineered TdT has an amino acid sequence comprising one or more residue differences as compared to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192, and/or 5246, that increases thermostability of the engineered TdT, as compared to a wild-type or engineered reference TdT, comprises an amino acid sequence selected from the even-numbered sequences of SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476.

[0211] In some embodiments, the engineered TdT has an amino acid sequence comprising one or more residue differences as compared to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, that increases the activity of the engineered TdT at high temperatures (by way of example and not limitation, 40 °C, 45 °C, 50 °C, 55 °C, 60 °C, or 65 °C), as compared to a wild-type or engineered reference TdT, comprises an amino acid sequence selected from the even-numbered sequences of SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476.

[0212] In some embodiments, the engineered TdT has an amino acid sequence comprising one or more residue differences as compared to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, that reduces the by-product formation of the engineered TdT, as compared to a wild-type or engineered reference TdT, comprises an amino acid sequence selected from the even-numbered sequences of SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476.

[0213] In some embodiments, the engineered TdT has an amino acid sequence comprising one or more residue differences as compared to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348,

1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, that increases specific activity of the engineered TdT on one or more NTP-3'-O-RBG or a natural or modified NTP substrates, as compared to a wild-type or engineered reference TdT, comprises an amino acid sequence selected from the even-numbered sequences of SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476.

[0214] In some embodiments, the engineered TdT has an amino acid sequence comprising one or more residue differences as compared to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, that increases specific activity of the engineered TdT on one or more oligo acceptor substrates, as compared to a wild-type or engineered reference TdT, comprises an amino acid sequence selected from the even-numbered sequences of SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476.

[0215] In some embodiments, the engineered TdT has an amino acid sequence comprising one or more residue differences as compared to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, that increases incorporation efficiency in extension of an oligo acceptor substrate by addition of an NTP or NQP of greater than 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. when compared to the incorporation efficiency of a wild-type or engineered reference TdT, and comprises an amino acid sequence selected from the even-numbered sequences of SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476

[0216] In some embodiments, the engineered TdT with improved properties has an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to a reference sequence of SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246 and at least one substitution or substitution set at amino acid positions selected from 26, 30, 38, 79, 81, 90, 92, 94, 101, 108, 137, 140 141, 142, 153, 155, 160, 163, 165, 177, 184, 189, 194, 196, 201, 203, 205, 213, 219, 231, 246, 248, 258, 263, 264, 266, 267, 301, 304, 307, 314, 318, 325, 333, 340, 344, 353, 362, 379, 390, 392, 394, 395, 396, 397, 398, 402, 403, 406, 408, 410, 411, 413, 414, 416, 425, 427, 429, 433, 434, 441, 442, 444, 446, 451, 455, 460, 461, 466, 468, 476, 481, 484, 485, 488, 495, 499, 501, 502, 506, 515, and 525, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246.

[0217] In some embodiments, the engineered TdT with improved properties has an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97%, 98%, 99% or more sequence identity to a reference sequence of SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246 and at least one substitution at amino acid position 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 41, 44, 45, 46, 47, 50, 53, 57, 58, 61, 62, 65, 70, 76, 77, 79, 80, 81, 85, 89, 90, 92, 93, 94, 97, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 116, 119, 121, 122, 123, 124, 126, 129, 130, 131, 132, 133, 134, 135, 137, 138, 139, 140, 141, 142, 144, 145, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 165, 168, 169, 171, 173, 174, 175, 177, 179, 183, 184, 185, 186, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 203, 204, 205, 206, 208, 213, 217, 219, 220, 223, 227, 231, 233, 235, 236, 243, 244, 245, 246, 248, 249, 251, 252, 253, 255, 258, 259, 260, 261, 262, 263, 264, 266, 267, 268, 269, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 284, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 297, 298, 299, 300, 301, 302, 303, 304, 306, 307, 308, 309, 310, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 324, 325, 326, 327, 328, 329, 330, 333, 334, 336, 338, 340, 342, 343, 344, 347, 350, 352, 353, 359, 360, 361, 362, 363, 365, 366, 367, 368, 369, 370, 371, 372, 373, 376, 378, 379, 380, 382, 383, 385, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 421, 425, 426, 427, 428, 429, 431, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 448, 451, 452, 454, 455, 456, 457, 459, 460, 461, 462, 464, 465, 466, 468, 469, 470, 472, 473, 474, 475, 476, 477, 479, 480, 481, 484, 485, 488, 490, 491, 492, 493, 494, 495, 499, 500, 501, 502, 503, 504, 506, 508, 509, 515, 518, 520, 522, 523, 525, 526, or 528, or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246.

[0218] In some embodiments, the engineered TdT with improved properties has an amino acid sequence having at least 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to a reference sequence of SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246 and at least a substitution or amino acid residue 12A/F/N/Q/S/V, 13E/G/K/R/S, 14G/N/Q/Y, 15A/F/L/Q, 16S/V, 17A/G, 18D/R/Y, 20H/P/T, 21E, 22G/L, 23C/E/P/T, 24A/G/M/P, 25N, 26A/G/I/Q/S/T/W, 27D/F/R, 28N/R, 29G/I/P/R, 30E/G/L/T/V, 31G/S/V, 32E, 33A/C/G/K/P, 34D/K/R/S, 35E/G/H/W, 36G/K, 37A/F/G/S/T/V, 38L/T, 39A/G, 41V, 44S, 45R, 46M, 47A, 50L, 53K, 53E, 57H/T, 58A/M/N/S, 61A/H/L, 62W, 65S, 70A, 76P, 77C/S, 79T, 80D, 81E, 85V, 89A, 90L/M/S, 92M/R/S/V/Y, 93V, 94E/K/N/R, 97D/T, 101E/G/V, 102L, 103M, 104G/I/P/V, 105N/W, 106D/G/H/K/S/V, 107R/W, 108D/G/K/M, 109M/N/T, 110M/V, 116A, 119F/Q, 121S, 122I, 123M/Q, 124E/G/I/M/S, 126C/V, 129G, 130A/M/Q/S, 131E/G/R/W, 132S, 133G/M/Q, 134M/W, 135C/E/H/K/R, 137A/E, 138A/Q, 139A/G,

140E/G/V, 141E/M/R, 142M/S/V, 144C, 145E, 147G/L, 148T, 149R, 150E/G/P, 151I/S, 152G/H/R, 153E/G/K/M/P/Q/V, 154G, 155E/T, 156D/E/M/Q, 157L/V, 158A/H/S, 159D, 160G/M/N/S/V, 161D/E/G/H/R/S, 162A/E/H, 163I/L/V, 165K/P, 168S, 169E/M/R, 171K, 173I/L, 174L, 175D/I/L/V, 177L/R/Y, 179A/K/L/R, 183R, 184S, 185C/F/L, 186A/C/D/G/L/T, 188I/M, 189E/L/Q/R/V, 190L/M/R, 190E, 191V, 192C/G/I/L/R, 193C/G/N/R/S/T/V, 194A/C/D/E/F/G/L/M/R/S/W, 196A/G/L/M/N/R/S/T/W/Y, 197G/L/M/R, 198A/E/L/P/S, 199A/E/G/H/I/M/Q/R/S/V, 200A/C/K/N/V, 201A/R/W, 203A/D/E/G/L/M/R/S, 204L, 205E/L/R, 206N, 208A/V, 213S, 217Q, 219L/R, 220R, 223Q, 227M, 231S/T, 233A/D/R, 235R, 236E/I/P/V, 243L/R/S/T, 244V, 245A, 246E/K/N, 248C/K/L/R/S/T/V, 249A/G/R/T, 251K/R, 252E/K, 253I/L, 255G, 258A/C/E/G/K/L/M/Q/S/W, 259N/V, 260K, 261A/G/R/V, 262I/V, 263H/I/M/R/S, 264A/C/E/L/R/S/T/V, 266F/I/K/R/T/V/Y, 267A/C/H/M/Q/R/V/Y, 268C/I/L/T, 269W, 271C, 272T, 273C/D/E/F/G/I/L/M/S/V/W/Y, 274A/G/I/M/N/P/Q/T/V, 275D/V, 276S, 277E, 278C/E/F/H/I/L/M/N/R/T/V/Y, 279Y, 280F/L/S, 281A/C/G/K/L/Q/S/T/V, 282C/G/H/Q/R/W, 284I/L/M, 286N/S, 287I, 288E/V, 289D, 290A/L/V, 291M/S/W, 292L/N/T, 293S/Q, 294T, 295S, 297C/D/F/L/M/P/Q/R/S/T/V, 298I/M, 299A/L/M/N/S/Y, 300H/P/R/T, 301G/Q/S/T/V/W, 302A/N/S, 303A/E/H/M/N/Q/S/V, 304D/L/M/T/V, 306F/I/M, 307A/E/G/H/K/L/M/S/V, 308A/F/G/H/K/L/N/S/V/W, 309F/G/I/L/M/V/W, 310A/G/H/R/S, 312M/T/V, 313A/I/L/M/Q/R/S, 314A/G/I/K/L/M/Q/R/V/Y, 315A/G/Q/S, 316A/C/I/L/T, 317G/T, 318E/R/S/T/V, 319G/R, 320N, 321C/K/S, 322A/K/Q, 324I/V, 325A/F/L/T/V/W, 326C/M/N/R/S/T, 327I/M, 328T, 329K/R, 330E/K/N, 333A/D/G/H/R, 334E/R/S, 336D, 338T, 340A/G/I/M/R/S/V, 342E/R/V/W, 343V, 344V, 347I/Q, 350L/R/S/W, 352G/P/R/V, 353A/H/K/M/Q/R/S, 354S, 359G/L/V, 360C/I/V, 361T, 362S/T/Y, 363C/I, 365A, 366V, 367A/D, 368R, 369G/M/N, 370D/G/M/N/S/T, 371D, 372E/G, 373C/G/H/N/R, 376H/V, 378L, 379C/I/L/M/V, 380D, 382F/L, 383R, 385R, 390C/I/L/V, 391G/L/R, 392A/C/K/R/V, 393I/R/V, 394A/E/F/G/L/M/R/S/T/V/Y, 395A/L/R/S/T/W/Y, 396A/R/S/T, 397A/D/F/Q/R/T/W, 398W, 399C/D/F/G/T, 400A/E/W, 401E/G, 402E/F/G/Q/S/V, 403A/E/F/G/L/P/R/S, 404D/E/F/S/W, 405G/L/N/Y, 406G/N/P/T/V, 407A/D/F/L/M/N/R/S/W, 408A/E/G/I/L/M/P/R/T/V/W, 409K/L/Q, 410E/F/G/I/Q/S/V/Y, 411A/E/F/G/I/N/P/Q/R/T, 412N, 413A/C/E/F/G/I/L/M/P/S/V, 414A/E/F/G/H/Y, 415A/F/L/S/W, 416G/N/Q/S, 417G/V/W, 418I, 419A/G/H/L/M, 421F/I/M, 425D/K/R/T, 426P, 427C/E/F/L/M/N/Q/R/W/Y, 428V, 429R, 431R/S, 433A/E/G/H/M/P/R/S/V, 434N, 435A/C/E/G/I/K/P/Q/S/T, 436S, 437A/G/K/P/Q/R/S, 438V, 439G/P, 440E/K/V, 441K/M/N/V, 442A/G/K, 443T, 444A/G/H/R/S, 445N, 446E/G/P, 448R, 451K, 452I/L, 454F/M/V, 455I/L, 456K/P/R/S/T, 457S/V, 459H/I/Q/R/V, 460E/G/P/V, 461A/G/N/Q/S/V, 462E/F/H/I/L/Q/R/W, 464Y, 465E, 466M, 468A/F/H/M/Q/S/T/W, 469F/Q/Y, 470S/T, 472A/G, 473A/D/G/K/M/P/Q/S/V, 474M, 475V, 476R/V, 477G/Q/S/T, 479F/V, 480A/E/G/H/K/L/S/W, 481A/D/E/L/M/S/T/V/W, 484A/E/H/L/M/R, 485D/E/S, 488N/S, 490E/H/L/R/V/W, 491I/M, 492S/T, 493E/Q/R/V/Y, 494A/C/G/L/R/V/W, 495C/G/S, 499L/M/R, 500N, 501A/N/R, 502G/R, 503A/E/M/Q/R/S/T/V, 504K/N/Q/R/S/W, 506E/P/S/T, 508D/S/T, 509G/K, 515E/V, 515A, 518D, 520D/P, 522L, 523E/H, 525F/H/Q/R/S, 526L/Y, or 528L, or any combinations thereof, wherein the amino acid positions are

numbered with reference to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246.

[0219] In some embodiments, the engineered TdT with improved properties has an amino acid sequence comprising a sequence selected from the even-numbered sequences of SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476. In some embodiments, the engineered TdT with improved properties has an amino acid sequence comprising a sequence selected from SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246.

[0220] In some embodiments, the engineered TdT, comprises an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to one of the sequences of SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, as provided in the Examples.

[0221] In addition to the residue positions specified above, any of the engineered TdT polypeptides disclosed herein can further comprise other residue differences relative to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, at other residue positions (i.e., residue positions other than those included herein).

Residue differences at these other residue positions can provide for additional variations in the amino acid sequence without adversely affecting the ability of the polypeptide to carry out the conversion of substrate to product. Accordingly, in some embodiments, in addition to the amino acid residue differences present in any one of the engineered TdTs polypeptides selected from the even-numbered sequences in the range of SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476, the sequence can further comprise 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-26, 1-30, 1-35, 1-40, 1-45, 1-50, 1-100, or 1-150 residue differences at other amino acid residue positions as compared to the SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246. In some embodiments, the number of amino acid residue differences as compared to the reference sequence can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 100, or 150 residue positions. In some embodiments, the number of amino acid residue differences as compared to the reference sequence can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, 21, 22, 23, 24, or 25 residue positions. The residue differences at these other positions can be conservative changes or non-conservative changes. In some embodiments, the residue differences can comprise conservative substitutions and non-conservative substitutions as compared to the TdT polypeptide of SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882,

1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246.

[0222] In some embodiments, the present invention also provides engineered polypeptides that comprise a fragment of any of the engineered TdT polypeptides described herein that retains the functional activity and/or improved property of that engineered TdT. Accordingly, in some embodiments, the present invention provides a polypeptide fragment capable of converting substrate to product under suitable reaction conditions, wherein the fragment comprises at least about 90%, 95%, 96%, 97%, 98%, or 99% of a full-length or truncated amino acid sequence of an engineered TdT of the present invention, such as an exemplary TdT polypeptide selected from the even-numbered sequences in the range of SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476. In some embodiments, the engineered TdT can have an amino acid sequence comprising a deletion in any one of the TdT polypeptide sequences described herein, such as the exemplary engineered polypeptides of the even-numbered sequences in the range of SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476.

[0223] Thus, for each and every embodiment of the engineered TdT polypeptides of the invention, the amino acid sequence can comprise deletions of one or more amino acids, 2 or more amino acids, 3 or more amino acids, 4 or more amino acids, 5 or more amino acids, 6 or more amino acids, 8 or more amino acids, 10 or more amino acids, 15 or more amino acids, or 20 or more amino acids, up to 10% of the total number of amino acids, up to 20% of the total number of amino acids, or up to 30% of the total number of amino acids of the TdT polypeptides, where the associated functional activity and/or improved properties of the engineered TdT described herein are maintained. In some embodiments, the deletions can comprise 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-15, 1-20, 1-21, 1-22, 1-23, 1-24, 1-25, 1-30, 1-35, 1-40, 1-45, or 1-50 amino acid residues. In some embodiments, the number of deletions can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 30, 35, 40, 45, or 50 amino acid residues. In some embodiments, the deletions can comprise deletions of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, 21, 22, 23, 24, or 25 amino acid residues.

[0224] In some embodiments, the engineered TdT polypeptide herein can have an amino acid sequence comprising an insertion as compared to any one of the engineered TdT polypeptides described herein, such as the exemplary engineered polypeptides of the even-numbered sequences in the range of SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476. Thus, for each and every embodiment of the TdT polypeptides of the invention, the insertions can comprise one or more amino acids, 2 or more amino acids, 3 or more amino acids, 4 or more amino acids, 5 or more amino acids, 6 or more amino acids, 8 or more amino acids, 10 or more amino acids, 15 or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, or 50 or more amino acids, where the associated functional activity and/or improved properties of the engineered TdT described herein is maintained. The insertions can be to amino or carboxy terminus, or internal portions of the TdT polypeptide.

[0225] In some embodiments, the engineered TdT described herein can have an amino acid sequence comprising a sequence selected from the even-numbered sequences in the range of SEQ ID NO: 4-1960,

2004-3920, 4048-5466, and 5476, and optionally one or several (e.g., up to 3, 4, 5, or up to 10) amino acid residue deletions, insertions and/or substitutions. In some embodiments, the amino acid sequence has optionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-15, 1-20, 1-21, 1-22, 1-23, 1-24, 1-25, 1-30, 1-35, 1-40, 1-45, 1-50, 1-75, 1-100, or 1-150 amino acid residue deletions, insertions and/or substitutions. In some embodiments, the amino acid sequence has optionally around 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acid residue deletions, insertions and/or substitutions. In some embodiments, the substitutions can be conservative or non-conservative substitutions.

[0226] In the above embodiments, the suitable reaction conditions for the engineered polypeptides are provided in Tables 6.1, 7.1, 8.1, 9.1, 10.1, 11.1, 12.1, 13.1, 14.1, 15.1, 16.1, 17.1, 18.1, 19.1, 20.1, 21.1, 22.1, 23.1, 24.1, 25.1, 26.1, 27.1, 28.1, 29.1, 30.1, 31.1, 32.1, 33.1, 34.1, 35.1, 36.1, 37.1, 38.1, 39.1, 40.1, 41.1, 42.1, 43.1, 44.1, 45.1, 46.1, 47.1, 48.1, 49.1, 50.1, 51.1, 52.1, 53.1, 54.1, 55.1, 56.1, 59.1, 60.1, 61.1, 63.1, 64.1, 65.1, 66.1, 67.1, 68.1, 69.1, 70.1, 71.1, 72.1, 73.1, 74.1, 75.1, 76.1, 77.1, 78.1, 79.1, and 80.1, as described in the Examples herein.

[0227] In some embodiments, the polypeptides of the present invention are fusion polypeptides in which the engineered polypeptides are fused to other polypeptides, such as, by way of example and not limitation, antibody tags (e.g., myc epitope), purification sequences (e.g., His tags for binding to metals), cell localization signals (e.g., secretion signals), and polypeptides with enzymatic activity. Thus, the engineered polypeptides described herein can be used with or without fusions to other polypeptides.

[0228] In one embodiment of the engineered TdT polypeptides of the present invention, the polypeptide further comprises an N-terminal truncation of 1-156 amino acids of the polypeptide sequence relative to any even-numbered sequence set forth in SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476. For example, a 156 amino acid truncated version of a TdT variant polypeptide SEQ ID NO: 5028 is prepared and demonstrated to have TdT activity in Examples 81 and 82.

[0229] In some embodiments, the engineered TdT polypeptides of the invention can be fused to another second polypeptide, such as a polypeptide with a different enzymatic activity. In some embodiments, the present provides a fusion polypeptide comprising an engineered TdT polypeptide fused to a second polypeptide with inorganic pyrophosphatase (IPP) activity. For example, synthetic genes encoding an N-terminal and C-terminal hexahistidine tagged version of a wild-type (WT) inorganic pyrophosphatase (IPP) polypeptide (e.g., polypeptide of SEQ ID NO: 3942 or 3944) can be fused to gene encoding a TdT variant polypeptide. Typically, the polypeptides (e.g., IPP and TdT) are fused via a polypeptide linker (e.g., a GSGGTG linker) introduced in the construct between the genes encoding the polypeptides. Such fusion proteins can be constructed using well-established techniques (e.g., Gibson assembly cloning) and expressed in *E. coli* (e.g., a strain derived from W3110). Exemplary IPP-TdT polypeptide fusion constructs (e.g., the fusion constructs of SEQ ID NO: 5468, 5470, 5472, and 5474) are provided and demonstrated in Examples 81 and 82. Although the Examples demonstrate a fusion of a particular engineered TdT polypeptide of the present invention with a second polypeptide with IPP activity, it is

contemplated that any of the embodiments an engineered TdT polypeptides of even-numbered sequence set forth in SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476 could be used in such a fusion with a second polypeptide.

[0230] It is to be understood that the polypeptides described herein are not restricted to the genetically encoded amino acids. In addition to the genetically encoded amino acids, the polypeptides described herein may be comprised, either in whole or in part, of naturally occurring and/or synthetic non-encoded amino acids. Certain commonly encountered non-encoded amino acids of which the polypeptides described herein may be comprised include, but are not limited to: the D-stereoisomers of the genetically-encoded amino acids; 2,3-diaminopropionic acid (Dpr); α -aminoisobutyric acid (Aib); ϵ -aminohexanoic acid (Aha); δ -aminovaleric acid (Ava); N-methylglycine or sarcosine (MeGly or Sar); ornithine (Orn); citrulline (Cit); t-butylalanine (Bua); t-butylglycine (Bug); N-methylisoleucine (MeIle); phenylglycine (Phg); cyclohexylalanine (Cha); norleucine (Nle); naphthylalanine (Nal); 2-chlorophenylalanine (Ocf); 3-chlorophenylalanine (Mcf); 4-chlorophenylalanine (Pcf); 2-fluorophenylalanine (Off); 3-fluorophenylalanine (Mff); 4-fluorophenylalanine (Pff); 2-bromophenylalanine (Obf); 3-bromophenylalanine (Mbf); 4-bromophenylalanine (Pbf); 2-methylphenylalanine (Omf); 3-methylphenylalanine (Mmf); 4-methylphenylalanine (Pmf); 2-nitrophenylalanine (Onf); 3-nitrophenylalanine (Mnf); 4-nitrophenylalanine (Pnf); 2-cyanophenylalanine (Ocf); 3-cyanophenylalanine (Mcf); 4-cyanophenylalanine (Pcf); 2-trifluoromethylphenylalanine (Otf); 3-trifluoromethylphenylalanine (Mtf); 4-trifluoromethylphenylalanine (Ptf); 4-aminophenylalanine (Paf); 4-iodophenylalanine (Pif); 4-aminomethylphenylalanine (Pamf); 2,4-dichlorophenylalanine (Opf); 3,4-dichlorophenylalanine (Mpcf); 2,4-difluorophenylalanine (Opff); 3,4-difluorophenylalanine (Mpff); pyrid-2-ylalanine (2pAla); pyrid-3-ylalanine (3pAla); pyrid-4-ylalanine (4pAla); naphth-1-ylalanine (1nAla); naphth-2-ylalanine (2nAla); thiazolylalanine (taAla); benzothienylalanine (bAla); thienylalanine (tAla); furylalanine (fAla); homophenylalanine (hPhe); homotyrosine (hTyr); homotryptophan (hTrp); pentafluorophenylalanine (5ff); styrylalanine (sAla); authylalanine (aAla); 3,3-diphenylalanine (Dfa); 3-amino-5-phenylpentanoic acid (Afp); penicillamine (Pen); 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic); β -2-thienylalanine (Thi); methionine sulfoxide (Mso); N(w)-nitroarginine (nArg); homolysine (hLys); phosphonomethylphenylalanine (pmPhe); phosphoserine (pSer); phosphothreonine (pThr); homoaspartic acid (hAsp); homoglutamic acid (hGlu); 1-aminocyclopent-(2 or 3)-ene-4 carboxylic acid; pipercolic acid (PA), azetidine-3-carboxylic acid (ACA); 1-aminocyclopentane-3-carboxylic acid; allylglycine (aGly); propargylglycine (pgGly); homoalanine (hAla); norvaline (nVal); homoleucine (hLeu), homovaline (hVal); homoisoleucine (hIle); homoarginine (hArg); N-acetyl lysine (AcLys); 2,4-diaminobutyric acid (Dbu); 2,3-diaminobutyric acid (Dab); N-methylvaline (MeVal); homocysteine (hCys); homoserine (hSer); hydroxyproline (Hyp) and homoproline (hPro). Additional non-encoded amino acids of which the polypeptides described herein may be comprised will be apparent to those of skill in the art (See e.g., the various amino acids provided in Fasman, CRC Practical Handbook of Biochemistry and Molecular

Biology, CRC Press, Boca Raton, FL, pp. 3-70 [1989], and the references cited therein, all of which are incorporated by reference). These amino acids may be in either the L- or D-configuration.

[0231] Those of skill in the art will recognize that amino acids or residues bearing side chain protecting groups may also comprise the polypeptides described herein. Non-limiting examples of such protected amino acids, which in this case belong to the aromatic category, include (protecting groups listed in parentheses), but are not limited to: Arg(tos), Cys(methylbenzyl), Cys (nitropyridinesulfonyl), Glu(δ -benzylester), Gln(xanthyl), Asn(N- δ -xanthyl), His(bom), His(benzyl), His(tos), Lys(fmoc), Lys(tos), Ser(O-benzyl), Thr (O-benzyl) and Tyr(O-benzyl).

[0232] Non-encoding amino acids that are conformationally constrained of which the polypeptides described herein may be composed include, but are not limited to, N-methyl amino acids (L-configuration); 1-aminocyclopent-(2 or 3)-ene-4-carboxylic acid; pipecolic acid; azetidine-3-carboxylic acid; homoproline (hPro); and 1-aminocyclopentane-3-carboxylic acid.

[0233] In some embodiments, the engineered polypeptides can be in various forms, for example, such as an isolated preparation, as a substantially purified enzyme, whole cells transformed with gene(s) encoding the enzyme, and/or as cell extracts and/or lysates of such cells. The enzymes can be lyophilized, spray-dried, precipitated or be in the form of a crude paste, as further discussed below.

[0234] In some embodiments, the engineered polypeptides can be in the form of a biocatalytic composition. In some embodiments, the biocatalytic composition comprises (a) a means for conversion of an NTP-3-O-RBG or natural or modified NTP substrate and an oligo acceptor compound to an oligo acceptor product extended by one nucleotide by contact with a TdT and (b) a suitable cofactor. The suitable cofactor may be cobalt, manganese, or any other suitable cofactor.

[0235] In some embodiments, the polypeptides described herein are provided in the form of kits. The enzymes in the kits may be present individually or as a plurality of enzymes. The kits can further include reagents for carrying out the enzymatic reactions, substrates for assessing the activity of enzymes, as well as reagents for detecting the products. The kits can also include reagent dispensers and instructions for use of the kits.

[0236] In some embodiments, the kits of the present invention include arrays comprising a plurality of different TdT polypeptides at different addressable position, wherein the different polypeptides are different variants of a reference sequence each having at least one different improved enzyme property. In some embodiments, a plurality of polypeptides immobilized on solid supports are configured on an array at various locations, addressable for robotic delivery of reagents, or by detection methods and/or instruments. The array can be used to test a variety of substrate compounds for conversion by the polypeptides. Such arrays comprising a plurality of engineered polypeptides and methods of their use are known in the art (See e.g., WO2009/008908A2).

[0237] **Polynucleotides Encoding Engineered Terminal Deoxynucleotidyl Transferases, Expression Vectors and Host Cells**

[0238] In another aspect, the present invention provides polynucleotides encoding the engineered TdT polypeptides described herein. The polynucleotides may be operatively linked to one or more heterologous regulatory sequences that control gene expression to create a recombinant polynucleotide capable of expressing the polypeptide. Expression constructs containing a heterologous polynucleotide encoding the engineered TdT are introduced into appropriate host cells to express the corresponding TdT polypeptide.

[0239] As will be apparent to the skilled artisan, availability of a protein sequence and the knowledge of the codons corresponding to the various amino acids provide a description of all the polynucleotides capable of encoding the subject polypeptides. The degeneracy of the genetic code, where the same amino acids are encoded by alternative or synonymous codons, allows an extremely large number of nucleic acids to be made, all of which encode the improved TdT enzymes. Thus, having knowledge of a particular amino acid sequence, those skilled in the art could make any number of different nucleic acids by simply modifying the sequence of one or more codons in a way which does not change the amino acid sequence of the protein. In this regard, the present invention specifically contemplates each and every possible variation of polynucleotides that could be made encoding the polypeptides described herein by selecting combinations based on the possible codon choices, and all such variations are to be considered specifically disclosed for any polypeptide described herein, including the amino acid sequences presented in Tables 5.1, 6.2, 7.2, 8.2, 9.2, 10.2, 11.2, 12.2, 13.2, 14.2, 15.2, 16.2, 17.2, 18.2, 19.2, 20.2, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 26.3, 26.4, 27.2, 27.3, 27.4, 27.5, 28.1, 28.2, 28.3, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35.2, 36.2, 37.2, 38.2, 39.2, 40.2, 41.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 49.2, 50.2, 51.2, 52.2, 53.2, 54.2, 55.2, 56.2, 56.3, 56.4, 61.2, 63.2, 64.2, 65.2, 66.2, 67.2, 68.2, 69.2, 70.2, 71.2, 72.2, 73.2, 74.2, 75.2, 76.2, 77.2, 78.2, 79.2, and 80.1, and disclosed in the sequence listing incorporated by reference herein as the even-numbered sequences in the range of SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476.

[0240] In various embodiments, the codons are preferably selected to fit the host cell in which the protein is being produced. For example, preferred codons used in bacteria are used to express the gene in bacteria; preferred codons used in yeast are used for expression in yeast; and preferred codons used in mammals are used for expression in mammalian cells. In some embodiments, all codons need not be replaced to optimize the codon usage of the TdT since the natural sequence will comprise preferred codons and because use of preferred codons may not be required for all amino acid residues. Consequently, codon optimized polynucleotides encoding the TdT enzymes may contain preferred codons at about 40%, 50%, 60%, 70%, 80%, or greater than 90% of codon positions of the full-length coding region.

[0241] In some embodiments, the polynucleotide comprises a codon optimized nucleotide sequence encoding the TdT polypeptide amino acid sequence, as represented by SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246. In some embodiments, the polynucleotide has a nucleic acid sequence comprising at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the codon

optimized nucleic acid sequences encoding the even-numbered sequences in the range of SEQ ID NOs: 4-1960, 2004-3920, 4048-5466, and 5476. In some embodiments, the polynucleotide has a nucleic acid sequence comprising at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to the codon optimized nucleic acid sequences in the odd-numbered sequences in the range of SEQ ID NOs: 3-1959, 2003-3919, 4047-5465, and 5475. In some embodiments, the codon optimized sequences of the odd-numbered sequences in the range of SEQ ID NOs: 3-1959, 2003-3919, 4047-5465, and 5475, enhance expression of the encoded TdT, providing preparations of enzyme capable of converting substrate to product.

[0242] In some embodiments, the polynucleotide sequence comprises at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the reference sequence of SEQ ID NOs: 1, 7, 15, 23, 35, 267, 647, 659, 881, 1099, 1335, 1347, 1595, 1653, 1829, 1949, 2007, 2253, 2513, 2523, 2637, 2803, 2811, 2955, 3173, 3221, 3669, 3673, 3795, 3869, 3917, 4265, 4441, 4653, 4849, 4855, 4903, 5001, 5027, 5191, and/or 5245 and/or a functional fragment thereof, wherein said polynucleotide sequence encodes an engineered polypeptide comprising at least one substitution at one or more amino acid positions.

[0243] In some embodiments, the polynucleotide sequence encodes at least one engineered terminal deoxynucleotidyl transferase comprising a sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the reference sequence of SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246.

[0244] In some embodiments, the polynucleotide sequence comprises SEQ ID NOs: 7, 15, 23, 35, 267, 647, 659, 881, 1099, 1335, 1347, 1595, 1653, 1829, 1949, 2007, 2253, 2513, 2523, 2637, 2803, 2811, 2955, 3173, 3221, 3669, 3673, 3795, 3869, 3917, 4265, 4441, 4653, 4849, 4855, 4903, 5001, 5027, 5191, and/or 5245.

[0245] In some embodiments, the polynucleotides are capable of hybridizing under highly stringent conditions to a reference sequence selected from the odd-numbered sequences in SEQ ID NOs: 3-1959, 2003-3919, 4047-5465, and 5475, or a complement thereof, and encode a TdT.

[0246] In some embodiments, as described above, the polynucleotide encodes an engineered TdT polypeptide with improved properties as compared to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, wherein the polypeptide comprises an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to a reference sequence selected from SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, and one or more residue differences as

compared to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, wherein the sequence is selected from the even-numbered sequences in the range of SEQ ID NOs: 4-1960, 2004-3920, 4048-5466, and 5476. In some embodiments, the reference amino acid sequence is selected from the even-numbered sequences in the range of SEQ ID NOs: 4-1960, 2004-3920, 4048-5466, and 5476. In some embodiments, the reference amino acid sequence is SEQ ID NO: 2, while in some other embodiments, the reference sequence is SEQ ID NO: 8, while in some other embodiments, the reference sequence is SEQ ID NO: 16. In some embodiments, the reference amino acid sequence is SEQ ID NO: 24, while in some other embodiments, the reference sequence is SEQ ID NO: 36, while in some other embodiments, the reference sequence is SEQ ID NO: 268. In some embodiments, the reference amino acid sequence is SEQ ID NO: 648, while in some other embodiments, the reference sequence is SEQ ID NO: 660, while in some other embodiments, the reference sequence is SEQ ID NO: 882. In some embodiments, the reference amino acid sequence is SEQ ID NO: 1100, while in some other embodiments, the reference sequence is SEQ ID NO: 1336, while in some other embodiments, the reference sequence is SEQ ID NO: 1348. In some embodiments, the reference amino acid sequence is SEQ ID NO: 1596, while in some other embodiments, the reference sequence is SEQ ID NO: 1654, while in some other embodiments, the reference sequence is SEQ ID NO: 1830. In some embodiments, the reference amino acid sequence is SEQ ID NO: 1950, while in some other embodiments, the reference sequence is SEQ ID NO: 2008, while in some other embodiments, the reference sequence is SEQ ID NO: 2254. In some embodiments, the reference amino acid sequence is SEQ ID NO: 2514, while in some other embodiments, the reference sequence is SEQ ID NO: 2524, while in some other embodiments, the reference sequence is SEQ ID NO: 2638. In some embodiments, the reference amino acid sequence is SEQ ID NO: 2804, while in some other embodiments, the reference sequence is SEQ ID NO: 2812, while in some other embodiments, the reference sequence is SEQ ID NO: 2956. In some embodiments, the reference amino acid sequence is SEQ ID NO: 3174, while in some other embodiments, the reference sequence is SEQ ID NO: 3222, while in some other embodiments, the reference sequence is SEQ ID NO: 3670. In some embodiments, the reference amino acid sequence is SEQ ID NO: 3674, while in some other embodiments, the reference sequence is SEQ ID NO: 3796, while in some other embodiments, the reference sequence is SEQ ID NO: 3870.

[0247] In some embodiments, the polynucleotide encodes a TdT polypeptide capable of converting one or more substrates to product with improved properties as compared to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, wherein the polypeptide comprises an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654,

1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246.

[0248] In some embodiments, the polynucleotide encoding the engineered TdT comprises a polynucleotide sequence selected from the odd-numbered sequences in the range of SEQ ID NOs: 3-1959, 2003-3919, 4047-5465, and 5475.

[0249] In some embodiments, the polynucleotides are capable of hybridizing under highly stringent conditions to a reference polynucleotide sequence selected from the odd-numbered sequences in the range of SEQ ID NOs: 3-1959, 2003-3919, 4047-5465, and 5475 or a complement thereof, and encode a TdT polypeptide with one or more of the improved properties described herein. In some embodiments, the polynucleotide capable of hybridizing under highly stringent conditions encodes a TdT comprising an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, that has an amino acid sequence comprising one or more residue differences as compared to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246, as described above and in the Examples, below.

[0250] In some embodiments, the polynucleotide capable of hybridizing under highly stringent conditions encodes an engineered TdT polypeptide with improved properties comprising an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246. In some embodiments, the polynucleotides encode the polypeptides described herein but have at least about 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more sequence identity at the nucleotide level to a reference polynucleotide encoding the engineered TdT. In some embodiments, the reference polynucleotide sequence is selected from SEQ ID NOs: 3-1959, 2003-3919, 4047-5465, and 5475.

[0251] In some embodiments, the polynucleotide capable of hybridizing under highly stringent conditions encodes an engineered TdT polypeptide with improved properties comprising an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246. In some embodiments, the polynucleotides encode the polypeptides described herein but have at least about 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more sequence identity at the

nucleotide level to a reference polynucleotide encoding the engineered TdT. In some embodiments, the reference polynucleotide sequence is selected from SEQ ID NOs: 3-1959, 2003-3919, 4047-5465, and 5475.

[0252] In some embodiments, an isolated polynucleotide encoding any of the engineered TdT polypeptides provided herein is manipulated in a variety of ways to provide for expression of the polypeptide. In some embodiments, the polynucleotides encoding the polypeptides are provided as expression vectors where one or more control sequences is present to regulate the expression of the polynucleotides and/or polypeptides. Manipulation of the isolated polynucleotide prior to its insertion into a vector may be desirable or necessary depending on the expression vector. The techniques for modifying polynucleotides and nucleic acid sequences utilizing recombinant DNA methods are well known in the art.

[0253] In some embodiments, the control sequences include among other sequences, promoters, leader sequences, polyadenylation sequences, propeptide sequences, signal peptide sequences, and transcription terminators. As known in the art, suitable promoters can be selected based on the host cells used. For bacterial host cells, suitable promoters for directing transcription of the nucleic acid constructs of the present application, include, but are not limited to the promoters obtained from the *E. coli* lac operon, *Streptomyces coelicolor* agarase gene (dagA), *Bacillus subtilis* levansucrase gene (sacB), *Bacillus licheniformis* alpha-amylase gene (amyL), *Bacillus stearothermophilus* maltogenic amylase gene (amyM), *Bacillus amyloliquefaciens* alpha-amylase gene (amyQ), *Bacillus licheniformis* penicillinase gene (penP), *Bacillus subtilis* xylA and xylB genes, and prokaryotic beta-lactamase gene (See e.g., Villa-Kamaroff et al., Proc. Natl Acad. Sci. USA 75: 3727-3731 [1978]), as well as the *tac* promoter (See e.g., DeBoer et al., Proc. Natl Acad. Sci. USA 80: 21-25 [1983]). Exemplary promoters for filamentous fungal host cells, include promoters obtained from the genes for *Aspergillus oryzae* TKA amylase, *Rhizomucor miehei* aspartic proteinase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (glaA), *Rhizomucor miehei* lipase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Aspergillus nidulans* acetamidase, and *Fusarium oxysporum* trypsin-like protease (See e.g., WO 96/00787), as well as the NA2-tpi promoter (a hybrid of the promoters from the genes for *Aspergillus niger* neutral alpha-amylase and *Aspergillus oryzae* triose phosphate isomerase), and mutant, truncated, and hybrid promoters thereof. Exemplary yeast cell promoters can be from the genes can be from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* galactokinase (GAL1), *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH2/GAP), and *Saccharomyces cerevisiae* 3-phosphoglycerate kinase. Other useful promoters for yeast host cells are known in the art (See e.g., Romanos et al., Yeast 8:423-488 [1992]).

[0254] In some embodiments, the control sequence is a suitable transcription terminator sequence, a sequence recognized by a host cell to terminate transcription. The terminator sequence is operably linked to the 3' terminus of the nucleic acid sequence encoding the polypeptide. Any terminator which is

functional in the host cell of choice finds use in the present invention. For example, exemplary transcription terminators for filamentous fungal host cells can be obtained from the genes for *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* glucoamylase, *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* alpha-glucosidase, and *Fusarium oxysporum* trypsin-like protease. Exemplary terminators for yeast host cells can be obtained from the genes for *Saccharomyces cerevisiae* enolase, *Saccharomyces cerevisiae* cytochrome C (CYC1), and *Saccharomyces cerevisiae* glyceraldehyde-3-phosphate dehydrogenase. Other useful terminators for yeast host cells are known in the art (See e.g., Romanos et al., *supra*).

[0255] In some embodiments, the control sequence is a suitable leader sequence, a non-translated region of an mRNA that is important for translation by the host cell. The leader sequence is operably linked to the 5' terminus of the nucleic acid sequence encoding the polypeptide. Any leader sequence that is functional in the host cell of choice may be used. Exemplary leaders for filamentous fungal host cells are obtained from the genes for *Aspergillus oryzae* TAKA amylase and *Aspergillus nidulans* triose phosphate isomerase. Suitable leaders for yeast host cells include but are not limited to those obtained from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* 3-phosphoglycerate kinase, *Saccharomyces cerevisiae* alpha-factor, and *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH2/GAP). The control sequence may also be a polyadenylation sequence, a sequence operably linked to the 3' terminus of the nucleic acid sequence and which, when transcribed, is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence which is functional in the host cell of choice may be used in the present invention. Exemplary polyadenylation sequences for filamentous fungal host cells include but are not limited to those from the genes for *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* glucoamylase, *Aspergillus nidulans* anthranilate synthase, *Fusarium oxysporum* trypsin-like protease, and *Aspergillus niger* alpha-glucosidase. Useful polyadenylation sequences for yeast host cells are also known in the art (See e.g., Guo and Sherman, *Mol. Cell. Bio.*, 15:5983-5990 [1995]).

[0256] In some embodiments, the control sequence is a signal peptide coding region that codes for an amino acid sequence linked to the amino terminus of a polypeptide and directs the encoded polypeptide into the cell's secretory pathway. The 5' end of the coding sequence of the nucleic acid sequence may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region that encodes the secreted polypeptide. Alternatively, the 5' end of the coding sequence may contain a signal peptide coding region that is foreign to the coding sequence. Any signal peptide coding region that directs the expressed polypeptide into the secretory pathway of a host cell of choice finds use for expression of the engineered TdT polypeptides provided herein. Effective signal peptide coding regions for bacterial host cells include but are not limited to the signal peptide coding regions obtained from the genes for *Bacillus* NCIB 11837 maltogenic amylase, *Bacillus stearothermophilus* alpha-amylase, *Bacillus licheniformis* subtilisin, *Bacillus licheniformis* beta-lactamase, *Bacillus stearothermophilus* neutral proteases (nprT, nprS, nprM), and *Bacillus subtilis* prsA.

Further signal peptides are known in the art (See e.g., Simonen and Palva, Microbiol. Rev., 57:109-137 [1993]). Effective signal peptide coding regions for filamentous fungal host cells include but are not limited to the signal peptide coding regions obtained from the genes for *Aspergillus oryzae* TKA amylase, *Aspergillus niger* neutral amylase, *Aspergillus niger* glucoamylase, *Rhizomucor miehei* aspartic proteinase, *Humicola insolens* cellulase, and *Humicola lanuginosa* lipase. Useful signal peptides for yeast host cells include but are not limited to those from the genes for *Saccharomyces cerevisiae* alpha-factor and *Saccharomyces cerevisiae* invertase.

[0257] In some embodiments, the control sequence is a propeptide coding region that codes for an amino acid sequence positioned at the amino terminus of a polypeptide. The resultant polypeptide is referred to as a “proenzyme,” “propeptide,” or “zymogen,” in some cases). A propeptide can be converted to a mature active polypeptide by catalytic or autocatalytic cleavage of the propeptide from the propeptide. The propeptide coding region includes but is not limited to the genes for *Bacillus subtilis* alkaline protease (aprE), *Bacillus subtilis* neutral protease (nprT), *Saccharomyces cerevisiae* alpha-factor, *Rhizomucor miehei* aspartic proteinase, and *Myceliophthora thermophila* lactase (See e.g., WO 95/33836). Where both signal peptide and propeptide regions are present at the amino terminus of a polypeptide, the propeptide region is positioned next to the amino terminus of a polypeptide and the signal peptide region is positioned next to the amino terminus of the propeptide region.

[0258] In some embodiments, regulatory sequences are also utilized. These sequences facilitate the regulation of the expression of the polypeptide relative to the growth of the host cell. Examples of regulatory systems are those which cause the expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. In prokaryotic host cells, suitable regulatory sequences include, but are not limited to the *lac*, *tac*, and *trp* operator systems. In yeast host cells, suitable regulatory systems include, but are not limited to the ADH2 system or GAL1 system. In filamentous fungi, suitable regulatory sequences include, but are not limited to the TKA alpha-amylase promoter, *Aspergillus niger* glucoamylase promoter, and *Aspergillus oryzae* glucoamylase promoter.

[0259] The present invention also provides recombinant expression vectors comprising a polynucleotide encoding an engineered TdT polypeptide, and one or more expression regulating regions such as a promoter and a terminator, a replication origin, etc., depending on the type of hosts into which they are to be introduced. In some embodiments, the various nucleic acid and control sequences described above are combined together to produce a recombinant expression vector which includes one or more convenient restriction sites to allow for insertion or substitution of the nucleic acid sequence encoding the variant TdT polypeptide at such sites. Alternatively, the polynucleotide sequence(s) of the present invention are expressed by inserting the polynucleotide sequence or a nucleic acid construct comprising the polynucleotide sequence into an appropriate vector for expression. In creating the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression.

[0260] The recombinant expression vector may be any vector (e.g., a plasmid or virus), that can be conveniently subjected to recombinant DNA procedures and can result in the expression of the variant TdT polynucleotide sequence. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vectors may be linear or closed circular plasmids.

[0261] In some embodiments, the expression vector is an autonomously replicating vector (i.e., a vector that exists as an extra-chromosomal entity, the replication of which is independent of chromosomal replication, such as a plasmid, an extra-chromosomal element, a minichromosome, or an artificial chromosome). The vector may contain any means for assuring self-replication. In some alternative embodiments, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Furthermore, a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the host cell, or a transposon may be used.

[0262] In some embodiments, the expression vector preferably contains one or more selectable markers, which permit easy selection of transformed cells. A “selectable marker” is a gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophy, and the like. Examples of bacterial selectable markers include but are not limited to the *dal* genes from *Bacillus subtilis* or *Bacillus licheniformis*, or markers, which confer antibiotic resistance such as ampicillin, kanamycin, chloramphenicol or tetracycline resistance. Suitable markers for yeast host cells include, but are not limited to ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3. Selectable markers for use in a filamentous fungal host cell include, but are not limited to, *amdS* (acetamidase), *argB* (ornithine carbamoyltransferases), *bar* (phosphinothricin acetyltransferase), *hph* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *pyrG* (orotidine-5'-phosphate decarboxylase), *sC* (sulfate adenylyltransferase), and *trpC* (anthranilate synthase), as well as equivalents thereof. In another aspect, the present invention provides a host cell comprising a polynucleotide encoding at least one engineered TdT polypeptide of the present invention, the polynucleotide being operatively linked to one or more control sequences for expression of the engineered TdT enzyme(s) in the host cell. Host cells for use in expressing the polypeptides encoded by the expression vectors of the present invention are well known in the art and include but are not limited to, bacterial cells, such as *E. coli*, *Vibrio fluvialis*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* and *Pichia pastoris* [ATCC Accession No. 201178]); insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, BHK, 293, and Bowes melanoma cells; and plant cells. Exemplary host cells are *Escherichia coli* strains (e.g., W3110 (Δ fhuA) and BL21).

[0263] In some embodiments, the host cell strain comprises a knockout of one or more genes, in particular phosphatase genes. In some embodiments, the host cell comprises a knockout or single gene deletion of *E. coli* genes *aphA*, *surE*, *phoA*, and/or *cpdB*, as described below in the Examples. In some

embodiments, the host cell comprising a knockout of one or more phosphatase genes has increased production of the product and/or decreased de-phosphorylation of the product or substrate.

[0264] Accordingly, in another aspect, the present invention provides methods for producing the engineered TdT polypeptides, where the methods comprise culturing a host cell capable of expressing a polynucleotide encoding the engineered TdT polypeptide under conditions suitable for expression of the polypeptide. In some embodiments, the methods further comprise the steps of isolating and/or purifying the TdT polypeptides, as described herein.

[0265] Appropriate culture media and growth conditions for the above-described host cells are well known in the art. Polynucleotides for expression of the TdT polypeptides may be introduced into cells by various methods known in the art. Techniques include, among others, electroporation, biolistic particle bombardment, liposome mediated transfection, calcium chloride transfection, and protoplast fusion.

[0266] The engineered TdTs with the properties disclosed herein can be obtained by subjecting the polynucleotide encoding the naturally occurring or engineered TdT polypeptide to mutagenesis and/or directed evolution methods known in the art, and as described herein. An exemplary directed evolution technique is mutagenesis and/or DNA shuffling (See e.g., Stemmer, Proc. Natl. Acad. Sci. USA 91:10747-10751 [1994]; WO 95/22625; WO 97/0078; WO 97/35966; WO 98/27230; WO 00/42651; WO 01/75767 and U.S. Pat. 6,537,746). Other directed evolution procedures that can be used include, among others, staggered extension process (StEP), in vitro recombination (See e.g., Zhao et al., Nat. Biotechnol., 16:258–261 [1998]), mutagenic PCR (See e.g., Caldwell et al., PCR Methods Appl., 3:S136-S140 [1994]), and cassette mutagenesis (See e.g., Black et al., Proc. Natl. Acad. Sci. USA 93:3525-3529 [1996]).

[0267] For example, mutagenesis and directed evolution methods can be readily applied to polynucleotides to generate variant libraries that can be expressed, screened, and assayed. Mutagenesis and directed evolution methods are well known in the art (See e.g., US Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, 5,837,458, 5,928,905, 6,096,548, 6,117,679, 6,132,970, 6,165,793, 6,180,406, 6,251,674, 6,265,201, 6,277,638, 6,287,861, 6,287,862, 6,291,242, 6,297,053, 6,303,344, 6,309,883, 6,319,713, 6,319,714, 6,323,030, 6,326,204, 6,335,160, 6,335,198, 6,344,356, 6,352,859, 6,355,484, 6,358,740, 6,358,742, 6,365,377, 6,365,408, 6,368,861, 6,372,497, 6,337,186, 6,376,246, 6,379,964, 6,387,702, 6,391,552, 6,391,640, 6,395,547, 6,406,855, 6,406,910, 6,413,745, 6,413,774, 6,420,175, 6,423,542, 6,426,224, 6,436,675, 6,444,468, 6,455,253, 6,479,652, 6,482,647, 6,483,011, 6,484,105, 6,489,146, 6,500,617, 6,500,639, 6,506,602, 6,506,603, 6,518,065, 6,519,065, 6,521,453, 6,528,311, 6,537,746, 6,573,098, 6,576,467, 6,579,678, 6,586,182, 6,602,986, 6,605,430, 6,613,514, 6,653,072, 6,686,515, 6,703,240, 6,716,631, 6,825,001, 6,902,922, 6,917,882, 6,946,296, 6,961,664, 6,995,017, 7,024,312, 7,058,515, 7,105,297, 7,148,054, 7,220,566, 7,288,375, 7,384,387, 7,421,347, 7,430,477, 7,462,469, 7,534,564, 7,620,500, 7,620,502, 7,629,170, 7,702,464, 7,747,391, 7,747,393, 7,751,986, 7,776,598, 7,783,428, 7,795,030, 7,853,410, 7,868,138, 7,783,428, 7,873,477, 7,873,499, 7,904,249, 7,957,912, 7,981,614, 8,014,961, 8,029,988, 8,048,674, 8,058,001, 8,076,138, 8,108,150, 8,170,806,

8,224,580, 8,377,681, 8,383,346, 8,457,903, 8,504,498, 8,589,085, 8,762,066, 8,768,871, 9,593,326, and all related US, as well as PCT and non-US counterparts; Ling et al., *Anal. Biochem.*, 254(2):157-78 [1997]; Dale et al., *Meth. Mol. Biol.*, 57:369-74 [1996]; Smith, *Ann. Rev. Genet.*, 19:423-462 [1985]; Botstein et al., *Science*, 229:1193-1201 [1985]; Carter, *Biochem. J.*, 237:1-7 [1986]; Kramer et al., *Cell*, 38:879-887 [1984]; Wells et al., *Gene*, 34:315-323 [1985]; Minshull et al., *Curr. Op. Chem. Biol.*, 3:284-290 [1999]; Christians et al., *Nat. Biotechnol.*, 17:259-264 [1999]; Crameri et al., *Nature*, 391:288-291 [1998]; Crameri, et al., *Nat. Biotechnol.*, 15:436-438 [1997]; Zhang et al., *Proc. Nat. Acad. Sci. U.S.A.*, 94:4504-4509 [1997]; Crameri et al., *Nat. Biotechnol.*, 14:315-319 [1996]; Stemmer, *Nature*, 370:389-391 [1994]; Stemmer, *Proc. Nat. Acad. Sci. USA*, 91:10747-10751 [1994]; WO 95/22625; WO 97/0078; WO 97/35966; WO 98/27230; WO 00/42651; WO 01/75767; and WO 2009/152336, all of which are incorporated herein by reference).

[0268] In some embodiments, the enzyme clones obtained following mutagenesis treatment are screened by subjecting the enzymes to a defined temperature (or other assay conditions, such as testing the enzyme's activity over a broad range of substrates) and measuring the amount of enzyme activity remaining after heat treatments or other assay conditions. Clones containing a polynucleotide encoding a TdT polypeptide are then sequenced to identify the nucleotide sequence changes (if any) and used to express the enzyme in a host cell. Measuring enzyme activity from the expression libraries can be performed using any suitable method known in the art (e.g., standard biochemistry techniques, such as HPLC analysis).

[0269] In some embodiments, the clones obtained following mutagenesis treatment can be screened for engineered TdTs having one or more desired improved enzyme properties (e.g., improved regioselectivity). Measuring enzyme activity from the expression libraries can be performed using the standard biochemistry techniques, such as HPLC analysis, LC-MS analysis, RapidFire-MS analysis, and/or capillary electrophoresis analysis.

[0270] When the sequence of the engineered polypeptide is known, the polynucleotides encoding the enzyme can be prepared by standard solid-phase methods, according to known synthetic methods. In some embodiments, fragments of up to about 100 bases can be individually synthesized, then joined (e.g., by enzymatic or chemical ligation methods, or polymerase mediated methods) to form any desired continuous sequence. For example, polynucleotides and oligonucleotides encoding portions of the TdT can be prepared by chemical synthesis as known in the art (e.g., the classical phosphoramidite method of Beaucage et al., *Tet. Lett.* 22:1859-69 [1981], or the method described by Matthes et al., *EMBO J.* 3:801-05 [1984]) as typically practiced in automated synthetic methods. According to the phosphoramidite method, oligonucleotides are synthesized (e.g., in an automatic DNA synthesizer), purified, annealed, ligated and cloned in appropriate vectors. In addition, essentially any nucleic acid can be obtained from any of a variety of commercial sources. In some embodiments, additional variations can be created by synthesizing oligonucleotides containing deletions, insertions, and/or substitutions, and combining the oligonucleotides in various permutations to create engineered TdTs with improved properties.

[0271] Accordingly, in some embodiments, a method for preparing the engineered TdT polypeptide comprises: (a) synthesizing a polynucleotide encoding a polypeptide comprising an amino acid sequence having at least about 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more sequence identity to an amino acid sequence selected from the even-numbered sequences of SEQ ID NOs: 4-1960, 2004-3920, 4048-5466, and 5476, and having one or more residue differences as compared to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246; and (b) expressing the TdT polypeptide encoded by the polynucleotide.

[0272] In some embodiments of the method, the polynucleotide encodes an engineered TdT that has optionally one or several (e.g., up to 3, 4, 5, or up to 10) amino acid residue deletions, insertions and/or substitutions. In some embodiments, the amino acid sequence has optionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-15, 1-20, 1-21, 1-22, 1-23, 1-24, 1-25, 1-30, 1-35, 1-40, 1-45, 1-50, 1-75, 1-100, or 1-150 amino acid residue deletions, insertions and/or substitutions. In some embodiments, the amino acid sequence has optionally around 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acid residue deletions, insertions and/or substitutions. In some embodiments, the substitutions can be conservative or non-conservative substitutions.

[0273] In some embodiments, any of the engineered TdT enzymes expressed in a host cell can be recovered from the cells and/or the culture medium using any one or more of the well-known techniques for protein purification, including, among others, lysozyme treatment, sonication, filtration, salting-out, ultra-centrifugation, and chromatography. Suitable solutions for lysing and the high efficiency extraction of proteins from bacteria, such as *E. coli*, are commercially available (e.g., CelLytic B™, Sigma-Aldrich, St. Louis MO).

[0274] Chromatographic techniques for isolation of the TdT polypeptide include, among others, reverse phase chromatography high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, and affinity chromatography. Conditions for purifying a particular enzyme will depend, in part, on factors such as net charge, hydrophobicity, hydrophilicity, molecular weight, molecular shape, etc., and will be apparent to those having skill in the art.

[0275] In some embodiments, affinity techniques may be used to isolate the improved TdT enzymes. For affinity chromatography purification, any antibody which specifically binds the TdT polypeptide may be used. For the production of antibodies, various host animals, including but not limited to rabbits, mice, rats, etc., may be immunized by injection with a TdT polypeptide, or a fragment thereof. The TdT polypeptide or fragment may be attached to a suitable carrier, such as BSA, by means of a side chain functional group or linkers attached to a side chain functional group. In some embodiments, the affinity purification can use a specific ligand bound by the TdT or dye affinity column (See e.g., EP0641862;

Stellwagen, "Dye Affinity Chromatography," *In Current Protocols in Protein Science*, Unit 9.2-9.2.16 [2001]).

[0276] Methods of Using the Engineered TdT Enzymes

[0277] In some embodiments, the TdT enzymes described herein find use in processes for conversion of one or more suitable substrates to a product.

[0278] In some embodiments, the engineered TdT polypeptides disclosed herein can be used in a process for the conversion of the oligo acceptor substrate and an NTP-3'-O-RBG or natural or modified NTP substrate to a product comprising an oligo acceptor substrate extended by one nucleotide.

[0279] In the embodiments provided herein and illustrated in the Examples, various ranges of suitable reaction conditions that can be used in the processes, include but are not limited to, substrate loading, co-substrate loading, pH, temperature, buffer, solvent system, cofactor, polypeptide loading, and reaction time. Further suitable reaction conditions for carrying out the process for biocatalytic conversion of substrate compounds to product compounds using an engineered TdT described herein can be readily optimized in view of the guidance provided herein by routine experimentation that includes, but is not limited to, contacting the engineered TdT polypeptide and one or more substrate compounds under experimental reaction conditions of concentration, pH, temperature, and solvent conditions, and detecting the product compound.

[0280] The oligo acceptor substrate may be any nucleotide chain or similar moiety with an exposed 3'-OH. In some embodiments, the acceptor substrate may be single stranded. In yet other embodiments, the acceptor substrate may be double stranded or partially doubled stranded. In some embodiments, the acceptor substrate may comprise a nucleotide chain consisting of 1-10 nucleotides, 5-20 nucleotides, 15-50 nucleotides, 30-100 nucleotides, or greater than 100 nucleotides. In some embodiments, the oligo acceptor substrate may comprise a chemical moiety that is not a nucleotide chain but contains a free -OH capable of being recognized as a substrate by a wild-type or engineered TdT.

[0281] In some embodiments, the oligo acceptor substrate may comprise one or more nucleotides with a 2' modification, as described herein. In some embodiments, the oligo acceptor substrate may comprise one or more nucleotides with a 2' modification selected from 2'-OH, 2'-H, 2'-O-methyl, 2'-fluoro, or 2'-methoxyethyl, 2'-OCH₂CH₂OCH₃, 2'-CO₂R' (where R' is any alkyl or aryl), or another 2' atom or chemical group. In some embodiments, the oligo acceptor substrate may comprise one or more additional modifications, such as a phosphothiorate linkage.

[0282] In some embodiments, the sugar may have other modifications at other positions, such as locked nucleotides or constrained ethyl nucleotides, as is known in the art. In some embodiments, "locked nucleoside" or "locked nucleotide" refers to nucleoside or nucleotide, respectively, in which the ribose moiety is modified with a bridge connecting the 2' oxygen and 4' carbon (see, e.g., Obika et al., *Tetrahedron Letters*, 1997, 38(50):8735-8738; Orum et al., *Current Pharmaceutical Design*, 2008, 14(11):1138-1142). Typically, the bridge is a methylene bridge. In some embodiments, the 3'-phosphate group of the NQP may act as a removable blocking group or protecting group that may be selectively

unblocked or removed to allow further modifications, reactions, or incorporation of the NQP into a growing oligonucleotide chain during template-dependent or template-independent oligonucleotide synthesis

[0283] In some embodiments, the oligo acceptor substrate comprises a nucleotide chain of repeating nucleotides. In other embodiments, the oligo acceptor substrate comprises a nucleotide chain of varied nucleotides that do not repeat. In some embodiments, the oligo acceptor substrate comprises a nucleotide chain with an odd number of nucleotides. In some embodiments, the oligo acceptor substrate comprises a nucleotide with an even number of nucleotides.

[0284] In some embodiments, the oligo acceptor substrate is secured to solid support. Suitable solid supports are known to those in the art and described, below, in this disclosure.

[0285] In some embodiments, the oligo acceptor substrate comprises one or more nucleotide sequences selected from the following 5'-6-FAM-T17ATCmC, 5'-6-FAM-T17AT*mC, 5'-6-FAM-T17ATC(2'dF)C, 5'-6-FAM-T12ATCAC*(2'dF)A, 5'-6-FAM-T12ATCAC*mC, 5'-6-FAM-T12ATCAC*mA, 5'-6-FAM-T15AmG*mC, 5'-6-FAM-T15AmG*mC, 5'-6-FAM-T12TATCAC*mC, 5'-6-FAM-T15AmU*mG, 5'-6-FAM-T15AmU*mG, 5'-6-FAM-T14ATCmC, 5'-6-FAM-T15AT*mG, 5'-6-FAM-T17mAmUmC, 5'-6-FAM-T17mUmUmC, 5'-6-FAM-T17mCmUmG, 5'-6-FAM-T15AT*mA, 5'-6-FAM-T15AT*mC, 5'-6-FAM-T15AT*mU, T14ATCmC, 5'-6-FAM-T15mUmGmA, 5'-6-FAM-T15mAmU*mG, 5'-6-FAM-T15mC*mG*mA, 5'-6-FAM-T17mGmUmC, 5'-6-FAM-T12mAmUmA, 5'-6-FAM-T22mAmUmU, 5'-6-FAM-T27mAmUmG, 5'-6-FAM-T57mUmUmC, 5'-6-FAM-T32mAmCmC, 5'-6-FAM-T37mAmGmC, 5'-6-FAM-T42mAmAmC, 5'-6-FAM-T47mGmUmC, 5'-6-FAM-T52mCmUmC, 5'-6-FAM-T15mAmU(2'dF)G, 5'-6-FAM-T15mAmG(MOE)C, 5'-6-FAM-T15mGmAmC, 5'-6-FAM-T22*(2'dF)A(2'dF)GmA, 5'-6-FAM-T22(2'dF)C(2'dF)G(2'dF)A, 5'-6-FAM-T27(2'dF)GmA(2'dF)U, 5'-6-FAM-T15mGmAmC, 5'-6-FAM-T11mCmGmA, 5'-6-FAM-T11mC*mA*mG, 5'-6-FAM-T15mA(2'dF)UmC, 5'-6-FAM-T15mCmUmG, 5'-6-FAM-T27(2'dF)C*(2'dF)G*(2'dF)A, 5'-6-FAM-T11mU*(2'dF)A*(2'dF)A, 5'-6-FAM-T48mG*mA*mC, 5'-6-FAM-T15mAmCmU, 5'-6-FAM-T17*(2'dF)A*(2'dF)A(2'dF)G, 5'-6-FAM-T15mAmU(2'dF)U, 5'-6-FAM-T15mAmU(2'dF)C, 5'-BiosG-T3(iFluorT)T9mAmUmA, 5'-BiosG-T3(iFluorT)T9mAmUmAmA, 5'-BiosG-T3(iFluorT)T9mAmUmAmAmG, 5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA, 5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA(2'dF)A, 5'-BiosG-T13mAmUmA, 5'-BiosG-T13mAmUmAmA, 5'-BiosG-T13mAmUmAmAmG, 5'-BiosG-T13mAmUmAmAmGmA, 5'-BiosG-T13mAmUmAmAmGmA(2'dF)A, as further described in the Examples (Table 4.1) and the accompanying sequence listing. These embodiments are intended to be non-limiting. Any suitable oligo acceptor substrate finds use in the present invention.

[0286] In some embodiments, the NTP-3'-O-RBG substrate comprises a deoxyribonucleoside triphosphate with a 3'-O-RBG. In other embodiments, the NTP-3'-O-RBG substrate may comprise a ribonucleoside triphosphate with a 3'-O-RBG. In yet other embodiments, the NTP-3'-O-RBG substrate may comprise a synthetic nucleoside triphosphate with a 3'-O-RBG. In some embodiments, the NTP-3'-

O-RBG substrate may comprise a sugar ring with a number of carbons that is not five. A non-limiting example of this is a threose nucleoside triphosphate.

[0287] A range of 3' removable blocking groups for the NTP-3'-O-RBG substrate useful in the present disclosure are known in the art and include but are not limited to, -O-NH₂, -O-NO₂, -O-PO₃. In some embodiments, the NTP-3'-O-RBG substrate with 3' removable blocking group can be selected from the group consisting of NTP-3'-O-NH₂, NTP-3'-O-NO₂, or NTP-3'-O-PO₃. In some embodiments, the NTP-3'-O-RBG substrate comprises another blocking group that would sterically hinder addition of a second NTP-3'-O-RBG substrate to the 3' end of the growing oligo acceptor substrate strand prior to removal of the removable blocking from the first round of addition.

[0288] In some embodiments, the deoxyribonucleoside triphosphate with a 3'-O-RBG or ribonucleoside triphosphate with a 3'-O-RBG further comprises a natural purine or pyrimidine base, such as adenine, guanine, cytosine, thymine, or uridine. In some embodiments, deoxyribonucleoside triphosphate with a 3'-O-RBG or ribonucleoside triphosphate with a 3'-O-RBG further comprises an unnatural base analog such as inosine, xanthine, hypoxanthine or another base analog, as is known in the art. In some embodiments, the deoxyribonucleoside triphosphate with a 3'-O-RBG or ribonucleoside triphosphate with a 3'-O-RBG further comprises a base with modifications, as is known in the art. In some embodiments, the deoxyribonucleoside triphosphate with a 3'-O-RBG or ribonucleoside triphosphate with a 3'-O-RBG further comprises a 2' modification or substitution. In some embodiments, the deoxyribonucleoside triphosphate with a 3'-O-RBG or ribonucleoside triphosphate with a 3'-O-RBG further comprises substitution of an oxygen for a sulfur atom for the creation of phosphorothioate linkages. In some embodiments, the deoxyribonucleoside triphosphate with a 3'-O-RBG or ribonucleoside triphosphate with a 3'-O-RBG further comprises substitution of two oxygens for sulfurs for the creation of phosphorodithioate linkages.

[0289] The substrate compound(s) in the reaction mixtures can be varied, taking into consideration, for example, the desired amount of product compound, the effect of each substrate concentration on enzyme activity, stability of enzyme under reaction conditions, and the percent conversion of each substrate to product. In some embodiments, the suitable reaction conditions comprise a substrate compound loading for each oligo acceptor substrate of at least about 0.1 μ M to 1 μ M, 1 μ M to 2 μ M, 2 μ M to 3 μ M, 3 μ M to 5 μ M, 5 μ M to 10 μ M, or 10 μ M or greater. In some embodiments, the suitable reaction conditions comprise a substrate compound loading for each oligo acceptor substrate of at least about 0.5 to about 25 g/L, 1 to about 25 g/L, 5 to about 25 g/L, about 10 to about 25 g/L, or 20 to about 25 g/L. In some embodiments, the suitable reaction conditions comprise a substrate compound loading for each oligo acceptor substrate of at least about 0.5 g/L, at least about 1 g/L, at least about 5 g/L, at least about 10 g/L, at least about 15 g/L, at least about 20 g/L, or at least about 30 g/L, or even greater.

[0290] In some embodiments, the suitable reaction conditions comprise a substrate compound loading for each NTP-3'-O-RBG or natural or modified NTP substrate of at least about 1 μ M to 5 μ M, 5 μ M to 10 μ M, 10 μ M to 25 μ M, 25 μ M to 50 μ M, 50 μ M to 100 μ M, 100 μ M to 200 μ M, 200 μ M to 300 μ M, or

300 μ M to 500 μ M. In some embodiments, the suitable reaction conditions comprise a substrate compound loading for each oligo acceptor substrate of at least about 0.5 g/L, at least about 1 g/L, at least about 5 g/L, at least about 10 g/L, at least about 15 g/L, at least about 20 g/L, or at least about 30 g/L, or even greater.

[0291] In carrying out the TdT-mediated synthesis processes described herein, the engineered polypeptide may be added to the reaction mixture in the form of a purified enzyme, partially purified enzyme, whole cells transformed with gene(s) encoding the enzyme, as cell extracts and/or lysates of such cells, and/or as an enzyme immobilized on a solid support. Whole cells transformed with gene(s) encoding the engineered TdT enzyme or cell extracts, lysates thereof, and isolated enzymes may be employed in a variety of different forms, including solid (e.g., lyophilized, spray-dried, and the like) or semisolid (e.g., a crude paste). The cell extracts or cell lysates may be partially purified by precipitation (ammonium sulfate, polyethyleneimine, heat treatment or the like, followed by a desalting procedure prior to lyophilization (e.g., ultrafiltration, dialysis, etc.). Any of the enzyme preparations (including whole cell preparations) may be stabilized by crosslinking using known crosslinking agents, such as, for example, glutaraldehyde or immobilization to a solid phase (e.g., Eupergit C, and the like).

[0292] The gene(s) encoding the engineered TdT polypeptides can be transformed into host cell separately or together into the same host cell. For example, in some embodiments one set of host cells can be transformed with gene(s) encoding one engineered TdT polypeptide, and another set can be transformed with gene(s) encoding another TdT. Both sets of transformed cells can be utilized together in the reaction mixture in the form of whole cells, or in the form of lysates or extracts derived therefrom. In other embodiments, a host cell can be transformed with gene(s) encoding multiple engineered TdT polypeptides. In some embodiments the engineered polypeptides can be expressed in the form of secreted polypeptides, and the culture medium containing the secreted polypeptides can be used for the TdT reaction.

[0293] In some embodiments, the improved activity of the engineered TdT polypeptides disclosed herein provides for processes wherein higher percentage conversion can be achieved with lower concentrations of the engineered polypeptide. In some embodiments of the process, the suitable reaction conditions comprise an engineered polypeptide amount of about 1% (w/w), 2% (w/w), 5% (w/w), 10% (w/w), 20% (w/w), 30% (w/w), 40% (w/w), 50% (w/w), 75% (w/w), 100% (w/w) or more of substrate compound loading.

[0294] In some embodiments, the engineered polypeptide is present at a molar ratio of engineered polypeptide to substrate of about 50 to 1, 25 to 1, 10 to 1, 5 to 1, 1 to 1, 1 to 5, 1 to 10, 1 to 25 or 1 to 50. In some embodiments, the engineered polypeptide is present at a molar ratio of engineered polypeptide to substrate from a range of about 50 to 1 to a range of about 1 to 50.

[0295] In some embodiments, the engineered polypeptide is present at about 0.01 g/L to about 50 g/L; about 0.01 to about 0.1 g/L; about 0.05 g/L to about 50 g/L; about 0.1 g/L to about 40 g/L; about 1 g/L to about 40 g/L; about 2 g/L to about 40 g/L; about 5 g/L to about 40 g/L; about 5 g/L to about 30 g/L; about

0.1 g/L to about 10 g/L; about 0.5 g/L to about 10 g/L; about 1 g/L to about 10 g/L; about 0.1 g/L to about 5 g/L; about 0.5 g/L to about 5 g/L; or about 0.1 g/L to about 2 g/L. In some embodiments, the TdT polypeptide is present at about 0.01 g/L, 0.05 g/L, 0.1 g/L, 0.2 g/L, 0.5 g/L, 1, 2 g/L, 5 g/L, 10 g/L, 15 g/L, 20 g/L, 25 g/L, 30 g/L, 35 g/L, 40 g/L, or 50 g/L.

[0296] In some embodiments, the suitable reaction conditions comprise a divalent metal cofactor. In some embodiments, the divalent metal cofactor is cobalt. In some embodiments, the cobalt (II) chloride is present at concentrations of about 1 to 1000 μ M; about 50 to 400 μ M; about 100 to 300 μ M; or about 200 to 600 μ M; about 500 to 1000 μ M. In some embodiments, the cobalt (II) chloride is present at concentrations of about 150 μ M; about 200 μ M; about 250 μ M, about 500 μ M; or about 1000 μ M.

[0297] In some embodiments of the reaction, a phosphatase is used to degrade inorganic phosphate and shift the reaction equilibrium toward the oligo acceptor extension product. In some embodiments, the phosphatase is an *E. coli* pyrophosphatase. In some embodiments, the phosphatase is present at a concentration of about 0.0001 to 0.01 units/uL; about 0.001 to 0.005 units/uL; or about 0.002 to 0.003 units/uL. In some embodiments, the phosphatase is present at a concentration of about 0.001 units/uL; about 0.002 units/uL; or about 0.003 units/uL. In some embodiments, the phosphatase is from *Geobacillus zalihae*. In some embodiments, the phosphatase is present at a concentration of about 0.01 to 10 μ M; about 0.01 to 0.1 μ M; or about 0.1 to 1 μ M; or about 0.1 to 10 μ M. In some embodiments, the phosphatase is present at a concentration of about 0.05 μ M; about 0.5 μ M; or about 5 μ M; or about 10 μ M.

[0298] During the course of the reaction, the pH of the reaction mixture may change. The pH of the reaction mixture may be maintained at a desired pH or within a desired pH range. This may be done by the addition of an acid or a base, before and/or during the course of the reaction. Alternatively, the pH may be controlled by using a buffer. Accordingly, in some embodiments, the reaction condition comprises a buffer. Suitable buffers to maintain desired pH ranges are known in the art and include, by way of example and not limitation, borate, phosphate, 2-(N-morpholino)ethanesulfonic acid (MES), 3-(N-morpholino)propanesulfonic acid (MOPS), acetate, triethanolamine (TEoA), and 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris), and the like. In some embodiments, the reaction conditions comprise water as a suitable solvent with no buffer present.

[0299] In the embodiments of the process, the reaction conditions comprise a suitable pH. The desired pH or desired pH range can be maintained by use of an acid or base, an appropriate buffer, or a combination of buffering and acid or base addition. The pH of the reaction mixture can be controlled before and/or during the course of the reaction. In some embodiments, the suitable reaction conditions comprise a solution pH from about 4 to about 10, pH from about 5 to about 10, pH from about 5 to about 9, pH from about 6 to about 9, pH from about 6 to about 8. In some embodiments, the reaction conditions comprise a solution pH of about 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10.

[0300] In the embodiments of the processes herein, a suitable temperature is used for the reaction conditions, for example, taking into consideration the increase in reaction rate at higher temperatures, and

the activity of the enzyme during the reaction time period. Accordingly, in some embodiments, the suitable reaction conditions comprise a temperature of about 10 °C to about 95 °C, about 10 °C to about 75 °C, about 15 °C to about 95 °C, about 20 °C to about 95 °C, about 20 °C to about 65 °C, about 25 °C to about 70 °C, or about 50 °C to about 70 °C. In some embodiments, the suitable reaction conditions comprise a temperature of about 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C, 50 °C, 55 °C, 60 °C, 65 °C, 70 °C, 75 °C, 80 °C, 85 °C, 90 °C or 95 °C. In some embodiments, the temperature during the enzymatic reaction can be maintained at a specific temperature throughout the course of the reaction. In some embodiments, the temperature during the enzymatic reaction can be adjusted over a temperature profile during the course of the reaction.

[0301] In some embodiments, the processes of the invention are carried out in a solvent. Suitable solvents include water, aqueous buffer solutions, organic solvents, polymeric solvents, and/or co-solvent systems, which generally comprise aqueous solvents, organic solvents and/or polymeric solvents. The aqueous solvent (water or aqueous co-solvent system) may be pH-buffered or unbuffered. In some embodiments, the processes using the engineered TdT polypeptides can be carried out in an aqueous co-solvent system comprising an organic solvent (e.g., ethanol, isopropanol (IPA), dimethyl sulfoxide (DMSO), dimethylformamide (DMF) ethyl acetate, butyl acetate, 1-octanol, heptane, octane, methyl t butyl ether (MTBE), toluene, and the like), ionic or polar solvents (e.g., 1-ethyl 4 methylimidazolium tetrafluoroborate, 1-butyl-3-methylimidazolium tetrafluoroborate, 1-butyl 3 methylimidazolium hexafluorophosphate, glycerol, polyethylene glycols, and the like). In some embodiments, the co-solvent can be a polar solvent, such as a polyol, dimethylsulfoxide (DMSO), or lower alcohol. The non-aqueous co-solvent component of an aqueous co-solvent system may be miscible with the aqueous component, providing a single liquid phase, or may be partly miscible or immiscible with the aqueous component, providing two liquid phases. Exemplary aqueous co-solvent systems can comprise water and one or more co-solvents selected from an organic solvent, polar solvent, and polyol solvent. In general, the co-solvent component of an aqueous co-solvent system is chosen such that it does not adversely inactivate the TdT enzyme under the reaction conditions. Appropriate co-solvent systems can be readily identified by measuring the enzymatic activity of the specified engineered TdT enzyme with a defined substrate of interest in the candidate solvent system, utilizing an enzyme activity assay, such as those described herein.

[0302] In some embodiments of the process, the suitable reaction conditions comprise an aqueous co-solvent, where the co-solvent comprises DMSO at about 1% to about 50% (v/v), about 1 to about 40% (v/v), about 2% to about 40% (v/v), about 5% to about 30% (v/v), about 10% to about 30% (v/v), or about 10% to about 20% (v/v). In some embodiments of the process, the suitable reaction conditions can comprise an aqueous co-solvent comprising ethanol at about 1% (v/v), about 5% (v/v), about 10% (v/v), about 15% (v/v), about 20% (v/v), about 25% (v/v), about 30% (v/v), about 35% (v/v), about 40% (v/v), about 45% (v/v), or about 50% (v/v).

[0303] In some embodiments, the reaction conditions comprise a surfactant for stabilizing or enhancing the reaction. Surfactants can comprise non-ionic, cationic, anionic and/or amphiphilic surfactants.

Exemplary surfactants, include by way of example and not limitation, nonyl phenoxypolyethoxylethanol (NP40), TRITON™ X-100 polyethylene glycol *tert*-octylphenyl ether, polyoxyethylene-stearylamine, cetyltrimethylammonium bromide, sodium oleylamidosulfate, polyoxyethylene-sorbitanmonostearate, hexadecyldimethylamine, etc. Any surfactant that may stabilize or enhance the reaction may be employed. The concentration of the surfactant to be employed in the reaction may be generally from 0.1 to 50 mg/mL, particularly from 1 to 20 mg/mL.

[0304] In some embodiments, the reaction conditions include an antifoam agent, which aids in reducing or preventing formation of foam in the reaction solution, such as when the reaction solutions are mixed or sparged. Anti-foam agents include non-polar oils (e.g., minerals, silicones, etc.), polar oils (e.g., fatty acids, alkyl amines, alkyl amides, alkyl sulfates, etc.), and hydrophobic (e.g., treated silica, polypropylene, etc.), some of which also function as surfactants. Exemplary anti-foam agents include Y-30® (Dow Corning), poly-glycol copolymers, oxy/ethoxylated alcohols, and polydimethylsiloxanes. In some embodiments, the anti-foam can be present at about 0.001% (v/v) to about 5% (v/v), about 0.01% (v/v) to about 5% (v/v), about 0.1% (v/v) to about 5% (v/v), or about 0.1% (v/v) to about 2% (v/v). In some embodiments, the anti-foam agent can be present at about 0.001% (v/v), about 0.01% (v/v), about 0.1% (v/v), about 0.5% (v/v), about 1% (v/v), about 2% (v/v), about 3% (v/v), about 4% (v/v), or about 5% (v/v) or more as desirable to promote the reaction.

[0305] The quantities of reactants used in the TdT reaction will generally vary depending on the quantities of product desired, and concomitantly the amount of substrates employed. Those having ordinary skill in the art will readily understand how to vary these quantities to tailor them to the desired level of productivity and scale of production.

[0306] In some embodiments, the order of addition of reactants is not critical. The reactants may be added together at the same time to a solvent (e.g., monophasic solvent, biphasic aqueous co-solvent system, and the like), or alternatively, some of the reactants may be added separately, and some together at different time points. For example, the cofactor, co-substrate and substrate may be added first to the solvent.

[0307] The solid reactants (e.g., enzyme, salts, etc.) may be provided to the reaction in a variety of different forms, including powder (e.g., lyophilized, spray dried, and the like), solution, emulsion, suspension, and the like. The reactants can be readily lyophilized or spray dried using methods and equipment that are known to those having ordinary skill in the art. For example, the protein solution can be frozen at -80°C in small aliquots, then added to a pre-chilled lyophilization chamber, followed by the application of a vacuum.

[0308] For improved mixing efficiency when an aqueous co-solvent system is used, the TdT, and co-substrate may be added and mixed into the aqueous phase first. The substrate may be added and mixed in, followed by the organic phase or the substrate may be dissolved in the organic phase and mixed in. Alternatively, the substrate may be premixed in the organic phase, prior to addition to the aqueous phase.

[0309] The processes of the present invention are generally allowed to proceed until further conversion of substrate to product does not change significantly with reaction time (e.g., less than 10% of substrate being converted, or less than 5% of substrate being converted). In some embodiments, the reaction is allowed to proceed until there is complete or near complete conversion of substrate to product.

Transformation of substrate to product can be monitored using known methods by detecting substrate and/or product, with or without derivatization. Suitable analytical methods include gas chromatography, HPLC, MS, and the like. In some embodiments, after suitable conversion to product, the reactants are separated from the oligo acceptor substrate extension product and additional reactants are added to the oligo acceptor substrate extension product to further extend the growing polynucleotide chain. The processes of the present invention may be used to iteratively extend the oligo acceptor extension product until a polynucleotide of a defined sequence and length is synthesized.

[0310] Any of the processes disclosed herein using the engineered polypeptides for the preparation of products can be carried out under a range of suitable reaction conditions, including but not limited to ranges of substrates, temperature, pH, solvent system, substrate loading, polypeptide loading, cofactor loading, and reaction time. In one example, the suitable reaction conditions comprise: (a) oligo acceptor substrate loading of about 0.1 – 5000 μM of substrate compound; (b) NTP-3'-O-RBG substrate or NTP loading of about 1 – 10000 μM of substrate compound; (c) of about 0.01 g/L to 5 g/L engineered polypeptide; (d) 100 to 5000 μM cobalt (II) chloride; (e) 5 to 100 mM triethanolamine buffer; (f) 0.05 to 10 μM pyrophosphatase; (g) pH at 5-9; and (h) temperature of about 15 $^{\circ}\text{C}$ to 70 $^{\circ}\text{C}$. In some embodiments, the suitable reaction conditions comprise: (a) oligo acceptor substrate loading of about 400 μM of substrate compound; (b) NTP-3'-O-RBG or NTP substrate loading of about 800 μM of substrate compound; (c) of about 0.06 g/L engineered polypeptide; (d) 600 μM cobalt (II) chloride; (e) 100 mM triethanolamine buffer; (f) 5 μM pyrophosphatase; (g) pH at 7.8; and (h) temperature of about 50 $^{\circ}\text{C}$. In some embodiments, the enzyme loading is between 1-30% w/w. In some embodiments, additional reaction components or additional techniques carried out to supplement the reaction conditions. These can include taking measures to stabilize or prevent inactivation of the enzyme, reduce product inhibition, shift reaction equilibrium to formation of the desired product.

[0311] In some embodiments, the present disclosure provides an engineered TdT, wherein said engineered TdT has improved activity on NTP-3'-RBGs or modified NTPs, such that NTP-3'-RBGs are incorporated with equivalent efficiency to native NTPs, as compared to another wild-type or engineered TdT. In some embodiments, the engineered TdT with improved activity on dNTP-3'-O- PO_3 , such that dNTP-3'-O- PO_3 is incorporated with equivalent efficiency to native dNTPs, is an engineered TdT polypeptide comprising an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246.

[0312] Methods of Using Engineered TdT's for Template-Independent Oligonucleotide Synthesis

[0313] As described in the above embodiments, modifications of an engineered TdT allow for improved oligonucleotide (with either a phosphodiester or phosphorothioate linkage) and 3'-O-blocked NTP (either with a triphosphate or an alpha-thio triphosphate) acceptance, which enable template-independent oligonucleotide synthesis. While these embodiments have several advantages over phosphoramidite chemistry, a preferred embodiment, described herein, dramatically reduces the amount of solid support and organic solvent used in the method, furthering enabling the production of high volumes of single stranded oligonucleotides necessary for siRNA therapeutics applications.

[0314] Traditionally, methods of oligonucleotide synthesis, including both phosphoramidite chemistry and newer methods of template-independent enzymatic synthesis, have relied upon immobilization of the growing oligonucleotide chain on a support, such as a solid support or a solution-based support. While this method allows addition and purification steps to proceed, a large volume of solid support is required and requires concomitant high volumes of NTPs and other reagents to drive the synthesis reaction. These methods generate substantial waste and are not feasible for the industrial scale production of kilograms of oligonucleotide necessary for siRNA therapeutics.

[0315] In some embodiments, the present disclosure provides a novel method of oligonucleotide synthesis wherein the engineered TdT or a template-independent polymerase is immobilized. Although enzyme immobilization is known in the art, template-independent oligonucleotide synthesis using an immobilized TdT or template-independent polymerase for iterative rounds of nucleotide addition required for oligonucleotide synthesis has not been reported. The described novel method includes various embodiments that overcome process challenges inherent in such a method.

[0316] In some embodiments, the immobilized TdT or template-independent polymerase is an engineered TdT, described above, comprising greater than 60% sequence identity to the even-numbered sequences of SEQ ID NOs: 4-1960, 2004-3920, 4048-5466, and 5476 and one or more substitutions or substitution sets in the amino acid sequence of the engineered TdT, as compared to a reference sequence. In some embodiments, the immobilized TdT or template-independent polymerase is another wild-type or engineered polymerase. Any suitable enzyme having template-independent polymerase activity may be used in these methods.

[0317] In one embodiment, the present disclosure provides a method for template-independent synthesis of an oligonucleotide, the method comprising: (a) providing at least one TdT or template-independent polymerase; (b) providing at least one oligo acceptor substrate, wherein the oligo acceptor substrate comprises a 3'-OH or equivalent; and (c) contacting the oligo acceptor substrate, the TdT or template-independent polymerase, and a nucleotide triphosphate, a modified nucleotide triphosphate, or a NTP-3'-O-RBG under conditions sufficient for the addition of the nucleotide, modified nucleotide, or nucleotide-3'-O-RBG to the 3' end. In some embodiments, step (c) optionally includes contacting the oligo acceptor substrate, the TdT or template-independent polymerase, and a nucleotide triphosphate, a modified nucleotide triphosphate, or NTP-3'-O-RBG with a phosphatase, such as an inorganic pyrophosphatase to

convert pyrophosphate to inorganic phosphate. In another embodiment, the method further comprises (d) deblocking the oligonucleotide formed in step (c) at the protected 3'-O-position of the oligonucleotide product. In some embodiments, the method comprises (e) deactivating unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs. In some embodiments, step (d) deblocking the oligonucleotide formed in step (c) at the protected 3'-O-position of the nucleotide-3'-O-RBG and step (e) deactivating unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs occur simultaneously. In some embodiments, step (d) deblocking the oligonucleotide formed in step (c) at the protected 3'-O-position of the nucleotide-3'-O-RBG and step (e) deactivating unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs occur simultaneously, wherein the NTP-3'-O-RBG comprises a 3' phosphate and a phosphatase is used to deblock the nucleotide-3'-O-RBG while simultaneously deactivating unreacted NTP-3'-O-RBGs by removing the 5' phosphates to leave nucleosides. In some embodiments, the method comprises an optional step (f) of removing excess nucleoside and/or excess inorganic phosphate and/or pyrophosphate from the reaction. In some embodiments, steps (a)-(c) or (a)-(d) or (a)-(e) or (a)-(f) are repeated until a desired oligonucleotide sequence is obtained. In another embodiment, the method further comprises (g) cleaving or releasing the growing or completed oligonucleotide chain from the oligo acceptor substrate.

[0318] In some embodiments of the described method for template-independent synthesis of an oligonucleotide, the oligo acceptor substrate and growing oligonucleotide chain are immobilized on a solid support. In some embodiments of the described method for template-independent synthesis of an oligonucleotide, the TdT or template-independent polymerase, oligo acceptor substrate and growing oligonucleotide chain are all in solution phase. The oligo substrate and growing oligo chain can be optionally substituted with a soluble tag that aids extended oligo product isolation and purification. In some embodiments of the described method for template-independent synthesis of an oligonucleotide, the TdT or template-independent polymerase is immobilized. In some embodiments of the described method for template-independent synthesis of an oligonucleotide, the TdT or template-independent polymerase is simultaneously purified and immobilized on a solid support. In some embodiments, the immobilized TdT or template-independent polymerase is an engineered TdT with greater than 60% sequence identity to SEQ ID NOs: 4-1960, 2004-3920, 4048-5466, and 5476 and one or more substitutions or substitution sets in the amino acid sequence of the engineered TdT. In some embodiments, the immobilized TdT or template-independent polymerase is immobilized on a solid support.

[0319] In some embodiments, the engineered TdT polypeptides can be provided on a solid support, such as a membrane, resin, solid carrier, or other solid phase material. A solid support can be composed of organic polymers such as polystyrene, polyethylene, polypropylene, polyfluoroethylene, polyethyleneoxy, and polyacrylamide, as well as co-polymers and grafts thereof. A solid support can also be inorganic, such as glass, silica, controlled pore glass (CPG), reverse phase silica or metal, such as gold or platinum. The configuration of a solid support can be in the form of beads, spheres, particles, granules, a gel, a membrane or a surface. Surfaces can be planar, substantially planar, or non-planar. Solid supports can be

porous or non-porous, and can have swelling or non-swelling characteristics. A solid support can be configured in the form of a well, depression, or other container, vessel, feature, or location.

[0320] In some embodiments, the engineered TdT polypeptides of the present invention can be immobilized on a solid support such that they retain their improved activity, and/or other improved properties relative to the reference polypeptide of SEQ ID NOs: 2, 8, 16, 24, 36, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192 and/or 5246. In such embodiments, the immobilized polypeptides can facilitate the biocatalytic conversion of the substrate compounds or other suitable substrates to the product and after the reaction is complete are easily retained (e.g., by retaining beads on which polypeptide is immobilized) and then reused or recycled in subsequent reactions. Such immobilized enzyme processes allow for further efficiency and cost reduction.

Accordingly, it is further contemplated that any of the methods of using the TdT polypeptides of the present invention can be carried out using the TdT polypeptides bound or immobilized on a solid support.

[0321] Methods of enzyme immobilization are well-known in the art. The engineered polypeptides can be bound non-covalently or covalently. Various methods for conjugation and immobilization of enzymes to solid supports (e.g., resins, membranes, beads, glass, etc.) are well known in the art (See e.g., Yi et al., *Proc. Biochem.*, 42(5): 895-898 [2007]; Martin et al., *Appl. Microbiol. Biotechnol.*, 76(4): 843-851 [2007]; Koszelewski et al., *J. Mol. Cat. B: Enzymatic*, 63: 39-44 [2010]; Truppo et al., *Org. Proc. Res. Dev.*, published online: [dx.doi.org/10.1021/op200157c](https://doi.org/10.1021/op200157c); Hermanson, *Bioconjugate Techniques*, 2nd ed., Academic Press, Cambridge, MA [2008]; Mateo et al., *Biotechnol. Prog.*, 18(3):629-34 [2002]; and “Bioconjugation Protocols: Strategies and Methods,” *In Methods in Molecular Biology*, Niemeyer (ed.), Humana Press, New York, NY [2004]; the disclosures of each which are incorporated by reference herein). Solid supports useful for immobilizing the engineered TdT of the present invention include but are not limited to beads or resins comprising polymethacrylate with epoxide functional groups, polymethacrylate with amino epoxide functional groups, styrene/DVB copolymer or polymethacrylate with octadecyl functional groups. Exemplary solid supports useful for immobilizing the engineered TdT polypeptides of the present invention include, but are not limited to, EnginZyme (including, EziG-1, EziG-1, and EziG-3), chitosan beads, Eupergit C, and SEPABEADs (Mitsubishi) (including EC-EP, EC-HFA/S, EXA252, EXE119 and EXE120).

[0322] In some embodiments of the described method for template-independent synthesis of an oligonucleotide, the TdT or template-independent polymerase is immobilized, and the method comprises an aqueous liquid phase. In some embodiments, the method for template-independent synthesis of an oligonucleotide comprising an aqueous phase and an immobilized TdT or template-independent polymerase further comprises a column solid support. In some embodiments, the method for template-independent synthesis of an oligonucleotide comprising an aqueous phase and an immobilized TdT or template-independent polymerase further comprises a batch method with a solid support. In some embodiments, the oligo acceptor substrate and/or growing oligonucleotide chain are provided in an

aqueous phase. In some embodiments, the nucleotide triphosphate, the modified nucleotide triphosphate, or the NTP-3'-O-RBG are provided in an aqueous phase. In some embodiments, the method further comprises removing unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs from the oligo acceptor substrate and/or growing oligonucleotide chain. In some embodiments of the described method for template-independent synthesis of an oligonucleotide, the oligo acceptor substrate and oligonucleotide are immobilized. In some embodiments, neither the oligo acceptor substrate nor the TdT of template-independent polymerase are immobilized.

[0323] In some further embodiments, the method for template-independent synthesis of an oligonucleotide comprising an immobilized TdT or template-independent polymerase and an aqueous liquid phase further comprises the steps of (a) providing at least one TdT or template-independent polymerase on a solid support; (b) providing at least one oligo acceptor substrate in an aqueous phase, wherein the oligo acceptor substrate comprises a 3'-OH or equivalent; and (c) contacting the oligo acceptor substrate, the TdT or template-independent polymerase, and a nucleotide triphosphate, a modified nucleotide triphosphate, or NTP-3'-O-RBG under aqueous conditions sufficient for the addition of the nucleotide, modified nucleotide, or nucleotide-3'-O-RBG to the 3' end. In some embodiments, step (c) optionally includes contacting the oligo acceptor substrate, the TdT or template-independent polymerase, and a nucleotide triphosphate, a modified nucleotide triphosphate, or NTP-3'-O-RBG with a phosphatase, such as an inorganic pyrophosphatase to convert pyrophosphate to inorganic phosphate. In another embodiment, the method further comprises (d) deblocking the oligonucleotide formed in step (c) at the protected 3'-O-position of the oligonucleotide product. In another embodiment, the method further comprises (c) deactivating the unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs. In some embodiments, step (d) deblocking the oligonucleotide formed in step (c) at the protected 3'-O-position of the nucleotide-3'-O-RBG and step (e) deactivating unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs occur simultaneously. In some embodiments, step (d) deblocking the oligonucleotide formed in step (c) at the protected 3'-O-position of the nucleotide-3'-O-RBG and step (e) deactivating unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs occur simultaneously, wherein the NTP-3'-O-RBG comprises a 3' phosphate and a phosphatase is used to deblock the nucleotide-3'-O-RBG while simultaneously deactivating unreacted NTP-3'-O-RBGs by removing the 5' phosphates to leave nucleosides. In some embodiments, the method comprises an optional step (f) of removing excess nucleoside and/or excess inorganic phosphate and/or pyrophosphate from the reaction. In some embodiments, steps (a)-(c) or (a)-(d) or (a)-(e) or (a)-(f) are repeated until a desired oligonucleotide sequence is obtained. In another embodiment, the method further comprises (g) cleaving or releasing the growing or completed oligonucleotide chain from the oligo acceptor substrate once a desired oligonucleotide sequence is obtained. In some embodiments, any of steps (a)-(g) are completed on a solid support. In some embodiments the solid support is a column. In some embodiments, any of steps (a)-(g) are completed in an aqueous phase passing over one or a series of in line columns. In some embodiments,

the solid support is used in a batch method. In some embodiments, any of the above-described methods comprise synthesis of an RNA oligonucleotide or a modified RNA oligonucleotide. In some embodiments, any of the above-described methods comprise synthesis of a DNA oligonucleotide. In any of the embodiments described herein, the aqueous phase may comprise an aqueous co-solvent or aqueous co-solvent system, as further described herein.

[0324] In some further embodiments, the method for template-independent synthesis of an oligonucleotide comprising an immobilized TdT or template-independent polymerase and an aqueous liquid phase further comprises the steps of (a) providing at least one TdT on a solid support, wherein said TdT comprises a polypeptide sequence comprising at least 60% identity to any of the even-numbered sequences of SEQ ID NOs: 4-1960, 2004-3920, 4048-5466, and 5476 and at least one substitution or substitution set in said polypeptide sequence as compared to a reference sequence of any of the even-numbered sequences of SEQ ID NOs: 4-1960, 2004-3920, 4048-5466, and 5476; (b) providing at least one oligo acceptor substrate in an aqueous phase, wherein the oligo acceptor substrate comprises a 3'-OH or equivalent; and (c) contacting the oligo acceptor substrate, the TdT or template-independent polymerase, and a nucleotide triphosphate, a modified nucleotide triphosphate, or NTP-3'-O-RBG under aqueous conditions sufficient for the addition of the nucleotide, modified nucleotide, or nucleotide-3'-O-RBG to the 3' end. In some embodiments, step (c) optionally includes contacting the oligo acceptor substrate, the TdT or template-independent polymerase, and a nucleotide triphosphate, a modified nucleotide triphosphate, or NTP-3'-O-RBG with a phosphatase, such as a pyrophosphatase to convert pyrophosphate to inorganic phosphate. In another embodiment, the method further comprises (d) deblocking the oligonucleotide formed in step (c) at the protected 3'-O-position of the oligonucleotide product. In another embodiment, the method further comprises (e) deactivating the unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs. In some embodiments, step (d) deblocking the oligonucleotide formed in step (c) at the protected 3'-O-position of the oligonucleotide product and step (e) deactivating unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs occur simultaneously. In some embodiments, step (d) deblocking the oligonucleotide formed in step (c) at the protected 3'-O-position of the oligonucleotide product and step (e) deactivating unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs occur simultaneously, wherein the NTP-3'-O-RBG comprises a 3' phosphate and a phosphatase is used to deblock the nucleotide-3'-O-RBG while simultaneously deactivating unreacted NTP-3'-O-RBGs by removing the 5' phosphates to leave nucleosides. In some embodiments, the method comprises an optional step (f) of removing excess nucleoside and/or excess inorganic phosphate and/or pyrophosphate from the reaction. In some embodiments, steps (a)-(c) or (a)-(d) or (a)-(e) or (a)-(f) are repeated until a desired oligonucleotide sequence is obtained. In another embodiment, the method further comprises (g) cleaving or releasing the growing or completed oligonucleotide chain from the oligo acceptor substrate once a desired nucleotide sequence is obtained. In some embodiments, any of steps (a)-(g) are completed on a solid support. In some embodiments the solid support is a column. In some embodiments, any of

steps (a)-(g) are completed in an aqueous phase passing over one or a series of in line columns. In some embodiments, the solid support is used in a batch method. In some embodiments, any of the above-described methods comprise synthesis of an RNA oligonucleotide or modified RNA oligonucleotide. In some embodiments, any of the above-described methods comprise synthesis of a DNA oligonucleotide.

[0325] In some further embodiments, the method for template-independent synthesis of an oligonucleotide comprises a nucleotide triphosphate, a modified nucleotide triphosphate, or an NTP-3'-O-RBG. The nucleotide triphosphate may comprise a deoxyribonucleotide triphosphate, a dideoxy ribonucleotide triphosphate, a ribonucleotide triphosphate, or any other modified nucleotide triphosphate, as is known in the art. Modifications may be at the 3' position, as is in the case of NTP-3'-O-RBG, or at the 2' position. Modifications may be at other positions of the sugar or to the base. Modifications may also be present as substitutions of one or more of the phosphate groups of the nucleotide triphosphate and may be incorporated into the phospho backbone of the growing oligonucleotide chain. Although specific examples of suitable modifications are provided herein, any modification to the nucleotide triphosphate may be used in the described methods. Various modifications may confer various desired properties to the oligonucleotide chain. For example, the use of phosphorothiate linkages and 2' modifications in RNA synthesis for RNA therapeutics protects the RNA strand from degradation in the body and extends the half-life of the therapeutic. Various photolabile or cleavable tags may also be present as modifications and may aid in visualization or purification of the oligonucleotide during the synthesis method.

[0326] In some embodiments, the method for template-independent synthesis of an oligonucleotide comprises a nucleotide triphosphate, a modified nucleotide triphosphate, or an NTP-3'-O-RBG comprising a 3' modification. In some embodiments, the 3' modification comprises -NH₂, -NO₂, -(CH₂)₂-CN, or -PO₃. In some embodiments, the 3' modification comprises carbonitriles, phosphates, carbonates, carbamates, esters, ethers, borates, nitrates, sugars, phosphoramidates, phenylsulfonates, and sulfates,

[0327] In some embodiments, the method for template-independent synthesis of an oligonucleotide comprises a nucleotide triphosphate, a modified nucleotide triphosphate, or an NTP-3'-O-RBG comprising a 2' modification. In some embodiments, the 2' modification comprises a 2'-F or 2'-O-alkyl. In some further embodiments, the 2'-F modified nucleotide comprises 2'-fluoro-2'-deoxyadenosine-5'-triphosphate, 2'-fluoro-2'-deoxycytidine-5'-triphosphate, 2'-fluoro-2'-deoxyguanosine-5'-triphosphate, and 2'-fluoro-2'-deoxyuridine-5'-triphosphate. In some further embodiments, the 2'-O-alkyl modified nucleotide comprises 2'-O-methyladenosine-5'-triphosphate, 2'-O-methylcytidine-5'-triphosphate, 2'-O-methylguanosine-5'-triphosphate, 2'-O-methyluridine-5'-triphosphate, and 2'-O-methylinosine-5'-triphosphate. In yet some further embodiments, any of the 2'-F modified nucleotides or 2'-O-alkyl modified nucleotides further comprise a 3'-O-removable blocking group. In yet some further embodiments, any of the 2'-F modified nucleotide triphosphates or 2'-O-alkyl modified nucleotide triphosphates further comprise a 3'-O-phosphate removable blocking group. In some embodiments, the modified nucleotide triphosphate comprises or further comprises a phosphorothioate group at the 5' alpha position.

[0328] In some embodiments, the method for template-independent synthesis of an oligonucleotide comprises optionally contacting the oligo acceptor substrate, the TdT or template-independent polymerase, and a nucleotide triphosphate, a modified nucleotide triphosphate, or NTP-3'-O-RBG with a phosphatase to convert pyrophosphate to inorganic phosphate. In some embodiments, the production of inorganic phosphate from pyrophosphate drives the extension reaction toward the N+1 product. In some embodiments, the phosphatase is an inorganic pyrophosphatase. In some embodiments, the inorganic pyrophosphatase is derived from *Thermocrinis ruber*, *Aquifex pyrophilus*, *Thermus oshimai*, *Sulfolobus sp. A20*, *Geobacillus zalihae*, *Bacillus thermozeamaize*, or *Bacillus smithii*. In some embodiments, the inorganic pyrophosphatase comprises a sequence selected from SEQ ID NOs: 3936, 3938, 3940, 3942, 3944, 3946, or 3948. In some embodiments, the inorganic pyrophosphatase may be immobilized on a solid support.

[0329] In some embodiments, the method for template-independent synthesis of an oligonucleotide comprises a of step deblocking the oligonucleotide at the protected 3'-O-position of the oligonucleotide product and/or a step of deactivating unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs to nucleosides using a phosphatase. In some embodiments, the phosphatase is an alkaline phosphatase. In some embodiments, the alkaline phosphatase is derived from *Pyrococcus furiosus*, *Thermotoga maritima*, *Thermotoga sp. 50_64*, *Pseudothermotoga lettingae*, *Thermotoga neapolitana*, *Thermoflexibacter ruber*, or *Bacillus licheniformis*. In some embodiments, the alkaline phosphatase comprises a sequence selected from SEQ ID NOs: 3922, 3924, 3926, 3928, 3930, 3932, or 3934. In some embodiments, the alkaline phosphatase may be immobilized on a solid support.

[0330] In some embodiments, the method for template-independent synthesis of an oligonucleotide comprises an optional step of removing excess inorganic phosphate or nucleoside from the reaction.

[0331] In some embodiments, the method for template-independent synthesis of an oligonucleotide comprises an optional step of cleaving or releasing the growing or completed oligonucleotide chain from the oligo acceptor substrate. In some embodiments, an exonuclease is used to cleave or release the growing or completed oligonucleotide chain from the oligo acceptor substrate.

[0332] In further embodiments, any of the above-described processes for the conversion of one or more substrate compounds to product compound can further comprise one or more steps selected from: extraction; isolation; purification; and crystallization of product compound. As is known to those skilled in the art, acidic compounds such as oligonucleotides, NTPs, modified NTPs, and NTP-3'-O-RBGs may exist in various salt forms that can be used interchangeably in the methods described herein. All such forms are specifically envisaged for use in the methods described herein. Methods, techniques, and protocols for extracting, isolating, purifying, and/or crystallizing the product from biocatalytic reaction mixtures produced by the above disclosed processes are known to the ordinary artisan and/or accessed through routine experimentation. Additionally, illustrative methods are provided in the Examples below.

[0333] Various features and embodiments of the invention are illustrated in the following representative examples, which are intended to be illustrative, and not limiting.

EXAMPLES

[0334] The following Examples, including experiments and results achieved, are provided for illustrative purposes only and are not to be construed as limiting the present invention. Indeed, there are various suitable sources for many of the reagents and equipment described below. It is not intended that the present invention be limited to any particular source for any reagent or equipment item.

[0335] In the experimental disclosure below, the following abbreviations apply: M (molar); mM (millimolar), μ M and uM (micromolar); nM (nanomolar); mol (moles); gm and g (gram); mg (milligrams); ug and μ g (micrograms); L and l (liter); ml and mL (milliliter); cm (centimeters); mm (millimeters); μ m and μ m (micrometers); sec. (seconds); min(s) (minute(s)); h(s) and hr(s) (hour(s)); U (units); MW (molecular weight); rpm (rotations per minute); psi and PSI (pounds per square inch); °C (degrees Celsius); RT and rt (room temperature); CV (coefficient of variability); CAM and cam (chloramphenicol); PMBS (polymyxin B sulfate); IPTG (isopropyl β -D-l-thiogalactopyranoside); LB (lysogeny broth); TB (terrific broth); SFP (shake flask powder); CDS (coding sequence); DNA (deoxyribonucleic acid); RNA (ribonucleic acid); nt (nucleotide; polynucleotide); aa (amino acid; polypeptide); E. coli W3110 (commonly used laboratory E. coli strain, available from the Coli Genetic Stock Center [CGSC], New Haven, CT); HTP (high throughput); HPLC (high pressure liquid chromatography); HPLC-UV (HPLC-Ultraviolet Visible Detector); ¹H NMR (proton nuclear magnetic resonance spectroscopy); FIOPC (fold improvements over positive control); Sigma and Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO; Difco (Difco Laboratories, BD Diagnostic Systems, Detroit, MI); Microfluidics (Microfluidics, Westwood, MA); Life Technologies (Life Technologies, a part of Fisher Scientific, Waltham, MA); Amresco (Amresco, LLC, Solon, OH); Carbosynth (Carbosynth, Ltd., Berkshire, UK); Varian (Varian Medical Systems, Palo Alto, CA); Agilent (Agilent Technologies, Inc., Santa Clara, CA); Infors (Infors USA Inc., Annapolis Junction, MD); and Thermotron (Thermotron, Inc., Holland, MI).

[0336] Abbreviations for modified nucleotides

"*" : "There is a phosphorothioate bond between these base pairs.",
'5'-6-FAM-T" : "n is a 5'-6-carboxyfluorescein-thymidine",
'ddG' : "n is a 2',3'-dideoxyguanosine",
"(2'dF)A" : "n is a 2'-deoxyfluoroadenosine",
"(2'dF)C" : "n is a 2'-deoxyfluorocytidine",
"(2'dF)G" : "n is a 2'-deoxyfluoroguanosine",
"(2'dF)U" : "n is a 2'-deoxyfluorouridine",
"(2'dF)A-3'P" : "n is a 2'-deoxyfluoroadenosine with a 3'-PO ₄ blocking group",
"(2'dF)C-3'P" : "n is a 2'-deoxyfluorocytidine with a 3'-PO ₄ blocking group",
"(2'dF)G-3'P" : "n is a 2'-deoxyfluoroguanosine with a 3'-PO ₄ blocking group",
"(2'dF)U-3'P" : "n is a 2'-O-methyluridine with a 3'-PO ₄ blocking group",
'mA' : "n is a 2'-O-methyladenosine",

'mC' : "n is a 2'-O-methylcytidine",
'mG' : "n is a 2'-O-methylguanosine",
'mU' : "n is a 2'-O-methyluridine",
"mA-3'P" : "n is a 2'-O-methyladenosine with a 3'-PO ₄ blocking group",
"mC-3'P" : "n is a 2'-O-methylcytidine with a 3'-PO ₄ blocking group",
"mG-3'P" : "n is a 2'-O-methylguanosine with a 3'-PO ₄ blocking group",
"mU-3'P" : "n is a 2'-O-methyluridine with a 3'-PO ₄ blocking group",
'rA' : "n is an adenosine ribonucleotide",
'rG' : "n is a guanosine ribonucleotide ",
'rG' : "n is a guanosine ribonucleotide ",
'rU' : "n is a uridine ribonucleotide"
'(MOE)A' : "n is a 2'-O-(2-methoxyethyl)adenosine",
'(MOE)C' : "n is a 2'-O-(2-methoxyethyl)cytidine",
'(MOE)G' : "n is a 2'-O-(2-methoxyethyl)guanosine",
'(MOE)U' : "n is a 2'-O-(2-methoxyethyl)uridine",
'5'-BiosG-T' : "n is a thymidine with a 5'-biotin modification",
'(iFluorT)' : "n is a thymidine with a 5'-fluroscein modification",

EXAMPLE 1

Terminal deoxynucleotidyl transferase (TdT) gene Acquisition and Construction of Expression Vectors

[0337] The wild-type (WT) terminal deoxynucleotidyl transferase (TdT) enzyme (SEQ ID NO:2) is a predicted splice variant encoded by the genome of species *Monodelphis domestica*. A synthetic gene (SEQ ID NO:1 encoding an N-terminal 6-histidine tagged version of the WT TdT was designed with codon optimization for *E. coli* expression, synthesized, and subcloned into the *E. coli* expression vector pCK100900i (See e.g., US Pat. No. 7,629,157 and US Pat. Appln. Publn. 2016/0244787, both of which are hereby incorporated by reference). This plasmid construct was transformed into an *E. coli* strain derived from W3110. Directed evolution techniques generally known by those skilled in the art were used to generate libraries of gene variants from these plasmids (See e.g., US Pat. No. 8,383,346 and WO 2010/144103, both of which are hereby incorporated by reference). The substitutions in the enzyme variants described herein are indicated with reference to the N-terminal 6-histidine tagged version of the WT TdT enzyme (i.e., SEQ ID NO: 2) or variants thereof, as indicated.

EXAMPLE 2

TdT expression and lysate processing for high throughput (HTP) screening

High Throughput (HTP) Growth of TdT Enzyme and Variants

[0338] Transformed *E. coli* cells were selected by plating onto LB agar plates containing 1% glucose and 30 µg/mL chloramphenicol. After overnight incubation at 37 °C, colonies were placed into the wells of 96-well shallow flat bottom NUNC™ (Thermo-Scientific) plates filled with 180 µl/well LB medium supplemented with 1% glucose and 30 µg/mL chloramphenicol. The cultures were allowed to grow overnight for 18-20 hours in a shaker (200 rpm, 30°C, and 85% relative humidity; Kuhner). Overnight growth samples (20 µL) were transferred into Costar 96-well deep plates filled with 380 µL of Terrific Broth supplemented with 30 µg/mL chloramphenicol. The plates were incubated for 120 minutes in a shaker (250 rpm, 30 C, and 85% relative humidity; Kuhner) until the OD₆₀₀ reached between 0.4-0.8. The cells were then induced with 40 µL of 10 mM IPTG in sterile water and incubated overnight for 18-20 hours in a shaker (250 rpm, 30 C, and 85% relative humidity; Kuhner). The cells were pelleted (4,000 rpm for 20 min), the supernatants were discarded, and the cells were frozen at -80 °C prior to analysis.

Method 1: Lysis of HTP Cell Pellets with Lysozyme (Examples 7-12)

[0339] For lysis, 400 µL lysis buffer containing 50 mM MOPS buffer, pH 7.4, and 0.2 g/L lysozyme were added to the cell pellet in each well. The cells were lysed at room temperature for 2 hours with shaking on a bench top shaker. The plate was then centrifuged for 15 min at 4,000 rpm and 4 °C. The clear supernatants were then used in biocatalytic reactions to determine their activity levels.

Method 2: Thermal lysis of HTP Cell Pellets with Lysozyme (Examples 13-25)

[0340] For lysis, 400 µL lysis buffer containing 50 mM triethanolamine buffer, pH 7.5, and 0.1 g/L lysozyme were added to the cell pellet in each well. The cells were shaken vigorously at room temperature for 5 minutes on a bench top shaker. A 100-µL aliquot of the re-suspended cells was transferred to a 96-well format 200 µL BioRad PCR plate, then briefly spun-down prior to 1 h heat treatment at the temperature indicated, typically 48-60 °C. Following heat-treatment, the cell debris was pelleted by centrifugation (4,000 rpm at 4 °C for 10 min), and clear supernatants were then used in biocatalytic reactions to determine their activity levels.

EXAMPLE 3

Shake Flask Expression and Purification of TdT

Shake Flask Expression

[0341] Selected HTP cultures grown as described above were plated onto LB agar plates with 1% glucose and 30 µg/mL chloramphenicol and grown overnight at 37 °C. A single colony from each culture was transferred to 5 mL of LB broth with 1% glucose and 30 µg/mL chloramphenicol. The cultures were grown for 20 h at 30 °C, 250 rpm, and subcultured at a dilution of approximately 1:50 into 250 mL of Terrific Broth with 30 µg/mL of chloramphenicol, to a final OD₆₀₀ of about 0.05. The cultures were incubated for approximately 195 min at 30 °C, 250 rpm, to an OD₆₀₀ of about 0.6, and then induced with the addition of IPTG at a final concentration of 1 mM. The induced cultures were incubated for 20 h at

30 °C, 250 rpm. Following this incubation period, the cultures were centrifuged at 4,000 rpm for 10 min. The culture supernatant was discarded, and the pellets were resuspended in 35 mL of 20 mM triethanolamine, pH 7.5. This cell suspension was chilled in an ice bath and lysed using a Microfluidizer cell disruptor (Microfluidics M-110L). The crude lysate was pelleted by centrifugation (11,000 rpm for 60 min at 4 °C), and the supernatant was then filtered through a 0.2 µm PES membrane to further clarify the lysate.

Purification of TdT from Shake Flask Lysates

[0342] TdT lysates were supplemented with 1/10th volume of SF elution buffer (50mM Tris-HCl, 500 mM NaCl, 250 mM imidazole, 0.02% v/v Triton X-100 reagent) per well. Lysates were then purified using an AKTA Start purification system and a 5mL HisTrap FF column (GE Healthcare) using the AC Step HiF setting (the run parameters are provided below). The SF wash buffer comprised 50mM Tris-HCl, 300 mM NaCl, 20 mM imidazole, 0.02% v/v Triton X-100 reagent.

Table 3.1 Purification Parameters	
Parameter	Volume
Column volume	5 mL
Flow rate	8 mL/min
Pressure limit	0.3 MPa
Sample volume	35 mL
Equilibration volume	5 column volume (CV) = 25mL
Wash Unbound volume	20 CV = 100 mLs
Elution	Isocratic (step)
Elution volume	5 CV = 25 mLs
Fraction volume	1.5 mLs
RE-equilibration volume	5 CV = 25 mLs

Elution fractions containing protein were identified by UV absorption (A280) and pooled, then dialyzed overnight in dialysis buffer (20 mM Tris-HCl, pH 7.4, 100 mM KCl, 0.1 mM EDTA, and 50% glycerol) in a 3.5K Slide-A-Lyzer™ dialysis cassette (Thermo Fisher) for buffer exchange. TdT concentrations in the preparations were measured by absorption at 280 nm.

EXAMPLE 4

Capillary electrophoresis (CE) analysis of oligonucleotides

Sample preparation for reaction analysis using CE:

[0343] For analysis of the reaction samples, capillary electrophoresis was performed using an ABI 3500xl Genetic Analyzer (ThermoFisher). Reactions (20 µL) were quenched by the addition of 60 µL of 35 mM aqueous EDTA. Reactions (1 µL) were quenched by the addition of 99 µL of 1 mM aqueous EDTA. Quenched reactions were diluted in water to 1.25 nM oligonucleotide, and a 2-µL aliquot of this solution was transferred to a new 96-well MicroAmp Optical PCR plate or 384-well MicroAmp Optical PCR plate containing 18 µL Hi-Di™ Formamide (ThermoFisher) containing an appropriate size standard (LIZ or Alexa633). The ABI3500xl was configured with POP6 polymer, 50 cm capillaries, and a 55 °C

oven temperature. Pre-run settings were 18KV for 50 sec. Injection was 10KV for 2 sec, and the run settings were 19KV for 620 sec. FAM-labeled oligo substrates and products were identified by their sizes relative to the sizing ladder.

List of oligonucleotide substrates and products

[0344] Oligonucleotide used as substrates and detected as products are listed in Table 4.1 below.

Table 4.1. List of substrate and product oligonucleotides	
Structural Description	SEQ ID NO:
5'-6-FAM-T17ATCmC	1961
5'-6-FAM-T17AT*mC	1962
5'-6-FAM-T17ATC(2'dF)C	1963
5'-6-FAM-T12ATCAC*(2'dF)A	1964
5'-6-FAM-T12ATCAC*mC	1965
5'-6-FAM-T12ATCAC*mA	1966
5'-6-FAM-T15AmG*mC	1967
5'-6-FAM-T12TATCAC*mC	1968
5'-6-FAM-T15AmU*mG	1969
5'-6-FAM-T15AmU*mG	1970
5'-6-FAM-T14ATCmC	1971
5'-6-FAM-T15AT*mG	1972
5'-6-FAM-T17mAmUmC	1973
5'-6-FAM-T17mUmUmC	1974
5'-6-FAM-T17mCmUmG	1975
5'-6-FAM-T15AT*mA	1976
5'-6-FAM-T15AT*mC	1977
5'-6-FAM-T15AT*mU	1978
T14ATCmC	1979
5'-6-FAM-T17ATCmCddG	1980
5'-6-FAM-T17AT*mCddG	1981
5'-6-FAM-T17ATC(2'dF)CmA	1982
5'-6-FAM-T17ATC(2'dF)CddG	1983
5'-6-FAM-T12ATCAC*(2'dF)A*rA	1984
5'-6-FAM-T12ATCAC*mC*rG	1985
5'-6-FAM-T12ATCAC*mA*rG	1986
5'-6-FAM-T12ATCAC*mC*rU	1987
5'-6-FAM-T15AmG*mC *rG	1988
5'-6-FAM-T15AmG*mCmG	1989

5'-6-FAM-T12TATCAC*mCmG	1990
5'-6-FAM-T15AmU*mGmA	1991
5'-6-FAM-T14ATCmC*ddG	1992
5'-6-FAM-T15AT*mGmA-3'P	1993
5'-6-FAM-T17mAmUmCmA	1994
5'-6-FAM-T17mUmUmCmA	1995
5'-6-FAM-T17mCmUmGmA	1996
5'-6-FAM-T15AT*mAmA-3'P	1997
5'-6-FAM-T15AT*mGmC-3'P	1998
5'-6-FAM-T15AT*mGmU-3'P	1999
5'-6-FAM-T14ATCmCddG	2000
T14ATCmCddG	2001
5'-6-FAM-T15mUmGmA	2002
5'-6-FAM-T15mAmU*mG	3949
5'-6-FAM-T15mC*mG*mA	3950
5'-6-FAM-T17mGmUmC	3951
5'-6-FAM-T12mAmUmA	3952
5'-6-FAM-T22mAmUmU	3953
5'-6-FAM-T27mAmUmG	3954
5'-6-FAM-T57mUmUmC	3955
5'-6-FAM-T32mAmCmC	3956
5'-6-FAM-T37mAmGmC	3957
5'-6-FAM-T42mAmAmC	3958
5'-6-FAM-T47mGmUmC	3959
5'-6-FAM-T52mCmUmC	3960
5'-6-FAM-T15mAmU(2'dF)G	3961
5'-6-FAM-T15mAmG(MOE)C	3962
5'-6-FAM-T15mGmAmC	3963
5'-6-FAM-T22*(2'dF)A(2'dF)GmA	3964
5'-6-FAM-T22(2'dF)C(2'dF)G(2'dF)A	3965
5'-6-FAM-T27(2'dF)GmA(2'dF)U	3966
5'-6-FAM-T15mGmAmC	3967
5'-6-FAM-T11mCmGmA	3968
5'-6-FAM-T11mC*mA*mG	3969
5'-6-FAM-T15mA(2'dF)UmC	3970
5'-6-FAM-T15mCmUmG	3971
5'-6-FAM-T27(2'dF)C*(2'dF)G*(2'dF)A	3972

5'-6-FAM-T11mU*(2'dF)A*(2'dF)A	3973
5'-6-FAM-T48mG*mA*mC	3974
5'-6-FAM-T15mAmCmU	3975
5'-6-FAM-T17*(2'dF)A*(2'dF)A(2'dF)G	3976
5'-6-FAM-T15mAmU(2'dF)U	3977
5'-6-FAM-T15mAmU(2'dF)C	3978
5'-BiosG-T3(iFluorT)T9mAmUmA	3979
5'-BiosG-T3(iFluorT)T9mAmUmAmA	3980
5'-BiosG-T3(iFluorT)T9mAmUmAmAmG	3981
5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA	3982
5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA(2'dF)A	3983
5'-BiosG-T13mAmUmA	3984
5'-BiosG-T13mAmUmAmA	3985
5'-BiosG-T13mAmUmAmAmG	3986
5'-BiosG-T13mAmUmAmAmGmA	3987
5'-BiosG-T13mAmUmAmAmGmA(2'dF)A	3988
5'-6-FAM-T15AT*mUmA-3'P	3989
5'-6-FAM-T15AmU*mGmA-3'P	3990
5'-6-FAM-T15mUmGmA	3991
5'-6-FAM-T15AmG*mCmA-3'P	3992
5'-6-FAM-T15mAmU*mGmA-3'P	3993
5'-6-FAM-T15mC*mG*mAmA-3'P	3994
5'-6-FAM-T17mGmUmCmA-3'P	3995
5'-6-FAM-T12mAmUmAmA-3'P	3996
5'-6-FAM-T17mAmUmCmA-3'P	3997
5'-6-FAM-T22mAmUmUmA-3'P	3998
5'-6-FAM-T27mAmUmGmA-3'P	3999
5'-6-FAM-T57mUmUmCmA-3'P	4000
5'-6-FAM-T32mAmCmCmA-3'P	4001
5'-6-FAM-T37mAmGmCmA-3'P	4002
5'-6-FAM-T42mAmAmCmA-3'P	4003
5'-6-FAM-T47mGmUmCmA-3'P	4004
5'-6-FAM-T52mCmUmCmA-3'P	4005
5'-6-FAM-T15mAmU(2'dF)GmA-3'P	4006
5'-6-FAM-T15mAmG(MOE)CmA-3'P	4007
5'-6-FAM-T15mGmAmC-3'P	4008
5'-6-FAM-T22*(2'dF)A(2'dF)GmA(2'dF)A-3'P	4009

5'-6-FAM-T22(2'dF)C(2'dF)G(2'dF)A(2'dF)A-3'P	4010
5'-6-FAM-T27(2'dF)GmA(2'dF)U(2'dF)A-3'P	4011
5'-6-FAM-T15mGmA(2'dF)A-3'P	4012
5'-6-FAM-T11mCmA(2'dF)A-3'P	4013
5'-6-FAM-T15mUmGmA-3'P	4014
5'-6-FAM-T11mC*mA*mG(2'dF)A-3'P	4015
5'-6-FAM-T11mC*mA*mGmA-3'P	4016
5'-6-FAM-T15mA(2'dF)UmCmA-3'P	4017
5'-6-FAM-T15mCmUmGmA-3'P	4018
5'-6-FAM-T27(2'dF)C*(2'dF)G*(2'dF)A(2'dF)A-3'P	4019
5'-6-FAM-T11mU*(2'dF)A*(2'dF)A(2'dF)A-3'P	4020
5'-6-FAM-T15mGmA(2'dF)U-3'P	4021
5'-6-FAM-T48mG*mA*mCmA-3'P	4022
5'-6-FAM-T15mAmCmU(2'dF)A-3'P	4023
5'-6-FAM-T15mGmA(2'dF)A-3'P	4024
5'-6-FAM-T15mAmCmU(2'dF)U-3'P	4025
5'-6-FAM-T15mAmCmUmC-3'P	4026
5'-6-FAM-T15mAmCmUmG-3'P	4027
5'-6-FAM-T15mAmCmUmA-3'P	4028
5'-6-FAM-T17*(2'dF)A*(2'dF)A(2'dF)G(2'dF)A-3'P	4029
5'-6-FAM-T15mGmA(2'dF)C-3'P	4030
5'-6-FAM-T15mAmU(2'dF)UmA-3'P	4031
5'-6-FAM-T15mGmA(2'dF)G-3'P	4032
5'-6-FAM-T15mAmCmU*mU-3'P	4033
5'-6-FAM-T15mGmA(2'dF)C*mU-3'P	4034
5'-6-FAM-T15mGmA(2'dF)C*mG-3'P	4035
5'-6-FAM-T15mAmCmU*mG-3'P	4036
5'-6-FAM-T15mAmU(2'dF)CmA-3'P	4037
5'-BiosG-T3(iFluorT)T9mAmUmAmA-3'P	4038
5'-BiosG-T3(iFluorT)T9mAmUmAmAmG-3'P	4039
5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA-3'P	4040
5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA(2'dF)A-3'P	4041
5'-BiosG-T13mAmUmAmA-3'P	4042
5'-BiosG-T13mAmUmAmAmG-3'P	4043
5'-BiosG-T13mAmUmAmAmGmA-3'P	4044
5'-BiosG-T13mAmUmAmAmGmA(2'dF)A-3'P	4045
5'-6-FAM-T15mAmU*mG	4046

S08437572	5475
5'-6-FAM-T15mAmUmCmU	5477
T15mAmUmCmU	5478
5'-6-FAM-T11mAmAmA(2'dF)U	5479
5'-6-FAM-T18mA(2'dF)GmA(2'dF)G	5480
5'-6-FAM-T11mCmCmU(2'dF)A	5481
5'-6-FAM-T16*(2'dF)C*mA(2'dF)A(2'dF)A	5482
5'-6-FAM-T32mAmAmA(2'dF)G	5483
5'-6-FAM-T17*mA*mGmA	5484
5'-6-FAM-T26mU(2'dF)GmU(2'dF)C	5485
5'-6-FAM-T31mU(2'dF)CmA(2'dF)U	5486
5'-6-FAM-T21mU(2'dF)CmU(2'dF)C	5487
5'-6-FAM-T47*mA(2'dF)A(2'dF)A	5488
5'-6-FAM-T37mG*mA*mC	5489
5'-6-FAM-T46mA(2'dF)GmU(2'dF)G	5490
5'-6-FAM-T41mA(2'dF)U(2'dF)C(2'dF)C	5491
5'-6-FAM-T31mAmAmA(2'dF)G	5492
5'-6-FAM-T57mUmU(2'dF)C	5493
5'-6-FAM-T42*mA*mCmU	5494
5'-6-FAM-T51mU*(2'dF)A*(2'dF)A(2'dF)G	5495
5'-6-FAM-T12mC*mA*mG	5496
5'-6-FAM-T22*mGmAmA	5497
5'-6-FAM-T27mAmAmA	5498
5'-6-FAM-T47*mCmUmU	5499
5'-6-FAM-T52mUmUmU	5500
5'-6-FAM-T16mC*mA*mGmA	5501
5'-6-FAM-T11AmC*mA*mG	5502
5'-6-FAM-T21*mA*mGmAmA	5503
5'-6-FAM-T26*mGmAmAmA	5504
5'-6-FAM-T36mAmA(2'dF)GmA	5505
5'-6-FAM-T41(2'dF)GmA(2'dF)GmU	5506
5'-6-FAM-T51(2'dF)GmU(2'dF)GmU	5507
5'-6-FAM-T56(2'dF)GmU(2'dF)CmU	5508
5'-6-FAM-T10(2'dF)CmU(2'dF)CmA	n/a
5'-6-FAM-T16mA(2'dF)GmA(2'dF)G	5509
5'-6-FAM-T31mU(2'dF)CmAmU	5510
5'-6-FAM-T36(2'dF)CmAmUmC	5511

5'-6-FAM-T41mAmUmCmU	5512
5'-6-FAM-T46mUmCmUmU	5513
5'-6-FAM-T51mCmUmUmA	5514
5'-6-FAM-T10mAmAmA(MOE)A	n/a
5'-6-FAM-T12mAmG(MOE)C	5515
5'-6-FAM-T26mCmCmU*mU	5516
5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA	5517
5'-6-FAM-T15mAmU*mG(2'dF)A-3'P	5518
5'-6-FAM-T15mAmUmCmU(2'dF)A-3'P	5519
T15mAmUmCmU(2'dF)A-3'P	5520
5'-6-FAM-T17*(2'dF)A*(2'dF)A(2'dF)GmA-3'P	5521
5'-6-FAM-T11mC*mA*mG*mU-3'P	5522
5'-6-FAM-T11mU*(2'dF)A*(2'dF)AmA-3'P	5523
5'-6-FAM-T11mAmAmA(2'dF)U(2'dF)U-3'P	5524
5'-6-FAM-T18mA(2'dF)GmA(2'dF)GmA-3'P	5525
5'-6-FAM-T11mCmCmU(2'dF)A(2'dF)U-3'P	5526
5'-6-FAM-T16*(2'dF)C*mA(2'dF)A(2'dF)AmU-3'P	5527
5'-6-FAM-T32mAmAmA(2'dF)GmA-3'P	5528
5'-6-FAM-T17*mA*mGmA*mU-3'P	5529
5'-6-FAM-T18mA(2'dF)GmA(2'dF)G(2'dF)U-3'P	5530
5'-6-FAM-T26mU(2'dF)GmU(2'dF)CmA-3'P	5531
5'-6-FAM-T31mU(2'dF)CmA(2'dF)U(2'dF)U-3'P	5532
5'-6-FAM-T21mU(2'dF)CmU(2'dF)CmU-3'P	5533
5'-6-FAM-T47*mA(2'dF)A(2'dF)AmA-3'P	5534
5'-6-FAM-T37mG*mA*mC*mU-3'P	5535
5'-6-FAM-T27(2'dF)GmA(2'dF)UmA-3'P	5536
5'-6-FAM-T26mU(2'dF)GmU(2'dF)C(2'dF)U-3'P	5537
5'-6-FAM-T46mA(2'dF)GmU(2'dF)GmA-3'P	5538
5'-6-FAM-T41mA(2'dF)U(2'dF)C(2'dF)C(2'dF)U-3'P	5539
5'-6-FAM-T31mAmAmA(2'dF)GmU-3'P	5540
5'-6-FAM-T57mUmU(2'dF)CmA-3'P	5541
5'-6-FAM-T42*mA*mCmU*mU-3'P	5542
5'-6-FAM-T47*mA(2'dF)A(2'dF)A(2'dF)U-3'P	5543
5'-6-FAM-T51mU*(2'dF)A*(2'dF)A(2'dF)GmA-3'P	5544
5'-6-FAM-T51mU*(2'dF)A*(2'dF)A(2'dF)G(2'dF)U-3'P	5545
5'-6-FAM-T46mA(2'dF)GmU(2'dF)GmU-3'P	5546
5'-6-FAM-T11mC*mA*mGmC-3'P	5547

5'-6-FAM-T11mC*mA*mG*mG-3'P	5548
5'-6-FAM-T12mC*mA*mG*mG-3'P	5549
5'-6-FAM-T57mUmU(2'dF)CmC-3'P	5550
5'-6-FAM-T57mUmU(2'dF)C*mG-3'P	5551
5'-6-FAM-T17*mA*mGmA*3'P	5552
5'-6-FAM-T17*mA*mGmA*mG-3'P	5553
5'-6-FAM-T22*mGmA*3'P	5554
5'-6-FAM-T22*mGmA*mG-3'P	5555
5'-6-FAM-T27mAmAmA*3'P	5556
5'-6-FAM-T27mAmAmA*mG-3'P	5557
5'-6-FAM-T32mAmAmA(2'dF)GmC-3'P	5558
5'-6-FAM-T32mAmAmA(2'dF)G*mG-3'P	5559
5'-6-FAM-T37mG*mA*mCmC-3'P	5560
5'-6-FAM-T37mG*mA*mC*mG-3'P	5561
5'-6-FAM-T42*mA*mCmUmC-3'P	5562
5'-6-FAM-T42*mA*mCmU*mG-3'P	5563
5'-6-FAM-T47*mCmUmUmC-3'P	5564
5'-6-FAM-T47*mCmUmU*mG-3'P	5565
5'-6-FAM-T52mUmUmUmC-3'P	5566
5'-6-FAM-T52mUmUmU*mG-3'P	5567
5'-6-FAM-T31mAmAmA(2'dF)GmA-3'P	5568
5'-6-FAM-T16mC*mA*mGmA-3'P	5569
5'-6-FAM-T11AmC*mA*mGmA-3'P	5570
5'-6-FAM-T21*mA*mGmA*3'P	5571
5'-6-FAM-T26*mGmA*3'P	5572
5'-6-FAM-T36mAmA(2'dF)GmA-3'P	5573
5'-6-FAM-T41(2'dF)GmA(2'dF)GmUmA-3'P	5574
5'-6-FAM-T51(2'dF)GmU(2'dF)GmUmU-3'P	5575
5'-6-FAM-T56(2'dF)GmU(2'dF)CmUmU-3'P	5576
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmU-3'P	n/a
5'-6-FAM-T16mA(2'dF)GmA(2'dF)GmU-3'P	5577
5'-6-FAM-T26mU(2'dF)GmU(2'dF)CmU-3'P	5578
5'-6-FAM-T31mU(2'dF)CmAmUmU-3'P	5579
5'-6-FAM-T36(2'dF)CmAmUmCmU-3'P	5580
5'-6-FAM-T41mAmUmCmU(2'dF)A-3'P	5581
5'-6-FAM-T46mUmCmUmU(2'dF)A-3'P	5582
5'-6-FAM-T51mCmUmUmA(2'dF)A-3'P	5583

5'-6-FAM-T10mAmAmA(MOE)A(2'dF)A-3'P	n/a
5'-6-FAM-T12mAmG(MOE)C(2'dF)A-3'P	5584
5'-6-FAM-T26mCmCmU*mUmC-3'P	5585
5'-6-FAM-T26mCmCmU*mU*mG-3'P	5586
5'-6-FAM-T26mCmCmU*mU*mU-3'P	5587
5'-6-FAM-T26mCmCmU*mU(2'dF)G-3'P	5588
5'-6-FAM-T26mCmCmU*mU(2'dF)U-3'P	5589
5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmAmC-3'P	5590
5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA*mG-3'P	5591
5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA*mU-3'P	5592
5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA(2'dF)G-3'P	5593
5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA(2'dF)U-3'P	5594
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA-3'P	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)G-3'P	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)G	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU-3'P	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)G-3'P	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)G	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU-3'P	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU(2'dF)C-3'P	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU(2'dF)C	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU(2'dF)CmU-3'P	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU(2'dF)CmU	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU(2'dF)CmU(2'dF)C-3'P	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU(2'dF)CmU(2'dF)C	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU*mG-3'P	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU*mG	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU(2'dF)C*mG-3'P	n/a
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU(2'dF)C*mG	n/a
5'-6-FAM-T15mGmAmC(2'dF)C-3'P	5595
5'-6-FAM-T15mGmAmC(2'dF)C	5596
5'-6-FAM-T15mGmAmC(2'dF)CmU-3'P	5597
5'-6-FAM-T15mGmAmC(2'dF)CmU	5598
5'-6-FAM-T15mGmAmC(2'dF)CmU(2'dF)C-3'P	5599

5'-6-FAM-T15mGmAmC(2'dF)CmU(2'dF)C	5600
5'-6-FAM-T15mGmAmC(2'dF)CmU(2'dF)CmA-3'P	5601
5'-6-FAM-T15mGmAmC(2'dF)CmU(2'dF)CmA	5602
5'-6-FAM-T15mGmAmC(2'dF)CmU(2'dF)CmAmU-3'P	5603
5'-6-FAM-T15mGmAmC(2'dF)CmU(2'dF)CmAmU	5604
5'-6-FAM-T15mGmAmCmG	5605
5'-6-FAM-T15mAmU(2'dF)GmA	5606
5'-6-FAM-T21mU(2'dF)CmU(2'dF)CmA-3'P	5607
5'-6-FAM-T21mU(2'dF)CmU(2'dF)CmA	5608
A4mC*mA*mG	n/a
A4mC*mA*mGmA-3'P	n/a
A4mC*mA*mGmA	n/a
5'-6-FAM-T15mAmG*mGmA-3'P	5609
5'-6-FAM-T15mAmG*mGmA	5610
T15mAmG*mGmA-3'P	5611
T15mAmG*mGmA	5612
5'-6-FAM-T17mAmCmCmA-3'P	5613
5'-6-FAM-T17mAmCmCmA	5614
T15mAmU(2'dF)G	5615

EXAMPLE 5**Isolated protein yield improvements over SEQ ID NO: 2**

Relative isolated protein yield of shake-flask purified TdT variants

[0345] TdT variants of SEQ ID NO: 2-38 were produced in shake flask and purified as described in

Example 3. TdT concentrations were measured by absorption at 280 nm.

[0346] Protein recovery relative to SEQ ID NO: 2 was calculated as the ratio of mg/mL protein recovered after purification of the variant compared with SEQ ID NO: 2. The results are shown in Table 5.1.

Table 5.1		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2)	Protein recovery after purification Relative to SEQ ID NO: 2
3/4	N80D/C121S/I174L/F185L/K190E/D244V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/C391G/I394R/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E	+++
5/6	N80D/C121S/I174L/F185L/K190E/D244V/L273V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/C391G/I394R/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E	+++

7/8	N80D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/C391G/S470T/Q474M/K499L/I522L/Q523E	+++
9/10	N80D/C121S/I174L/F185L/K190E/E193G/E196Y/D244V/L273V/F284L/N288E/K289D/I290V/D293S/K297R/I313A/C315G/S317T/V324I/L336D/T342E/E352P/F359L/Q376H/N380D/C391G/I394R/L401G/Q415S/L419A/E428V/R431S/M435T/E441M/Y462F/S470T/Q474M/K499L/I522L/Q523E	+++
11/12	N80D/C121S/F185L/K190E/K289D/I290V/D293S/I313A/C315G/L336D/T342E/F359L/C391G/F414H/S470T/Q474M/K499L/I522L/Q523E	+++
13/14	N80D/C121S/I174L/F185L/K190E/D244V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/L419A/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E	+++
15/16	N80D/C121S/I174L/F185L/K190E/D244V/L273V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/L419A/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E	+++
17/18	N80D/C121S/I174L/F185L/K190E/E193G/D244V/L273V/F284L/N288E/K289D/I290V/D293S/K297R/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/Q415S/L419A/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E	+++
19/20	N80D/C121S/I174L/Q179K/F185L/K190E/G236P/D244V/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/S363I/C391G/I394R/I408P/H426P/Y462F/S470T/Q474M/K499L/I522L/Q523E	++
21/22	N80D/C121S/I174L/F185L/K190E/D244V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E	++
23/24	N80D/C121S/I174L/F185L/K190E/E196R/D244V/T266K/L273V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/V324I/L336D/T342E/E352P/F359L/C391G/I394R/T397R/L401G/L419A/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E	++
25/26	N80D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/K300R/I313A/C315G/S317T/L336D/T342E/F359L/C391G/S470T/Q474M/K499L/I522L/Q523E	++
27/28	N80D/C121S/F185L/K190E/D244V/L273V/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/L419A/M435T/S470T/Q474M/K499L/I522L/Q523E	++
29/30	N80D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/N380D/C391G/L401G/L419A/S470T/Q474M/K499L/I522L/Q523E	++
31/32	N80D/C121S/I174L/F185L/K190E/G236P/D244V/L273V/M282R/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/E395R/L419A/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E	++

33/34	N80D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/C391G/E395W/S470T/Q474M/K499L/I522L/Q523E	++
35/36	N80D/C121S/F185L/K190E/K289D/I290V/D293S/I313A/T342E/K499L	++
37/38	N80D/C121S/F185L/K190E/E196R/D244V/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/C391G/S470T/Q474M/K499L/I522L/Q523E	++
39/40	N80D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/F359L/C391G/D392R/S470T/Q474M/K499L/I522L/Q523E	++
41/42	N80D/C121S/I174L/F185L/E186G/K190E/G236P/D244V/L273V/F284L/N288E/K289D/I290V/D293S/I313A/C315G/S317T/L336D/T342E/E352P/F359L/C391G/I394R/E395R/L419A/E428V/R431S/Y462F/S470T/Q474M/K499L/I522L/Q523E	++
43/44	N80D/C121S/K190E/K289D/I290V	+
45/46	N80D/K106D/C121S/F185L/K190E/M205R/K289D/I290V/D293S/I313A/T342E/S470T/Q474M/K499L/Q523E	+
47/48	N80D/C121S/F185L/K190E/K289D/I290V/D293S/I313A/L336D/T342E/F359L/C391G/S470T/Q474M/K499L/I522L/Q523E	+
49/50	C121S/F185L/K190E/C213S/K289D/I290V/D293S	+
51/52	N80D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/I313A/L336D/T342E/F359L/C391G/F414H/S470T/Q474M/K499L/I522L/Q523E	+
53/54	N80D/C121S/F185L/C315G	+
55/56	N80D/C121S/K131E/F185L/K190E/K289D/I290V/D293S/I313A/T342E/S470T/Q474M/K499L/I522L/Q523E	+
57/58	N80D/F185L/G236P/K289D/D293S	+
59/60	N80D/K106D/C121S/F185L/K190E/M205R/K289D/I290V/D293S/I313A/C315G/L336D/T342E/F359L/C391G/S470T/Q474M/K499L/I522L/Q523E	+
61/62	N80D/C121S/K131E/F185L/K190E/M205R/D244V/K289D/I290V/D293S/I313A/C315G/L336D/T342E/F359L/C391G/F414H/S470T/Q474M/K499L/I522L/Q523E	+
63/64	N80D/C121S/K131E/F185L/K190E/M205R/D244V/K289D/I290V/D293S/I313A/L336D/T342E/F359L/C391G/F414H/S470T/Q474M/K499L/I522L/Q523E	+
65/66	F185L/K289D/I290V/D293S	+
67/68	N80D/K106D/C121S/F185L/K190E/D244V/K289D/I290V/D293S/C307K/T342E/F359L/S470T/Q474M/K499L	+
69/70	N80D/C121S/F185L/K190E/C201R/K289D/I290V/D293S/I313A/T342E/S470T/Q474M/K499L/I522L	+
Levels of increased protein recovery relative to the reference polypeptide of SEQ ID NO: 2 and defined as follows: “+” 1.00 to 3.20-fold increased activity; “++” > 3.20-fold increased activity; “+++” > 6.50-fold increased activity.		

EXAMPLE 6

Thermal stability improvements over SEQ ID NO: 36Relative stability measurements of shake-flask purified TdT variants

[0347] TdT variants SEQ ID NO: 4, 8, 16, 36, and 48 were produced in shake flask and purified as described in Example 3. TdT concentrations were measured by absorption at 280 nm.

[0348] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1 μ M oligonucleotide, 25 μ M nucleotide triphosphate, 1 μ M TdT, 20 mM triethanolamine (pH 7.8), and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for NTP, were pre-mixed in a single solution, and 20 μ L of this solution was aliquoted into each well of the 96-well plate; (ii) the plate was heated at 39.8 °C or 63.1 °C as indicated; (iii) 15 μ L of the heat-treated solution was transferred into a 96-well plate containing 5 μ L of NTP solution (4x concentration in water); (iii) the solution was mixed well, spun down, and reacted at 45 °C for 15 minutes. Reaction plates were heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature, then held at 4 °C until the reaction was quenched. Reactions were quenched and diluted for analytical analysis by CE as described in Example 4.

Table 6.1 – Reaction conditions

Reaction buffer-20 mM TEA, 0.25 mM CoCl₂, pH 7.8; Preincubation Temp (C) - 39.8 °C or 63.1 °C; Preincubation Time (min)- 15; Reaction temperature (C)- 45 °C; Reaction Time (min)- 15; Reaction volume (μ L) - 20; TdT concentration (μ M)- 1; NTP- ddGTP; NTP conc (μ M)-1; Oligonucleotide-5'-6-FAM-T14ATCmC; Oligonucleotide conc (μ M)- 1; Product- 5'-6-FAM-T14ATCmCddG.

[0349] Relative activity for each variant was calculated as the percent product of the variant as measured after a 39.8 °C pre-incubation relative to the percent product measured after pre-incubation at 63.1 °C multiplied by 100. The results are shown in Table 6.2.

Table 6.2 (relative % activity)

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 36)	Stability FIOP percent activity at 63.1 vs. activity at 39.8 Relative to SEQ ID NO: 36
15/16	I174L/D244V/L273V/F284L/N288E/C315G/S317T/L336D/E352P/F359L/C391G/I394R/L419A/E428V/R431S/Y462F/S470T/Q474M/I522L/Q523E	++
3/4	I174L/D244V/F284L/N288E/C315G/S317T/L336D/F359L/C391G/I394R/E428V/R431S/Y462F/S470T/Q474M/I522L/Q523E	++
47/48	L336D/F359L/C391G/S470T/Q474M/I522L/Q523E	+
7/8	D244V/C315G/S317T/L336D/F359L/C391G/S470T/Q474M/I522L/Q523E	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 36 and defined as follows: “+” 0.50 to 6.05-fold increased activity; “++” > 6.05-fold increased activity; “+++” > 15-fold increased activity.		

EXAMPLE 7**Improvements over SEQ ID NO: 8 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0350] SEQ ID NO: 8 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 7.1.

[0351] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 7.1-Reaction conditions

Lysis buffer-MOPS (50 mM, pH 7.4), 0.2 g/L lysozyme; Reaction buffer-20 mM MOPS, 50 mM KOAc, 0.25 mM CoCl₂, pH 7.2; Lysate concentration (vol%)-25; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-15; Reaction temperature (°C)-50 °C; Reaction Time (min)-90; Reaction volume (μ L) -20; NTP (μ M)-ddGTP; NTP conc (μ M)-25; Oligonucleotide-5'-6-FAM-T17ATCmC; Substrate conc (μ M)-1; Product -5'-6-FAM-T17ATCmCddG.

[0352] Activity relative to SEQ ID NO: 8 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 8 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 7.2.

Table 7.2

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 8)	FIOP Product Peak Area Relative to SEQ ID NO: 8
33/34	E395W	+++
39/40	D392R	+++
71/72	E395T	+++
25/26	K300R	+++
73/74	E395R	+++
37/38	E196R	+++
75/76	E395Y	+++
77/78	E395S	+++
79/80	V456R	+++

81/82	L42II	+++
83/84	D392C	+++
85/86	Q415S	+++
87/88	I343V	+++
89/90	E196G	+++
91/92	K297T	+++
93/94	K318E	++
95/96	R481V	++
97/98	R477T	++
99/100	K300P	++
101/102	K195W	++
103/104	V456K	++
105/106	L421M	++
107/108	V316C	++
109/110	R281A	++
111/112	E186A	++
113/114	L411R	++
115/116	C417G	++
117/118	L360V	++
119/120	K303M	++
121/122	V316I	++
123/124	K297Q	++
125/126	K195R	++
127/128	L360C	++
129/130	K318S	++
131/132	D183R	++
133/134	M282R	++
135/136	E186T	+
137/138	K303E	+
139/140	E193V	+
141/142	L454V	+
143/144	Q415A	+
145/146	D392A	+
147/148	E186L	+
149/150	T397R	+
151/152	D129G/E196G	+
153/154	K318T	+
155/156	E395L	+
157/158	K318V	+
159/160	D492T	+
161/162	E193N	+
163/164	E186G	+
165/166	T173L	+
167/168	E193G	+
169/170	C417V	+
171/172	E395A	+
173/174	L411A	+

175/176	E196A	+
177/178	E196W	+
179/180	L411G	+
181/182	E320N	+
183/184	E196Y	+
185/186	E193C	+
187/188	V268C	+
189/190	K297R	+
191/192	R481E	+
193/194	V324I	+
195/196	K303A	+
197/198	K297F	+
199/200	V316T	+
201/202	T266K	+
203/204	K263R	+
205/206	M282Q	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 8 and defined as follows: "+" 1.01 to 1.50-fold increased activity; "++" > 1.50-fold increased activity; "+++" > 2.25-fold increased activity.		

EXAMPLE 8

Improvements over SEQ ID NO: 16 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0353] SEQ ID NO: 16 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and reactions prepared as described in Table 8.1.

[0354] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 8.1-Reaction conditions
Lysis buffer-MOPS (50 mM, pH 7.4), 0.2 g/L lysozyme; Reaction buffer-20 mM MOPS, 50 mM KOAc, 0.25 mM CoCl ₂ , pH 7.2; Lysate concentration (vol%)-12.5; Preincubation Temp (C)-50 °C; Preincubation Time (min)-15; Reaction temperature (C)-50 °C; Reaction Time (min)-15; Reaction

volume (μL) -20; NTP (μM)-ddGTP; NTP conc (μM)-25; Oligonucleotide-5'-6-FAM-T17AT*mC;
Substrate conc (μM)-1; Product -5'-6-FAM-T17AT*mCddG.

[0355] Activity relative to SEQ ID NO: 16 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 16 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate).

The results are shown in Table 8.2.

Table 8.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 16)	FIOP Product Peak Area Relative to SEQ ID NO: 16
9/10	E193G/E196Y/K297R/V324I/Q376H/N380D/L401G/Q415S/M435T/E441M	+++
17/18	E193G/K297R/Q415S	+++
41/42	E186G/G236P/E395R	+++
23/24	E196R/T266K/V324I/T397R/L401G	+++
31/32	G236P/M282R/E395R	+++
207/208	Q415S	+++
209/210	K297R/N380D/L401G/E441M	++
211/212	E193N/E196Y/V324I	++
213/214	G236P/M282R	++
215/216	Q376H/L401G/E441M	++
217/218	M435T/E441M	++
219/220	E186G	++
221/222	E196Y/K297R/V324I/M435T	++
223/224	K297R/M435T	++
225/226	E193G/K297R/V324I/Q376H/M435T	++
227/228	T266K/K297R/N380D/T397R/L401G	++
229/230	K318S/E395R	+
231/232	E193G/E196Y	+
233/234	E193G/M435T	+
235/236	E186G/G236P/K318S	+
237/238	E186G/M282R/K318S	+
239/240	E193G/E196Y/T266K/V324I/Q376H/N380D	+
241/242	M282R/K318S	+
243/244	E193G/E196R/K297R	+
245/246	E193G/E196Y/Q376H/N380D	+
247/248	M282R/R481E	+
249/250	G236P/K318S/R481E	+
251/252	M282R	+
253/254	E193G/E196Y/V324I/T397R/L401G/E441M	+
255/256	E196R/T266K	+
257/258	E196R	+
259/260	E193N/K297R/V324I/N380D	+

Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 16 and defined as follows: “+” 1.24 to 1.57-fold increased activity; “++” > 1.57-fold increased activity; “+++” > 2.34-fold increased activity.

EXAMPLE 9

Improvements over SEQ ID NO: 24 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0356] SEQ ID NO: 24 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 9.1.

[0357] Reactions were performed in 96-well format 200 µL BioRad PCR plates. Reactions included 1-10 µM oligonucleotide, 25-50 µM nucleotide triphosphate, 20 mM buffer, and 250 µM cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 9.1-Reaction conditions
Lysis buffer-MOPS (50 mM, pH 7.4), 0.2 g/L lysozyme; Reaction buffer-20 mM MOPS, 50 mM KOAc, 0.25 mM CoCl ₂ , pH 7.2; Lysate concentration (vol%)-12.5; Preincubation Temp (°C)-47 °C; Preincubation Time (min)-15; Reaction temperature (°C)-47 °C; Reaction Time (min)-30; Reaction volume (µL) -20; NTP (µM)-mATP; NTP conc (µM)-25; Oligonucleotide-5'-6-FAM-T17ATC(2'dF)C; Substrate conc (µM)-1; Product -5'-6-FAM-T17ATC(2'dF)CmA.

[0358] Activity relative to SEQ ID NO: 24 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 24 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 8.2.

Table 9.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 24)	FIOP Product Peak Area Relative to SEQ ID NO: 24
261/262	D21E	+++
263/264	G92R	+++
265/266	V124E	+++
267/268	K53E	+++
269/270	K24G	+++

271/272	T101E	+++
273/274	I155E	+++
275/276	I34S	+++
277/278	I34D	+++
279/280	L153E	+++
281/282	T58S	+++
283/284	T108K	+++
285/286	D142M	+++
287/288	G92Y	+++
289/290	K161E	+++
291/292	M37F	+++
293/294	M37A	+++
295/296	A150E	+++
297/298	T107R	+++
299/300	T162E	+++
301/302	V141E	+++
303/304	L156D	+++
305/306	K161S	+++
307/308	L156E/K294T	+++
309/310	H33K	++
311/312	M30G	++
313/314	T58A	++
315/316	M104I	++
317/318	I155T	++
319/320	K161D	++
321/322	L156Q	++
323/324	K106S	++
325/326	M12S	++
327/328	L156M	++
329/330	M104P	++
331/332	R14G	++
333/334	T152R	++
335/336	L153Q	++
337/338	M37T	++
339/340	N145E	++
341/342	D142S	++
343/344	S20T	++
345/346	R14Q	++
347/348	D94E	++
349/350	T160G	++
351/352	H13S	++
353/354	G92M	++
355/356	L153K	++
357/358	S35E	++
359/360	L153G	++
361/362	L153P	++
363/364	T159D	++

365/366	M37G	++
367/368	G92V	++
369/370	I18D	++
371/372	K106H	++
373/374	V141R	++
375/376	M37V	++
377/378	K133G	++
379/380	D31S	+
381/382	M30V	+
383/384	I34K	+
385/386	M30E	+
387/388	T17A	+
389/390	D147L	+
391/392	T152G	+
393/394	I41V	+
395/396	H13R	+
397/398	T149R	+
399/400	M137A	+
401/402	I34R	+
403/404	L153V	+
405/406	T61H	+
407/408	K29P	+
409/410	K103M	+
411/412	A97D	+
413/414	M104G	+
415/416	H13K	+
417/418	L153M	+
419/420	H33P	+
421/422	T108D	+
423/424	S139A	+
425/426	T17G	+
427/428	M37S	+
429/430	I18R	+
431/432	G23P	+
433/434	T107W	+
435/436	K126V	+
437/438	F22G	+
439/440	H33G	+
441/442	G92S	+
443/444	Q135R	+
445/446	I163V	+
447/448	K29R	+
449/450	S20H	+
451/452	K161R	+
453/454	D94R	+
455/456	S35G	+
457/458	R140E	+

459/460	A57H	+
461/462	T61L	+
463/464	K106G	+
465/466	F22L	+
467/468	Q27D	+
469/470	V124I	+
471/472	K161G	+
473/474	E138Q	+
475/476	G23E	+
477/478	V141M	+
479/480	H102L	+
481/482	E105N	+
483/484	A144C	+
485/486	A150G	+
487/488	R140G	+
489/490	R26G	+
491/492	I163L	+
493/494	N154G	+
495/496	H13G	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 24 and defined as follows: “+” > 1.00-fold increased activity; “++” > 1.30-fold increased activity; “+++” > 1.75-fold increased activity.		

EXAMPLE 10

Improvements over SEQ ID NO: 24 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0359] SEQ ID NO: 24 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 10.1.

[0360] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 10.1-Reaction conditions

Lysis buffer-MOPS (50 mM, pH 7.4), 0.2 g/L lysozyme; Reaction buffer-20 mM MOPS, 50 mM KOAc, 0.25 mM CoCl₂, pH 7.2; Lysate concentration (vol%)-25; Preincubation Temp (C)-58 °C; Preincubation Time (min)-15; Reaction temperature (C)-58 °C; Reaction Time (min)-30; Reaction volume (μL) -20; NTP (μM)-ddGTP; NTP conc (μM)-25; Oligonucleotide-5'-6-FAM-T17ATC(2'dF)C; Substrate conc (μM)-1; Product -5'-6-FAM-T17ATC(2'dF)CddG.

[0361] Activity relative to SEQ ID NO: 24 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 24 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 10.2.

Table 10.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 24)	FIOP Product Peak Area Relative to SEQ ID NO: 24
271/272	T101E	+++
265/266	V124E	+++
497/498	V124G	+++
499/500	T101V	+++
501/502	H7Y/Q135C	+++
361/362	L153P	+++
503/504	K29I	+++
505/506	R16V	+++
507/508	Q109M	+++
509/510	I85V	+++
511/512	K24A	+++
513/514	K131W	+++
515/516	Q135E	+++
517/518	F110V	+++
519/520	R14Y	+++
521/522	S35W	+++
523/524	T101G	+++
525/526	K123M	+++
527/528	F110M	++
529/530	V124M	++
531/532	G23C	++
533/534	K24M	++
535/536	R14N	++
537/538	H44S	++
539/540	H13E	++
541/542	D65S	++
543/544	T108M	++
545/546	K123Q	++
547/548	H33C	++
549/550	A57T	++
551/552	T130M	++

553/554	K133M	++
555/556	D94N	++
475/476	G23E	++
557/558	G23T	++
559/560	E105W	++
561/562	G132S	++
563/564	E77C	++
565/566	V124S	++
567/568	E77S	++
569/570	Q135K	++
571/572	S35H	++
409/410	K103M	++
303/304	L156D	++
573/574	Y134M	++
575/576	Q135H	++
577/578	M137E	++
579/580	N32E	+
581/582	K131G	+
583/584	N89A	+
585/586	K133Q	+
587/588	T108G	+
589/590	Q27F	+
591/592	I119Q	+
593/594	Y134W	+
595/596	K29G	+
597/598	K25N	+
397/398	T149R	+
469/470	V124I	+
599/600	K24P	+
335/336	L153Q	+
279/280	L153E	+
377/378	K133G	+
601/602	S139G	+
603/604	T130A	+
605/606	K126C	+
607/608	S20P	+
609/610	E45R	+
467/468	Q27D	+
479/480	H102L	+
611/612	I15L	+
613/614	T130Q	+
615/616	T130S	+
481/482	E105N	+
617/618	A97T	+
619/620	K28R	+
489/490	R26G	+
621/622	Q109N	+

623/624	F46M	+
625/626	K106V	+
627/628	I119F	+
629/630	I18Y	+
631/632	H33A	+
331/332	R14G	+
633/634	D31V	+
359/360	L153G	+
635/636	Q109T	+
275/276	I34S	+
399/400	M137A	+
295/296	A150E	+
637/638	E138A	+
639/640	S93V	+
641/642	T17A/K131R	+
643/644	M12F	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 24 and defined as follows: “+” 1.21 to 1.42-fold increased activity; “++” > 1.42-fold increased activity; “+++” > 1.69-fold increased activity.		

EXAMPLE 11

Improvements over SEQ ID NO: 268 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0362] SEQ ID NO: 268 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 11.1.

[0363] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 11.1-Reaction conditions
Lysis buffer-MOPS (50 mM, pH 7.4), 0.2 g/L lysozyme; Reaction buffer-20 mM MOPS, 50 mM KOAc, 0.25 mM CoCl ₂ , pH 7.2; Lysate concentration (vol%)-12.5; Preincubation Temp (C)-47 °C; Preincubation Time (min)-15; Reaction temperature (C)-47 °C; Reaction Time (min)-30; Reaction

volume (μL) -20; NTP (μM)-mATP; NTP conc (μM)-25; Oligonucleotide-5'-6-FAM-T17ATC(2'dF)C; Substrate conc (μM)-1; Product -5'-6-FAM-T17ATC(2'dF)CmA.

[0364] Activity relative to SEQ ID NO: 268 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 268 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 11.2.

Table 11.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 268)	FIOP Product Peak Area Relative to SEQ ID NO: 268
645/646	K300P/E395Y/A419L	++
647/648	K106V/K300P/Q415A/A419L/V456R	++
649/650	R140G	+
651/652	R14G/E53K/A419L	+
653/654	R14G/E53K/K300P	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 268 and defined as follows: "+" 1.02 to 1.70-fold increased activity; "++" > 1.70-fold increased activity.		

EXAMPLE 12

Improvements over SEQ ID NO: 648 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0365] SEQ ID NO: 648 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 12.1.

[0366] Reactions were performed in 96-well format 200 μL BioRad PCR plates. Reactions included 1-10 μM oligonucleotide, 25-50 μM nucleotide triphosphate, 20 mM buffer, and 250 μM cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 12.1-Reaction conditions
Lysis buffer-MOPS (50 mM, pH 7.4), 0.2 g/L lysozyme; Reaction buffer-20 mM MOPS, 50 mM KOAc, 0.25 mM CoCl_2 , pH 7.2; Lysate concentration (vol%)-3.125; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-15; Reaction temperature (°C)-50 °C; Reaction Time (min)-30; Reaction

volume (μL) -20; NTP (μM)-ddGTP; NTP conc (μM)-25; Oligonucleotide-5'-6-FAM-T17AT*mC;
Substrate conc (μM)-1; Product -5'-6-FAM-T17AT*mCddG.

[0367] Activity relative to SEQ ID NO: 648 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 648 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate).

The results are shown in Table 12.2.

Table 12.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 648)	FIOP Product Peak Area Relative to SEQ ID NO: 648
655/656	R14G/I34D/D94E/V106K/T108K/V141E	+++
657/658	I34D/M37A/G92R	+++
659/660	M12S/R14G/I34D/M37A/V106K/R140G/D142M/A150E/T152R/L153E	+++
661/662	I34D/G92R/D94E/V141E/D142M	+++
663/664	M12S/M37A/D94E/V141E/A150E/T152R/L153E	+++
665/666	M12S/I34D/G92R/R140G	+++
667/668	I34D/A150E/T152R/L153E	+++
669/670	M12S/R14G/I34D/M37F/D94E/R140G/V141E/N145E	+++
671/672	T101E/V106S/V124E/I155E	+++
673/674	G92R/D94E/V106K/D142S/N145E	+++
675/676	S20T/D21E/K24G/H33K/T58A/M104P/V106S/V124E/I155E/L156E	+++
677/678	M12S/R14G/I34D/M37A/A150E	+++
679/680	S20T/D21E/H33K/T58A/T101E/M104P/V106K/V124E/I155E	+++
681/682	I34S/M37A/A150E/L153E	+++
683/684	M12S/R14G/V106K/T108K/R140G/V141E/N145E/A150E	+++
685/686	M12S/R14G/I34D/R140G/A150E	+++
687/688	R14G/I34S/A150E/L153E	+++
689/690	I34D/M37A	+++
691/692	M30G/M104P/V106S/I155E/L156E	+++
693/694	M12S/R14G/I34D/M37A/V141E/D142S	+++
695/696	R14G/D31G/I34D/M37A/R140G/V141E/N145E/K161E/T162E	+++
697/698	V106K/T108K/R140G/V141E/T152R/L153E	+++
699/700	M30G/T101E/M104P/V106S/I155E/L156E	+++
701/702	D21E/T101E/M104P/V106S/L156E	++
703/704	D21E/H33K/T58A/T101E/V106K	++
705/706	R14G/I34S/M37A	++
707/708	M104P/V106S	++
709/710	M12S/M37F/R140G/V141E/A150E/T162E	++
711/712	M12S/R14G/G92R/D94E	++
713/714	M12S/I34D/A150E/T152R	++
715/716	T58S/T101E/M104P/V106S/L156E	++

717/718	R14G/V141E/K161E	++
719/720	R14G/I34D/A150E/L153E	++
721/722	R14G/I34S/M37A/T152R	++
723/724	V106K/T108K/R140G/D142S/A150E/L153E	++
725/726	M12S/R14G/I34D/R140G/D142S	++
727/728	D21E/H33K/T101E/M104P/V106S	++
729/730	R14G/K161E	++
731/732	T58A/T101E/V106K/I155E	++
733/734	M12S/R14G/D94E/A150E/T152R	++
735/736	M12S/R14G/I34D/M37F/A150E/L153E	++
737/738	T101E/V106K/I155E/L156E	++
739/740	M104P/V106S/I155E	++
741/742	M30G/M104P/V106S/I155E	++
743/744	M12S/R14G/I34S/M37A/D142M/K161E	++
745/746	M30G/V106K/I155E	++
747/748	M12S/R14G/A150E/T152R/L153E	++
749/750	S20T/T58S/T101E/M104P/V106S/L156E	++
751/752	M30G/H33K/T58S/M104P/V106S/I155E/L156E	++
753/754	T101E/V106K	++
755/756	M104P/V106K/I155E	++
757/758	M30G/H33K/M104I/V106S/I155E/L156E	++
759/760	M12S/R14G/I34S/D142S/A150E/L153E	++
761/762	D21E/M104P/V106S	++
763/764	D21E/T58S/T101E/M104I/V106S/I155E	+
765/766	K161E	+
767/768	D21E/M104P/V106S/L156E	+
769/770	M12S/R14G/I34D	+
771/772	R14G/L153E	+
773/774	M12S/R14G/L153E	+
775/776	S20T/T58A/T101E/V106K	+
777/778	R140G/N145E	+
779/780	S20T/H33K/M104P/V106K/V124E/L156E	+
781/782	D21E/H33K/T58S/V106S/I155E/L156E	+
783/784	D21E/T58A/V106K/I155E/L156E	+
785/786	H33K/T58A/M104I/V106S	+
787/788	I34S/M37F/R140G/V141E/D142M/N145E	+
789/790	M12S/R14G/V141E/D142S	+
791/792	R140G/N145E/A150E/T152R	+
793/794	H33K/T101E/M104P/V106K/I155E	+
795/796	T101E/M104I/V106K/L156E	+
797/798	T101E/M104I/V106K	+
799/800	M104I/V106K	+
801/802	M12S/R14G/V106K/T108K/T152R	+
803/804	M12S/R14G/I34D/M37F/D142S/N145E	+
805/806	M37A/V141E/D142S	+
807/808	I34D/V141E/D142S/N145E	+
809/810	M104I/V106K/V124E	+

811/812	T101E/M104I/V106S/L156E	+
813/814	M104I/V106S/I155E/L156E	+
815/816	V141E/T152R	+
817/818	M30G/H33K/T101E/V106S/L156E	+
819/820	R14G/I34S/M37F/N145E	+
821/822	S20T/T101E/V106K/L156E	+
823/824	V141E	+
825/826	S20T/D21E/T58A/M104I/V106S/I155E/L156E	+
827/828	M12S/K161E	+
829/830	R14G	+
831/832	M37F/L153E	+
833/834	T101E/M104P/V106K/I155E/L156E	+
835/836	M104I/V106S	+
837/838	D21E/H33K/V106S	+
839/840	I34D/M37F/V141E/D142S	+
841/842	M104P/V106K/L156E	+
843/844	T58S/M104I/V106K/I155E/L156E	+
845/846	V106K/T107R/T108K/D142S/S220R	+
847/848	M37F/G92R/D142M	+
849/850	V106K/L156E	+
851/852	R14G/V106K	+
853/854	D21E/M104I/V106S/V124E	+
855/856	R14G/R140G	+
857/858	R14G/D142M	+
859/860	R140G/V141E/D142M	+
861/862	M104P/V106K	+
863/864	R14G/D142S/K161E/T162E	+
865/866	M12S/R14G/V106K/T107R/V141E/D142M	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 648 and defined as follows: “+” 1.19 to 3.50-fold increased activity; “++” > 3.50-fold increased activity; “+++” > 8.50-fold increased activity.		

EXAMPLE 13

Improvements over SEQ ID NO: 660 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0368] SEQ ID NO: 660 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 13.1.

[0369] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution

was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 13.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-50 °C; Preincubation Time (min)-60; Reaction temperature (C)-50 °C; Reaction Time (min)-30; Reaction volume (μL) -20; NTP (μM)-*rATP; NTP conc (μM)-25; Oligonucleotide-5'-6-FAM-T12ATCAC*(2'dF)A; Substrate conc (μM)-1; Product -5'-6-FAM-T12ATCAC*(2'dF)A*rA.

[0370] Activity relative to SEQ ID NO: 660 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 660 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 13.2.

Table 13.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 660)	FIOP Product Peak Area Relative to SEQ ID NO: 660
867/868	R16V/K29I/M30E/T101E/M104V	+++
869/870	T101E/V141E/I155E/L156E	+++
871/872	T101E/M137E/I155E	+++
873/874	G92R/T101E/M137A/I155E/E476R	+++
875/876	T101E/M104V	++
877/878	T101E	++
879/880	R16V/K29I/M30E	++
881/882	G92R/D94E/T108K/V141E/I155E/D392R	++
883/884	T58A	++
885/886	T108K	+
887/888	D94E/T101E/L156E/E476R	+
889/890	R16V/M30L/M104V	+
891/892	K29I/M30E	+
893/894	R16V/K29I/M30E/H33K/E153P	+
895/896	R16V/H33K	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 660 and defined as follows: "+" 1.20 to 1.56-fold increased activity; "++" > 1.56-fold increased activity; "+++" > 2.42-fold increased activity.		

EXAMPLE 14

Improvements over SEQ ID NO: 660 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0371] SEQ ID NO: 660 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 14.1.

[0372] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 14.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-48 °C; Preincubation Time (min)-60; Reaction temperature (C)-48 °C; Reaction Time (min)-30; Reaction volume (μ L) -20; NTP (μ M)-*rGTP; NTP conc (μ M)-25; Oligonucleotide-5'-6-FAM-T12ATCAC*mA; Substrate conc (μ M)-1; Product -5'-6-FAM-T12ATCAC*mA*rG.

[0373] Activity relative to SEQ ID NO: 660 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 660 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 14.2.

Table 14.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 660)	FIOP Product Peak Area Relative to SEQ ID NO: 660
897/898	E342V	+++
899/900	F204L/E342W	+++
901/902	L264R	+++
903/904	E310A	+++
905/906	K303V	+++
907/908	V290L	+++
909/910	G391R	+++
911/912	L421F	+++
913/914	Y308F	+++
915/916	L306F	+++
917/918	L454M	+++
919/920	K278V	+++
921/922	K303A	+++
923/924	K278E	+++

925/926	V316L	+++
927/928	R281T	+++
929/930	E310R	+++
931/932	K278C	+++
933/934	R281C	+++
935/936	K278I	++
937/938	K297V	++
939/940	R456S	++
941/942	S525F	++
943/944	L360I	++
945/946	G236E/K297L	++
947/948	Q385R	++
949/950	E310G	++
951/952	R281G	++
953/954	R281L	++
955/956	G391L	++
957/958	K303N	++
959/960	V268L	++
961/962	L419G	++
963/964	K448R	++
965/966	M282G	++
967/968	K278T	++
969/970	K297D	++
971/972	E342R	++
973/974	P300R	++
975/976	R281S	++
977/978	R281V	++
979/980	K303S	++
981/982	K297S	++
983/984	H413F	++
985/986	M282W	++
987/988	E476V	++
989/990	R281A	++
991/992	H413C	+
993/994	H413V	+
995/996	R258S	+
997/998	Y308W	+
999/1000	M205E	+
1001/1002	R258W	+
1003/1004	G315S	+
1005/1006	M282C	+
1007/1008	R291S	+
1009/1010	K297C	+
1011/1012	L264A	+
1013/1014	Y309F	+
1015/1016	L264E	+
1017/1018	R473V	+

1019/1020	A276S	+
1021/1022	F353K	+
1023/1024	T344V	+
1025/1026	R258G	+
1027/1028	L264S	+
1029/1030	R258A	+
1031/1032	V290A	+
1033/1034	R258L	+
1035/1036	E310S	+
1037/1038	M282H	+
1039/1040	G315A	+
1041/1042	V316A	+
1043/1044	R258M	+
1045/1046	S261V	+
1047/1048	A515V	+
1049/1050	N197M	+
1051/1052	L419H	+
1053/1054	K303Q	+
1055/1056	L312V	+
1057/1058	F353M	+
1059/1060	F353A	+
1061/1062	Y280F	+
1063/1064	S261R	+
1065/1066	M205L	+
1067/1068	E310H	+
1069/1070	F262I	+
1071/1072	S525H	+
1073/1074	K278R	+
1075/1076	R258C	+
1077/1078	S261G	+
1079/1080	F353R	+
1081/1082	A410E	+
1083/1084	K195E	+
1085/1086	F353S	+
1087/1088	F269W	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 660 and defined as follows: “+” 1.08 to 1.94-fold increased activity; “++” > 1.94-fold increased activity; “+++” > 3.10-fold increased activity.		

EXAMPLE 15**Improvements over SEQ ID NO: 882 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0374] SEQ ID NO: 882 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination

of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 15.1.

[0375] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 15.1-Reaction conditions	
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-50 °C; Preincubation Time (min)-60; Reaction temperature (C)-50 °C; Reaction Time (min)-30; Reaction volume (μ L)-20; NTP (μ M)-*rGTP; NTP conc (μ M)-25; Oligonucleotide-5'-6-FAM-T12ATCAC*mC; Substrate conc (μ M)-1; Product -5'-6-FAM-T12ATCAC*mC*rG.	

[0376] Activity relative to SEQ ID NO: 882 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 882 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 15.2.

Table 15.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 882)	FIOP Product Peak Area Relative to SEQ ID NO: 882
1089/1090	T203A	+++
1091/1092	R394E	+++
1093/1094	R394T	+++
1095/1096	D314R	+++
1097/1098	D199R	+++
1099/1100	R461G	+++
1101/1102	L403F/F462H	+++
1103/1104	T484R	+++
1105/1106	D199V	+++
1107/1108	T379C	+++
1109/1110	S325F	+++
1111/1112	R481D	+++
1113/1114	D314V	+++
1115/1116	F462I	++
1117/1118	T484M	++
1119/1120	D314K	++
1121/1122	A321K	++

1123/1124	D314L	++
1125/1126	T203S	++
1127/1128	I208V	++
1129/1130	D199M	++
1131/1132	F462R	++
1133/1134	I408A	++
1135/1136	C457V	++
1137/1138	T203R	++
1139/1140	T203L	++
1141/1142	R477T	++
1143/1144	A321C	++
1145/1146	D314Y	++
1147/1148	F462W	++
1149/1150	A495S	+
1151/1152	Q329R/F462E	+
1153/1154	T317G	+
1155/1156	A321S	+
1157/1158	A313I	+
1159/1160	T275V	+
1161/1162	D314G	+
1163/1164	S325V	+
1165/1166	D199G	+
1167/1168	R406V	+
1169/1170	D199S	+
1171/1172	C457S	+
1173/1174	R406G	+
1175/1176	R196G	+
1177/1178	R481M	+
1179/1180	D199Q	+
1181/1182	R397T	+
1183/1184	S325T	+
1185/1186	R196Y	+
1187/1188	R397D	+
1189/1190	S325W	+
1191/1192	N175L	+
1193/1194	R481T	+
1195/1196	W469Q	+
1197/1198	T203G	+
1199/1200	R461V	+
1201/1202	I408T	+
1203/1204	D322A	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 882 and defined as follows: “+” 1.01 to 1.60-fold increased activity; “++” > 1.60-fold increased activity; “+++” > 2.85-fold increased activity.		

EXAMPLE 16

Improvements over SEQ ID NO: 882 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0377] SEQ ID NO: 882 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 16.1.

[0378] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 16.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl₂, pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-50 °C; Preincubation Time (min)-60; Reaction temperature (C)-50 °C; Reaction Time (min)-30; Reaction volume (μ L) -20; NTP (μ M)-*rUTP; NTP conc (μ M)-25; Oligonucleotide-5'-6-FAM-T12ATCAC*mC; Substrate conc (μ M)-1; Product -5'-6-FAM-T12ATCAC*mC*rU.

[0379] Activity relative to SEQ ID NO: 882 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 882 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 16.2.

Table 16.2

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 882)	FIOP Product Peak Area Relative to SEQ ID NO: 882
1205/1206	R394L	+++
1207/1208	R394T	+++
1209/1210	R461S	+++
1211/1212	R394M	+++
1213/1214	R461G	+++
1215/1216	R461A	+++
1217/1218	F462L	+++
1219/1220	R477Q	+++
1221/1222	T203M	+++
1223/1224	R394S	+++
1225/1226	D199R	+++

1227/1228	A495G	+++
1229/1230	D199A	+++
1231/1232	T484R	+++
1233/1234	I408L	+++
1235/1236	T484M	++
1237/1238	D492S	++
1239/1240	V273W	++
1241/1242	D314I	++
1243/1244	C201A	++
1245/1246	D199G	++
1247/1248	N175V	++
1249/1250	D492T	++
1251/1252	A313R	++
1253/1254	D199H	++
1255/1256	T275D	++
1257/1258	A495S	++
1259/1260	A313Q	++
1261/1262	D199Q	++
1263/1264	C307M	++
1265/1266	G350S	++
1267/1268	Q179R	++
1269/1270	S325V	++
1271/1272	D199I	+
1273/1274	R481W	+
1275/1276	D199V	+
1277/1278	D199E	+
1279/1280	C307V	+
1281/1282	T203R	+
1283/1284	A319R	+
1285/1286	G272T	+
1287/1288	A321K	+
1289/1290	N175D	+
1291/1292	S325A	+
1293/1294	V273M	+
1295/1296	D322K	+
1297/1298	F462Q	+
1299/1300	I408G	+
1301/1302	C201W	+
1303/1304	N175I	+
1305/1306	R196G	+
1307/1308	P404F	+
1309/1310	I408R	+
1311/1312	E523H	+
1313/1314	D322Q	+
1315/1316	D314G	+
1317/1318	A319G	+
1319/1320	A313S	+

1321/1322	R406V	+
1323/1324	L491I	+
1325/1326	R461Q	+
1327/1328	Q376V	+
1329/1330	A313M	+
1331/1332	P404W	+
1333/1334	I324V	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 882 and defined as follows: "+" 1.10 to 1.82-fold increased activity; "++" > 1.82-fold increased activity; "+++" > 3.14-fold increased activity.		

EXAMPLE 17

Improvements over SEQ ID NO: 1336 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0380] SEQ ID NO: 1336 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 17.1.

[0381] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 17.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-48 °C; Preincubation Time (min)-60; Reaction temperature (C)-48 °C; Reaction Time (min)-30; Reaction volume (μ L) -1; NTP (μ M)-*rGTP; NTP conc (μ M)-25; Oligonucleotide-5'-6-FAM-T15AmG*mC ; Substrate conc (μ M)-1; Product -5'-6-FAM-T15AmG*mC*rG.
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[0382] Activity relative to SEQ ID NO: 1336 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1336 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 17.2.

Table 17.2

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1336)	FIOP Product Peak Area Relative to SEQ ID NO: 1336
1337/1338	T203A/T344V/R394E	+++
1339/1340	T203A/K297D/D314R/R394E/E395W	+++
1341/1342	K195E/T203A/K278E/R394E/E395W	+++
1343/1344	T344V/R394E/E395W	+++
1345/1346	K195E/T203A/R394E	+++
1347/1348	D314R/R394E/E395W	++
1349/1350	R394E	++
1351/1352	D199R/T203A/K297D/R394E/E395W	++
1353/1354	T203A/R394E	++
1355/1356	K195E/R394E/E395W	++
1357/1358	T203A/K278E/K297D/R394E	++
1359/1360	I15F/D199R/T203A/R394E	++
1361/1362	K195E/D199R/T203A/K278E/D314R/F353K/R394E	++
1363/1364	K195E/K278E/K297D/R394E/E395W	+
1365/1366	K297D/R394E	+
1367/1368	K195E/D199R/T203A/K278E/K297D/R394E	+
1369/1370	K195E/T203A/K297D/D314R/R394E	+
1371/1372	T203A/R394E/E395W	+
1373/1374	K195E/K278E/K297D/R394E	+
1375/1376	K195E/T203A/K297D/R394E/L419M	+
1377/1378	K195E/D314R/T344V	+
1379/1380	K28N/T344V/F353K/E395W	+
1381/1382	T203A/E310R/D314R/R394E/E395W/L419M	+
1383/1384	T203A/F353K	+
1385/1386	I15F/D199R/R394E	+
1387/1388	F353K	+

Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1336 and defined as follows: “+” 1.15 to 3.70-fold increased activity; “++” > 3.70-fold increased activity; “+++” > 5.18-fold increased activity.

EXAMPLE 18

Improvements over SEQ ID NO: 1336 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0383] SEQ ID NO: 1336 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 18.1.

[0384] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution

was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 18.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-50 °C; Preincubation Time (min)-60; Reaction temperature (C)-48 °C; Reaction Time (min)-30; Reaction volume (μL) -1; NTP (μM)-mGTP; NTP conc (μM)-50; Oligonucleotide-5'-6-FAM-T12TATCAC*mC; Substrate conc (μM)-1; Product -5'-6-FAM-T12TATCAC*mCmG.

[0385] Activity relative to SEQ ID NO: 1336 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1336 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 18.2.

Table 18.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1336)	FIOP Product Peak Area Relative to SEQ ID NO: 1336
1389/1390	D330E	+++
1391/1392	W333H	+++
1393/1394	W333R	+++
1395/1396	L340S	+++
1397/1398	S292L/L411P	+++
1399/1400	M490R	+++
1401/1402	I503E	+++
1403/1404	H177L	+++
1405/1406	M435C	+++
1407/1408	D460G	+++
1409/1410	W333G	+++
1411/1412	W333A	+++
1413/1414	T200C/H425K	+++
1415/1416	L326T	+++
1417/1418	L340R	+++
1419/1420	V219R	+++
1421/1422	D338T	+++
1423/1424	T160M	+++
1425/1426	S446P	+++
1427/1428	L340M/G438V	+++
1429/1430	Q373H	+++
1431/1432	T334R	++
1433/1434	L326N	++

1435/1436	T334S	++
1437/1438	L340A	++
1439/1440	S363C	++
1441/1442	I503Q	++
1443/1444	H425R	++
1445/1446	I503V	++
1447/1448	E369N	++
1449/1450	M435T	++
1451/1452	S292T	++
1453/1454	M435A	++
1455/1456	L340M	++
1457/1458	W333D	++
1459/1460	D400A/G401E/K402F	++
1461/1462	Y459Q	++
1463/1464	M435K	++
1465/1466	Q373N	++
1467/1468	T160S	++
1469/1470	E217Q	++
1471/1472	M490V	++
1473/1474	K427Q	++
1475/1476	E369M	++
1477/1478	M435E	++
1479/1480	M435S	++
1481/1482	T61A	++
1483/1484	T160N	++
1485/1486	F504W	++
1487/1488	A444R	++
1489/1490	T162A	++
1491/1492	C213S	+
1493/1494	L326C	+
1495/1496	M435G	+
1497/1498	S446E	+
1499/1500	Q329K	+
1501/1502	Q370G	+
1503/1504	I503R	+
1505/1506	D372G	+
1507/1508	D223Q	+
1509/1510	T200V	+
1511/1512	K502G	+
1513/1514	T160V	+
1515/1516	L326M	+
1517/1518	H425T	+
1519/1520	T295S	+
1521/1522	K383R	+
1523/1524	L248S	+
1525/1526	E441N	+
1527/1528	T437S	+

1529/1530	S442G	+
1531/1532	K506E	+
1533/1534	M490H	+
1535/1536	M490E	+
1537/1538	L340G	+
1539/1540	G236I	+
1541/1542	T334E	+
1543/1544	T437Q	+
1545/1546	M435Q	+
1547/1548	M435S/I503M	+
1549/1550	G236V	+
1551/1552	K427E	+
1553/1554	H425D	+
1555/1556	K501A	+
1557/1558	F504R	+
1559/1560	V219L	+
1561/1562	K502R	+
1563/1564	E246K	+
1565/1566	E443T	+
1567/1568	L248C	+
1569/1570	R152H/I503S	+
1571/1572	T200C	+
1573/1574	G236P	+
1575/1576	E369G	+
1577/1578	L326S	+
1579/1580	N440V	+
1581/1582	K488S	+
1583/1584	T200K	+
1585/1586	Q329R	+
1587/1588	N440E	+
1589/1590	Q165K	+
1591/1592	M490W	+
1593/1594	F504N	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1336 and defined as follows: “+” 1.00 to 1.31-fold increased activity; “++” > 1.31-fold increased activity; “+++” > 1.67-fold increased activity.		

EXAMPLE 19**Improvements over SEQ ID NO: 1348 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0386] SEQ ID NO: 1348 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 19.1.

[0387] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 19.1-Reaction conditions	
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-50 °C; Preincubation Time (min)-60; Reaction temperature (C)-50 °C; Reaction Time (min)-30; Reaction volume (μ L) -1; NTP (μ M)-mGTP; NTP conc (μ M)-50; Oligonucleotide-5'-6-FAM-T15AmG*mC; Substrate conc (μ M)-1; Product -5'-6-FAM-T15AmG*mCmG.	

[0388] Activity relative to SEQ ID NO: 1348 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1348 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 19.2.

Table 19.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1348)	FIOP Product Peak Area Relative to SEQ ID NO: 1348
1595/1596	C201A/C213S/W333A/T344V/R397Q/H425D/R481W/H485D	+++
1597/1598	N175D/C201A/W333A/D412N/H425D/C457V/H485D	+++
1599/1600	W333A/T344V/E369N/H485D	+++
1601/1602	N175D/W333A/H485D	+++
1603/1604	N175D/H485D	+++
1605/1606	C201A/W333A/T344V/C457V/R481W	+++
1607/1608	C201A/W333A/R481W	++
1609/1610	H3Q/N175D/C213S/A313R/S325T/L340R/C457V/R481W/H485D	++
1611/1612	H3Q/C307M/A321K/L340R/F353K/R406G/I408A/K445N	++
1613/1614	P148T/N175D/C201A/C457V/H485D	++
1615/1616	T344V/H485D	++
1617/1618	C307M/Q373H/R406G/I408A/T484M	++
1619/1620	W333G	++
1621/1622	D199H/C307M/A321K/L340R/R406G/I408A/K445N/T484M	++
1623/1624	L340R/T484M	++
1625/1626	N175D/W333A	+
1627/1628	A321K/W333G/L340R	+

1629/1630	C213S/W333A/R397Q	+
1631/1632	C307M/W333G/L340R/I408A/K445N/F462L/K502G	+
1633/1634	S325T/H425D/C457V/R481W	+
1635/1636	S325T/W333A/E369N/H425D	+
1637/1638	C201R/R406G/I408A/F462L/T484M/K502G	+
1639/1640	R406G/I408A	+
1641/1642	F353K/R406G	+
1643/1644	N175D/W333A/E369N/R481W	+
1645/1646	Q373H	+
1647/1648	W333A/T344V/E369N/R397Q	+
1649/1650	C213S	+
1651/1652	N175D/S325T/R397Q	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1348 and defined as follows: “+” 1.33 to 2.18-fold increased activity; “++” > 2.18-fold increased activity; “+++” > 3.12-fold increased activity.		

EXAMPLE 20

Improvements over SEQ ID NO: 1596 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0389] SEQ ID NO: 1596 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 20.1.

[0390] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 20.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-50 °C; Preincubation Time (min)-60; Reaction temperature (C)-50 °C; Reaction Time (min)-30; Reaction volume (μ L) -1; NTP (μ M)-mATP; NTP conc (μ M)-50; Oligonucleotide-5'-6-FAM-T15AmU*mG ; Substrate conc (μ M)-10; Product -5'-6-FAM-T15AmU*mG mA.

[0391] Activity relative to SEQ ID NO: 1596 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1596 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 20.2.

Table 20.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1596)	FIOP Product Peak Area Relative to SEQ ID NO: 1596
1653/1654	R406G/I408A/S442G/S446P	++
1655/1656	R406G/I408A	++
1657/1658	T160M/V219R/D460G/I503E/K506E	++
1659/1660	S446P	++
1661/1662	I408A	+
1663/1664	V219R/C307M/L326T	+
1665/1666	R406G/I408A/M490R	+
1667/1668	A252K/A333H	+
1669/1670	A252K	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1596 and defined as follows: “+” 1.12 to 1.50-fold increased activity; “++” > 1.50-fold increased activity.		

EXAMPLE 21

Improvements over SEQ ID NO: 1596 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0392] SEQ ID NO: 1596 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 21.1.

[0393] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 21.1.-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Precubation Temp (C)-50 °C; Precubation Time (min)-60; Reaction temperature (C)-50 °C; Reaction Time (min)-30; Reaction volume (μL) -1; NTP (μM)-mATP; NTP conc (μM)-50; Oligonucleotide-5'-6-FAM-T15AmU*mG; Substrate conc (μM)-10; Product -5'-6-FAM-T15AmU*mGmA.

[0394] Activity relative to SEQ ID NO: 1596 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1596 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 21.2.

Table 21.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1596)	FIOP Product Peak Area Relative to SEQ ID NO: 1596
1671/1672	T484E	+++
1673/1674	Q179K	+++
1675/1676	F353Q	+++
1677/1678	W382L	+++
1679/1680	C307L	+++
1681/1682	M205R	+++
1683/1684	K402Q	+++
1685/1686	K488N	+++
1687/1688	H413G	++
1689/1690	Q165P	++
1691/1692	Q376H	++
1693/1694	M490L	++
1695/1696	Q329K	++
1697/1698	L393I	++
1699/1700	R406P	++
1701/1702	D400E	++
1703/1704	R171K	++
1705/1706	I163V	++
1707/1708	S325L	++
1709/1710	S261A	++
1711/1712	S442A	++
1713/1714	D330E	+
1715/1716	S405N	+
1717/1718	E371D	+
1719/1720	K508D	+
1721/1722	A410Q	+
1723/1724	A495G	+
1725/1726	A252E	+
1727/1728	L188M	+

1729/1730	I208A	+
1731/1732	E520D	+
1733/1734	K506S	+
1735/1736	V253I	+
1737/1738	T162H	+
1739/1740	L419A	+
1741/1742	D460E	+
1743/1744	K407A	+
1745/1746	E441M	+
1747/1748	E233D	+
1749/1750	H177Y	+
1751/1752	T200A	+
1753/1754	F464Y	+
1755/1756	D277E	+
1757/1758	Q260K	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1596 and defined as follows: "+" 1.00 to 1.31-fold increased activity; "++" > 1.31-fold increased activity; "+++" > 1.58-fold increased activity.		

EXAMPLE 22**Improvements over SEQ ID NO: 1596 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors****HTP Screening for Improved TdT Variants**

[0395] SEQ ID NO: 1596 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 22.1.

[0396] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 22.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl₂, pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-57 °C; Preincubation Time (min)-60; Reaction temperature (C)-57 °C; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-*ddGTP; NTP conc (μ M)-50; Oligonucleotide-5'-6-FAM-T14ATCmC; Substrate conc (μ M)-1; Product -5'-6-FAM-T14ATCmC*ddG.

[0397] Activity relative to SEQ ID NO: 1596 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1596 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 22.2.

Table 22.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1596)	FIOP Product Peak Area Relative to SEQ ID NO: 1596
1671/1672	T484E	+++
1759/1760	S325L	+++
1761/1762	M205R	+++
1763/1764	Q165P	+++
1765/1766	M490L	+++
1675/1676	F353Q	+++
1745/1746	E441M	+++
1709/1710	S261A	+++
1767/1768	K488N	+++
1769/1770	D460E	++
1771/1772	A252E	++
1773/1774	C307L	++
1775/1776	F414H	++
1673/1674	Q179K	++
1719/1720	K508D	++
1729/1730	I208A	++
1777/1778	R406P	++
1779/1780	G518D	++
1705/1706	I163V	++
1755/1756	D277E	++
1781/1782	Q370T	++
1783/1784	E371D	++
1785/1786	N380D	++
1721/1722	A410Q	++
1787/1788	K402Q	+
1789/1790	L306F	+
1791/1792	Q376H	+
1793/1794	K506S	+
1695/1696	Q329K	+
1795/1796	K368R	+
1727/1728	L188M	+
1797/1798	H413G	+
1799/1800	S442A	+
1801/1802	K161R	+
1803/1804	V253I	+
1805/1806	L419A	+
1807/1808	A321K	+

1753/1754	F464Y	+
1809/1810	Q251K	+
1747/1748	E233D	+
1811/1812	H426P	+
1813/1814	L393I	+
1723/1724	A495G	+
1815/1816	G231T	+
1817/1818	L327I	+
1701/1702	D400E	+
1819/1820	T162H	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1596 and defined as follows: “+” 1.00 to 1.84-fold increased activity; “++” > 1.84-fold increased activity; “+++” > 3.00-fold increased activity.		

EXAMPLE 23

Improvements over SEQ ID NO: 1654 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0398] SEQ ID NO: 1654 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 23.1.

[0399] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 23.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-50 °C; Preincubation Time (min)-60; Reaction temperature (C)-50 °C; Reaction Time (min)-45; Reaction volume (μ L) -1; NTP (μ M)-3'P-mATP; NTP conc (μ M)-50; Oligonucleotide-5'-6-FAM-T15AT*mG; Substrate conc (μ M)-10; Product -5'-6-FAM-T15AT*mGmA-3'P.

[0400] Activity relative to SEQ ID NO: 1654 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1654 (where

the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 23.2.

Table 23.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1654)	FIOP Product Peak Area Relative to SEQ ID NO: 1654
1821/1822	T160M/Q165P/M205R/V219R/E441M	++
1823/1824	M205R/C307L/E441M/D460G/K488N	++
1825/1826	E441M	++
1827/1828	T160M/T203E/M205R/E441M	+
1829/1830	T160M/Q165P/T203E/M205R/V219R/F353Q/D460G/K488N	+
1831/1832	Q179K/F353Q	+
1833/1834	T160M/V219R/D330K/T484E	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1654 and defined as follows: "+" 1.47 to 1.83-fold increased activity; "++" > 1.83-fold increased activity.		

EXAMPLE 24

Improvements over SEQ ID NO: 1654 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0401] SEQ ID NO: 1654 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 24.1.

[0402] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 24.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-57 °C; Preincubation Time (min)-60; Reaction temperature (C)-57 °C; Reaction Time (min)-60; Reaction volume (μ L) -1; NTP (μ M)-*ddGTP; NTP conc (μ M)-50; Oligonucleotide-5'-6-FAM-T14ATCmC; Substrate conc (μ M)-1; Product -5'-6-FAM-T14ATCmC*ddG.

[0403] Activity relative to SEQ ID NO: 1654 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1654 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 24.2.

Table 24.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1654)	FIOP Product Peak Area Relative to SEQ ID NO: 1654
1835/1836	Q165P/T203E/M205R/C307L/F414Y/E441M/T484E/A495G/I503E/K506E	+++
1837/1838	Q165P/Q179K/T203E/M205R/T484E/I503E	+++
1839/1840	T160M/Q179K/T203E/M205R/I208A/V219R/E371D/F414H/T484E/K506E	+++
1841/1842	T160M/M205R/I208A/V219R/L326T/E441M/T484E/K488N/I503E	+++
1843/1844	T160M/I208A/E371D/E441M/T484E/K506E	+++
1845/1846	T160M/Q179K/I208A/V219R/C307L/H413G/F414H/I503E/K506E	+++
1847/1848	T160M/Q179K/C307L/Q376H/E441M/K488N/I503E	+++
1829/1830	T160M/Q165P/T203E/M205R/V219R/F353Q/D460G/K488N	+++
1833/1834	T160M/V219R/D330K/T484E	+++
1849/1850	Q179K/M205R/I208A/F353Q/F414H/E441M/D460G/T484E/K488N	+++
1851/1852	T160M/Q165P/C307L/F353Q/H413G/F414H/E441M/K488N/A495G	+++
1853/1854	Q165P/Q179K/I208A/F353Q/H413G/F414H/I503E	+++
1821/1822	T160M/Q165P/M205R/V219R/E441M	++
1855/1856	Q165P/T203E/M205R/T484E/K488N	++
1857/1858	T160M/T203E/M205R/I208A/H413G/D460G/T484E/K488N	++
1859/1860	M205R/L326T/K488N/I503E/K506E	++
1861/1862	T160M/I208A/L326T/Q376H/F414H/E441M/T484E/K488N	++
1863/1864	T160M/V219R/C307L/E371D/K506E	++
1865/1866	H3Q/T160M/M205R/I208A/V219R/C307L/F353Q/E371D/Q376H/H413G/F414H/E441M/K488N	++
1867/1868	M205R/V219R/C307L/F353Q	++
1869/1870	Q165P/I208A/L326T/H413G/F414H/T484E/A495G/K506E	++
1823/1824	M205R/C307L/E441M/D460G/K488N	++
1871/1872	T160M/Q165P/Q179K/T203E/M205R/I208A/V219R/F353Q/Q376H/H413G/F414H/E441M/K488N	++
1873/1874	M205R/C307L	++
1875/1876	T203E/I208A/V219R/E441M	++
1877/1878	T160M/T203E/L326T/F353Q/H413G/F414H/T484E/A495G	++
1879/1880	T160M/I208A/F414H/E441M/V452I/R480H/K488N/A495G	++
1881/1882	Q165P/M205R	++
1831/1832	Q179K/F353Q	++
1883/1884	T160M/T203E/M205R/I208A/V219R/F414H/D460G/K506E	++
1885/1886	T160M/T203E/I208A/H413G/F414H/E441M/T484E	++

1887/1888	Q165P/Q179K/T203E/M205R/V219R/F414H/F418I/E441M/K488N/I503E	+
1889/1890	T203E/M205R/I208A/F353Q	+
1891/1892	T160M/Q165P/M205R	+
1893/1894	T203E/I208A/V219R/Q376H/E441M	+
1895/1896	T160M/Q165P/Q179K/T203E/C307L/Q376H/H413G/A495G	+
1897/1898	Q179K/T203E/I208A/L326T/F353Q/Q376H/T484E	+
1899/1900	T160M/Q165P/M205R/F414H/E441M/A495G	+
1901/1902	Q165P/Q179K/M205R/H413G/E441M	+
1903/1904	I208A/K488N/K506E	+
1905/1906	Q179K/I208A/F353Q/D460G	+
1907/1908	T160M/Q165P/T203E/M205R/C307L/E371D/Q376H/H413G/F414H	+
1909/1910	T160M/T203E/I208A/E371D/H413G/F414H	+
1911/1912	T160M/T203E/I208A/C307L/F353Q/A495G	+
1913/1914	T160M	+
1827/1828	T160M/T203E/M205R/E441M	+
1915/1916	T203E/I208A/L326T/F353Q	+
1917/1918	M205R/I208A/C307L/F353Q/Q376H/H413G	+
1919/1920	T203E/M205R/I208A/C307L/E441M	+
1921/1922	Q179K/M205R/F353Q	+
1923/1924	T203E/H413G/I503E/K506E	+
1925/1926	T160M/Q165P/T203E/I208A/E371D/Q376H/H413G/F414H	+
1927/1928	M205R/I208A/F414H	+
1929/1930	Q165P/T203E/M205R	+
1931/1932	M205R/C307L/Q376H/F414H/E441M/A495G	+
1933/1934	T160M/Q179K/I208A/C307L/E371D/Q376H/F414H	+
1935/1936	T203E/M205R/I208A/C307L/D330N/F353Q/E441M/D460G/I503E/K506E	+
1937/1938	Q165P/T203E/I208A/L326T/Q376H/I503E	+
1939/1940	T160M/Q165P/M205R/I208A	+
1941/1942	Q179K/T203E/M205R	+
1943/1944	Q179K	+
1945/1946	L326T/F353Q/E371D/Q376H/F414H	+
1825/1826	E441M	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1654 and defined as follows: “+” 1.09 to 2.31-fold increased activity; “++” > 2.31-fold increased activity; “+++” > 3.89-fold increased activity.		

EXAMPLE 25

Improvements over SEQ ID NO: 1830 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0404] SEQ ID NO: 1830 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination

of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 25.1.

[0405] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1-10 μ M oligonucleotide, 25-50 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 25.1-Reaction conditions	
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-50 °C; Preincubation Time (min)-60; Reaction temperature (C)-50 °C; Reaction Time (min)-60; Reaction volume (μ L) -1; NTP (μ M)-3'P-mATP; NTP conc (μ M)-50; Oligonucleotide-5'-6-FAM-T15AT*mG; Substrate conc (μ M)-10; Product -5'-6-FAM-T15AT*mGmA-3'P.	

[0406] Activity relative to SEQ ID NO: 1830 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1830 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 25.2.

Table 25.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1830)	FIOP Product Peak Area Relative to SEQ ID NO: 1830
1947/1948	I163V/Q179K/D277E/D338T/L340A	++
1949/1950	I163V/F414H/E441M	++
1951/1952	H177L	+
1953/1954	R171K/T200V/T334R/G406P/M490L	+
1955/1956	S292T/G406P	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1830 and defined as follows: "+" 1.52 to 2.05-fold increased activity; "++" > 2.05-fold increased activity.		

EXAMPLE 26

Activity improvement over SEQ ID NO: 1100

Activity with 2'OMe modified oligonucleotides and nucleotide triphosphates of shake-flask purified TdT variants

[0407] TdT variants of SEQ ID NO: 1100, 1336, and 1958 were produced in shake flask and purified as described in Example 3.

[0408] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 1 μ M oligonucleotide, 25 μ M nucleotide triphosphate, 1 μ M TdT, 20 mM triethanolamine (pH 7.8), and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 26.1

Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Preincubation Temp (C) – none; Preincubation Time (min)- none; Reaction temperature (C)- 50 °C; Reaction Time (min)- 60; Reaction volume (μ L)- 1; TdT concentration (μ M)- 4; NTP- mATP; NTP conc (μ M)-25; Oligonucleotide-5'-6-FAM-T17mAmUmC, 5'-6-FAM-T17mUmUmC, 5'-6-FAM-T17mCmUmG ; Oligonucleotide conc (μ M)- 10; Product- 5'-6-FAM-T17mAmUmCmA, 5'-6-FAM-T17mUmUmCmA, 5'-6-FAM-T17mCmUmGmA .
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[0409] Activity relative to SEQ ID NO: 1100 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1100 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Tables 26.2, 26.3, and 26.4.

Table 26.2 Product 5'-6-FAM-T17mAmUmCmA

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1100)	FIOP Product Peak Area Relative to SEQ ID NO: 1100
1957/1958	L264R	+
1335/1336	T101E/M137A/L264R/E476R/S525F	++
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1100 and defined as follows: “+” 1.15 to 1.30-fold increased activity; “++” > 1.30-fold increased activity.		

Table 26.3 Product 5'-6-FAM-T17mUmUmCmA

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1100)	FIOP Product Peak Area Relative to SEQ ID NO: 1100
1957/1958	L264R	+
1335/1336	T101E/M137A/L264R/E476R/S525F	++
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1100 and defined as follows: “+” 1.15 to 1.30-fold increased activity; “++” > 1.30-fold increased activity.		

Table 26.4 Product 5'-6-FAM-T17mCmUmGmA

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1100)	FIOP Product Peak Area Relative to SEQ ID NO: 1100
1957/1958	L264R	+
1335/1336	T101E/M137A/L264R/E476R/S525F	++

Table 26.4 Product 5'-6-FAM-T17mCmUmGmA

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1100)	FIOP Product Peak Area Relative to SEQ ID NO: 1100
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1100 and defined as follows: "+" 1.15 to 1.30-fold increased activity; "++" > 1.30-fold increased activity.		

EXAMPLE 27**Activity improvement over SEQ ID NO: 1654**

Activity of shake-flask purified TdT variants with 3'-phosphorylated nucleotide triphosphates

[0410] TdT variants of SEQ ID NO: 1654, 1830, and 1950 were produced in shake flask and purified as described in Example 3.

[0411] Reactions were performed in 96-well format 200 µL BioRad PCR plates. Reactions included 1 µM oligonucleotide, 25 µM nucleotide triphosphate, 1 µM TdT, 20 mM triethanolamine (pH 7.8), and 250 µM cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 27.1

Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Preincubation Temp (C) – none; Preincubation Time (min)- none; Reaction temperature (C)- 50°C; Reaction Time (min)- 60; Reaction volume (µL)- 1; TdT concentration (µM)- 4; NTP- 3'-phos-mATP; NTP conc (µM)-50; Oligonucleotide-5'-6-FAM-T15AT*mG, 5'-6-FAM-T15AT*mA, 5'-6-FAM-T15AT*mC, 5'-6-FAM-T15AT*mU; Oligonucleotide conc (µM)- 25; Product- -5'-6-FAM-T15AT*mGmA-3'phos, 5'-6-FAM-T15AT*mAmA-3'phos, 5'-6-FAM-T15AT*mCmA-3'phos, 5'-6-FAM-T15AT*mUmA-3'phos.
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[0412] Activity relative to SEQ ID NO: 1654 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1654 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Tables 27.2, 27.3, 27.4, and 27. 5.

Table 27.2 Product 5'-6-FAM-T15AT*mGmA-3'phos

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1654)	FIOP Product Peak Area Relative to SEQ ID NO: 1654
1829/1830	T160M/Q165P/T203E/M205R/V219R/F353Q/D460G/K488N	+

Table 27.2 Product 5'-6-FAM-T15AT*mGmA-3'phos

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1654)	FIOP Product Peak Area Relative to SEQ ID NO: 1654
1949/1950	T160M/I163V/Q165P/T203E/M205R/V219R/F353Q/F414H/E441M/D460G/K488N	++

Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1654 and defined as follows: "+" 1.46 to 2.20-fold increased activity; "++" > 2.20-fold increased activity; "+++" > 4.05-fold increased activity.

Table 27.3 Product 5'-6-FAM-T15AT*mAmA-3'phos

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1654)	FIOP Product Peak Area Relative to SEQ ID NO: 1654
1829/1830	T160M/Q165P/T203E/M205R/V219R/F353Q/D460G/K488N	++
1949/1950	T160M/I163V/Q165P/T203E/M205R/V219R/F353Q/F414H/E441M/D460G/K488N	+++

Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1654 and defined as follows: "+" 1.46 to 2.20-fold increased activity; "++" > 2.20-fold increased activity; "+++" > 4.05-fold increased activity.

Table 27.4 Product 5'-6-FAM-T15AT*mCmA-3'phos

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1654)	FIOP Product Peak Area Relative to SEQ ID NO: 1654
1829/1830	T160M/Q165P/T203E/M205R/V219R/F353Q/D460G/K488N	++
1949/1950	T160M/I163V/Q165P/T203E/M205R/V219R/F353Q/F414H/E441M/D460G/K488N	++

Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1654 and defined as follows: "+" 1.46 to 2.20-fold increased activity; "++" > 2.20-fold increased activity; "+++" > 4.05-fold increased activity.

Table 27.5 5'-6-FAM-T15AT*mUmA-3'phos

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1654)	FIOP Product Peak Area Relative to SEQ ID NO: 1654
1829/1830	T160M/Q165P/T203E/M205R/V219R/F353Q/D460G/K488N	+
1949/1950	T160M/I163V/Q165P/T203E/M205R/V219R/F353Q/F414H/E441M/D460G/K488N	++

Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1654 and defined as follows: "+" 1.46 to 2.20-fold increased activity; "++" > 2.20-fold increased activity; "+++" > 4.05-fold increased activity.

EXAMPLE 28**Activity of immobilized TdT enzymes**Activity of shake-flask purified and immobilized TdT variants

[0413] TdT variants of SEQ ID NO: 1596, 1654, 1822, 1824, 1826, 1828, 1830, and 1834 were produced in shake flask and purified as described in Example 3.

[0414] Enzyme immobilization was performed in 1 mL round bottom costar 96-well plate. A slurry of 25 mg of controlled porosity glass (CPG) with either hydrophilic surface (EziG-1 and EziG-3, EnginZyme) or hydrophobic surface (EziG-2) was prepared in 1 mL of water. 1 mg of EziG (1, 2, or 3) (40 μ L of slurry) was transferred to the plate, centrifuged and the supernatant was removed. 0.1 mg of TdT protein solutions (41 μ M) were transferred to wells containing solid support. Enough storage buffer (20 mM Tris pH 7.4, 100 mM KCl, 0.1 mM EDTA) was added to reach a 50 μ L volume. The plate was sealed and gently shaken at room temperature. After 24 hours, the contents were centrifuged, and the supernatant was removed. The immobilized variants were washed twice with reaction buffer (20 mM triethanolamine pH 7.8), centrifuged, and the supernatant was removed. To each well was added 40 μ L of reaction mixture including: 50 μ M 18-mer oligonucleotide (99% unlabeled T14-ATC-mC and 1% FAM- T14-ATC-mC), 100 μ M 2',3'-dideoxyguanosine-5'-triphosphate ddGTP, 0.5 mU/ μ L *E. coli* pyrophosphatase (New England Biolabs), 20 mM triethanolamine (pH 7.8), and 250 μ M cobalt (II) chloride. The plate was sealed and shaken at 500 rpm at 50 °C for 90 minutes. Reactions were quenched by the addition of 120 μ L of 35 mM EDTA. Quenched reactions were analyzed by capillary electrophoresis as described in Example 4.

[0415] Activity relative to SEQ ID NO: 1596 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1596 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Tables 28.1, 28.2, and 28.3.

Table 28.1 Activity with EziG-1		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1596)	FIOP Product Peak Area Relative to SEQ ID NO: 1596
1653/1654	R406G/I408A/S442G/S446P	++
1821/1822	T160M/Q165P/M205R/V219R/R406G/I408A/E441M/S442G/S446P	+++
1833/1834	T160M/V219R/D330K/R406G/I408A/S442G/S446P/T484E	+++
1827/1828	T160M/T203E/M205R/R406G/I408A/E441M/S442G/S446P	++
1829/1830	T160M/Q165P/T203E/M205R/V219R/F353Q/R406G/I408A/S442G/S446P/D460G/K488N	+++
1825/1826	R406G/I408A/E441M/S442G/S446P	++
1823/1824	M205R/C307L/R406G/I408A/E441M/S442G/S446P/D460G/K488N	++
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1596 and defined as follows: “+” 1.0 to 1.5-fold increased activity; “++” > 1.5-fold increased activity; “+++” > 2.5-fold increased activity.		

Table 28.2 Activity with EziG-2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1596)	FIOP Product Peak Area Relative to SEQ ID NO: 1596
1821/1822	T160M/Q165P/M205R/V219R/R406G/I408A/E441M/S442G/S446P	+
1833/1834	T160M/V219R/D330K/R406G/I408A/S442G/S446P/T484E	+

1827/1828	T160M/T203E/M205R/R406G/I408A/E441M/S442G/S446P	+
1829/1830	T160M/Q165P/T203E/M205R/V219R/F353Q/R406G/I408A/S442G/S446P/D460G/K488N	+
1823/1824	M205R/C307L/R406G/I408A/E441M/S442G/S446P/D460G/K488N	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1596 and defined as follows: “+” 1.0 to 1.5-fold increased activity; “++” > 1.5-fold increased activity; “+++” > 2.5-fold increased activity.		

Table 28.3 Activity with EziG-3		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1596)	FIOP Product Peak Area Relative to SEQ ID NO: 1596
1653/1654	R406G/I408A/S442G/S446P	+
1821/1822	T160M/Q165P/M205R/V219R/R406G/I408A/E441M/S442G/S446P	++
1833/1834	T160M/V219R/D330K/R406G/I408A/S442G/S446P/T484E	++
1827/1828	T160M/T203E/M205R/R406G/I408A/E441M/S442G/S446P	++
1829/1830	T160M/Q165P/T203E/M205R/V219R/F353Q/R406G/I408A/S442G/S446P/D460G/K488N	++
1825/1826	R406G/I408A/E441M/S442G/S446P	++
1823/1824	M205R/C307L/R406G/I408A/E441M/S442G/S446P/D460G/K488N	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1596 and defined as follows: “+” 1.0 to 1.5-fold increased activity; “++” > 1.5-fold increased activity; “+++” > 2.5-fold increased activity.		

EXAMPLE 29

Improvements over SEQ ID NO: 1950 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0416] SEQ ID NO: 1950 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 29.1.

[0417] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 29.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (μL)-1; NTP (μM)-mATP-3'P; NTP conc (μM)-100; Oligonucleotide-5'-6-FAM-T15AT*mG; Substrate conc (μM)-10; Product -5'-6-FAM-T15AT*mGmA-3'P.

[0418] Activity relative to SEQ ID NO: 1950 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1950 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 29.2.

Table 29.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1950)	FIOP % product Relative to SEQ ID NO: 1950
2003/2004	V455L	+++
2005/2006	T362S	+++
2007/2008	R258W	+++
2009/2010	G468Q	+++
2011/2012	G468W	+++
2013/2014	K274V	+++
2015/2016	H414G	+++
2017/2018	R258Q	+++
2019/2020	K407S	+++
2021/2022	S405Y	+++
2023/2024	L411Q/H414F	+++
2025/2026	E399G	+++
2027/2028	Y192C	+++
2029/2030	Y192R	+++
2031/2032	H494A	+++
2033/2034	K274M	+++
2035/2036	T286N	+++
2037/2038	E399D	+++
2039/2040	H413A/H414F	+++
2041/2042	E193S	+++
2043/2044	D409Q/H414F	+++
2045/2046	L411R/H414F	+++
2047/2048	G245A	+++
2049/2050	A410V/H414F	+++
2051/2052	L466M	++
2053/2054	E399F	++
2055/2056	S405L	++
2057/2058	K407N	++
2059/2060	S509K	++
2061/2062	K278N	++

2063/2064	A410Y/H414F	++
2065/2066	F398W	++
2067/2068	F194M	++
2069/2070	L359V	++
2071/2072	K278C	++
2073/2074	H414F/C417W	++
2075/2076	H413I/H414F	++
2077/2078	D409K/H414F	++
2079/2080	K274Q	++
2081/2082	A410S/H414F	++
2083/2084	A410I/H414F	++
2085/2086	V316C	++
2087/2088	K278L	++
2089/2090	L185F	++
2091/2092	F194C	++
2093/2094	P352R	++
2095/2096	Y259N	++
2097/2098	A304L	++
2099/2100	F194W	++
2101/2102	R291M	++
2103/2104	R258K	++
2105/2106	K274N	++
2107/2108	S261G	++
2109/2110	G468S	++
2111/2112	P352G	++
2113/2114	S509G	++
2115/2116	L411G/H414F	++
2117/2118	L185C	++
2119/2120	K274P	++
2121/2122	V378L	++
2123/2124	K274T	++
2125/2126	R258G	+
2127/2128	R258L	+
2129/2130	K263S	+
2131/2132	F194A	+
2133/2134	R258E	+
2135/2136	Y280S	+
2137/2138	S405G	+
2139/2140	T362Y	+
2141/2142	Y308N	+
2143/2144	Y192L	+
2145/2146	H414F/A415W	+
2147/2148	L284I	+
2149/2150	G468M	+
2151/2152	V163I/Q169E	+
2153/2154	A465E	+
2155/2156	H494G	+

2157/2158	H494L	+
2159/2160	D198L	+
2161/2162	L411A/H414F	+
2163/2164	K263I	+
2165/2166	E399C	+
2167/2168	L306I	+
2169/2170	V271C	+
2171/2172	V455I	+
2173/2174	L411I/H414F	+
2175/2176	H413G/H414F	+
2177/2178	N197G	+
2179/2180	F194G	+
2181/2182	Y192I	+
2183/2184	Q251R	+
2185/2186	V316A	+
2187/2188	F194D	+
2189/2190	K297P	+
2191/2192	S396T	+
2193/2194	Y259V	+
2195/2196	N197R	+
2197/2198	H494C	+
2199/2200	F347Q	+
2201/2202	H494W	+
2203/2204	A410F/H414F	+
2205/2206	H413F/H414F	+
2207/2208	E186C	+
2209/2210	K297L	+
2211/2212	E399T	+
2213/2214	R291W	+
2215/2216	E193R	+
2217/2218	T286S	+
2219/2220	Y280L	+
2221/2222	L306M	+
2223/2224	Y192G	+
2225/2226	L411T/H414F	+
2227/2228	L284M	+
2229/2230	V163I/Q169R	+
2231/2232	N197L	+
2233/2234	K407F	+
2235/2236	V290A	+
2237/2238	D198A	+
2239/2240	A415F	+
2241/2242	S261A	+
2243/2244	L393R	+
2245/2246	L411F/H414F	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO:1950 and defined as follows: "+" 1.21 to 1.91, "++" > 1.91, "+++" > 3.34.		

EXAMPLE 30**Improvements over SEQ ID NO: 2008 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0419] SEQ ID NO: 2008 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 30.1.

[0420] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 30.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15AT*mG; Substrate conc (μ M)-10; Product -5'-6-FAM-T15AT*mGmA-3'P.
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[0421] Activity relative to SEQ ID NO: 2008 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2008 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 30.2.

Table 30.2

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2008)	FIOP % Product Relative to SEQ ID NO: 2008
2247/2248	H413A/H414G/G468Q	+++
2249/2250	H413A/H414G	+++
2251/2252	L411G/H413A/G468Q	+++
2253/2254	K263H/L411G/H413A/H414G/G468Q	+++
2255/2256	H413A/G468Q	++
2257/2258	G468Q	++
2259/2260	P158H/K274V/L411G/H413A/H414G	++
2261/2262	K274V/L411G/G468Q	++

2263/2264	L411G/H414G	++
2265/2266	H413A	++
2267/2268	L185F/L411G/H413A/H414G	++
2269/2270	K274V/T286N/L411G/H413A/C417W/G468Q	+
2271/2272	H414G	+
2273/2274	K263H/H413A/H414G	+
2275/2276	K278N/L411G/H413A/G468Q	+
2277/2278	L185F/K274V/H413A/H414G	+
2279/2280	K274V/L411G/C417W/G468Q	+
2281/2282	L411G/H413A/C417W/G468Q	+
2283/2284	K274V/G468Q	+
2285/2286	L411G/H413A/C417W	+
2287/2288	H413A/C417W/G468Q	+
2289/2290	K274V/H413A/H414G/C417W/G468Q	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2008 and defined as follows: "+" 1.05 to 1.28, "++" > 1.28, "+++" > 1.67.		

EXAMPLE 31

Improvements over SEQ ID NO: 2008 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0422] SEQ ID NO: 2008 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 31.1.

Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 31.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (C)-50; Preincubation Time (min)-60; Reaction temperature (C)-50; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15AT*mU; Substrate conc (μ M)-10; Product - 5'-6-FAM-T15AT*mUmA-3'P.

[0423] Activity relative to SEQ ID NO: 2008 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2008 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 31.2.

Table 31.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2008)	FIOP % Product Relative to SEQ ID NO: 2008
2291/2292	R26A/Q27R	+++
2293/2294	R26G	+++
2295/2296	E246N	+++
2297/2298	L248R	+++
2299/2300	R26I	+++
2301/2302	G8P	+++
2303/2304	H3G	+++
2305/2306	S36G	++
2307/2308	R26Q	++
2309/2310	T58N	++
2311/2312	N76P	++
2313/2314	R26T	++
2315/2316	T58M	++
2317/2318	S36K	++
2319/2320	V253L	++
2321/2322	F62W	++
2323/2324	G151S	++
2325/2326	T58S	+
2327/2328	S116A	+
2329/2330	P157V	+
2331/2332	S12V	+
2333/2334	E249R	+
2335/2336	D147G	+
2337/2338	I50L	+
2339/2340	H3A	+
2341/2342	N90L	+
2343/2344	I15A	+
2345/2346	L248V	+
2347/2348	E249A	+
2349/2350	N255G	+
2351/2352	G11C	+
2353/2354	E249G	+
2355/2356	N90S	+
2357/2358	Y39A	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2008 and defined as follows: "+" 1.16 to 1.26, "++" > 1.26, "+++" > 1.41.		

EXAMPLE 32

Improvements over SEQ ID NO: 2008 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0424] SEQ ID NO: 2008 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 32.1.

Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 32.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-mATP; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15mUmGmA; Substrate conc (μ M)-10; Product -5'-6-FAM-T15mUmGmA.
--

[0425] Activity relative to SEQ ID NO: 2008 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2008 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 32.2.

Table 32.2

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2008)	FIOP % Product Relative to SEQ ID NO: 2008
2359/2360	N90M	+++
2361/2362	I15L	+++
2363/2364	K70A	+++
2365/2366	S12A	+++
2367/2368	R26S	++
2369/2370	S81E	++
2371/2372	I38T	++
2373/2374	S79T	++
2375/2376	R16S	++
2377/2378	I38L	++
2379/2380	G9T	+

2381/2382	S12Q	++
2383/2384	G11S	+
2385/2386	P157L	+
2387/2388	I15Q	+
2389/2390	G9A	+
2391/2392	R26W	+
2393/2394	S12N	+
2395/2396	T58A	+
2397/2398	E249T	+
2399/2400	Y39G	+
2401/2402	P158A	+
2403/2404	G151I	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2008 and defined as follows: "+" 1.03 to 1.07, "++" > 1.07, "+++" > 1.12.		

EXAMPLE 33

Improvements over SEQ ID NO: 2008 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0426] SEQ ID NO: 2008 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 33.1.

Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 33.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15AT*mU; Substrate conc (μ M)-10; Product -5'-6-FAM-T15AT*mU-mA-3'P.

[0427] Activity relative to SEQ ID NO: 2008 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2008 (where

the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 33.2.

Table 33.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2008)	FIOP % Product Relative to SEQ ID NO: 2008
2405/2406	R473S	+++
2407/2408	R473G	+++
2409/2410	R473A	+++
2411/2412	A189R	+++
2413/2414	N493E	+++
2415/2416	R480E	+++
2417/2418	H413E	+++
2419/2420	H413G	+++
2421/2422	R473Q	+++
2423/2424	A189Q	+++
2425/2426	H413S	++
2427/2428	Q353R	++
2429/2430	R196T	++
2431/2432	R480S	++
2433/2434	R480G	++
2435/2436	R473P	++
2437/2438	R480A	++
2439/2440	A410E	++
2441/2442	R206N	++
2443/2444	C307S	++
2445/2446	R196S	++
2447/2448	C307H	++
2449/2450	R473D	++
2451/2452	A408E	++
2453/2454	C307E	++
2455/2456	Q397A	+
2457/2458	H413L	+
2459/2460	K318R	+
2461/2462	I324V	+
2463/2464	W481V	+
2465/2466	W469Y	+
2467/2468	R196A	+
2469/2470	H413M	+
2471/2472	C307L	+
2473/2474	A408W	+
2475/2476	N493Q	+
2477/2478	W481S	+
2479/2480	W469F	+
2481/2482	R196Y	+
2483/2484	R314M	+
2485/2486	R314A	+

2487/2488	A408L	+
2489/2490	F262V	+
2491/2492	A189E	+
2493/2494	W481L	+
2495/2496	R205E	+
2497/2498	R314Q	+
2499/2500	W481A	+
2501/2502	L491M	+
2503/2504	R480W	+
2505/2506	R480L	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2008 and defined as follows: "+" 1.08 to 1.42, "++" > 1.42, "+++" > 1.71.		

EXAMPLE 34

Improvements over SEQ ID NO: 2254 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0428] SEQ ID NO: 2254 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 34.1.

Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 $^{\circ}$ C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 34.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (C)-50 $^{\circ}$ C; Preincubation Time (min)-60; Reaction temperature (C)-50 $^{\circ}$ C; Reaction Time (min)-30; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15AT*mU; Substrate conc (μ M)-10; Product -5'-6-FAM-T15AT*mUmA-3'P.
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[0429] Activity relative to SEQ ID NO: 2254 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2254 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 34.2.

Table 34.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2254)	FIOP % product Relative to SEQ ID NO: 2254
2507/2508	F398W/L466M	+++
2509/2510	E399D/G411Q/K416Q	+++
2511/2512	T362S/K407S/V455L	++
2513/2514	S396T/F398W/A410V/L466M	++
2515/2516	L284M/F398W/L466M	+
2517/2518	N197R/V455L	+
2519/2520	N197R/K407S/V455L	+
2521/2522	L466M	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2254 and defined as follows: "+" 1.13 to 1.20 (first 50%), "++" > 1.20, "+++" > 1.39.		

EXAMPLE 35

Improvements over SEQ ID NO: 2514 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0430] SEQ ID NO: 2514 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 35.1.

[0431] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 35.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15AmU*mG; Substrate conc (μ M)-10; Product -5'-6-FAM-T15AmU*mGmA-3'P.

[0432] Activity relative to SEQ ID NO: 2514 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2514 (where

the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 35.2.

Table 35.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2514)	FIOP % product Relative to SEQ ID NO: 2514
2523/2524	R26Q/N90L/E246N/L248R/T362S/V455L	+++
2525/2526	R26Q/N90L/E246N/L248R/V455L	+++
2527/2528	R26I/L248R/V455L	+++
2529/2530	R26G/N90L/L248V/V455L/Y459H	+++
2531/2532	R26Q/N90L/E246N/V455L	+++
2533/2534	R26Q/N90L/L248R/K266V/V455L	+++
2535/2536	N90L/E246N/L248R/S261A/K266V/T362S/V455L	+++
2537/2538	R26G/N90L/K266V	+++
2539/2540	R26Q/T173I/L248R	+++
2541/2542	R26Q/E246N/L248V/T362S	++
2543/2544	R26Q/L248V	++
2545/2546	R26Q/L248R	++
2547/2548	F62W/E249R	++
2549/2550	L248V/K266V/T362S	++
2551/2552	R26G/N90L/L248V/V455L	++
2553/2554	N90L/E246N/L248R/K266V/T362S/V455L	++
2555/2556	R26I/N90L/E246N/L248R	++
2557/2558	R26G/E246N/L248R/T362S	++
2559/2560	R26I/L248R/K266V/T362S	++
2561/2562	R26Q/N90L/E246N/L248R/S261A	++
2563/2564	R26G/E246N/L248V/V455L	++
2565/2566	R26Q/T362S	++
2567/2568	R26G/N90L/E246N/K266V/T362S	++
2569/2570	L248R	++
2571/2572	R26I/E246N/L248R/T362S/V455L	+
2573/2574	R26Q/L248R/S261A/K266V/T362S/V455L	+
2575/2576	L248R/V455L	+
2577/2578	E246N/L248R/T362S	+
2579/2580	R26G/L248V/S261A/K266V	+
2581/2582	R26Q/L248R/T362S/V455L	+
2583/2584	R26Q/N90L/E246N/L248V/T362S/V455L	+
2585/2586	R26Q/L248V/T362S/V455L	+
2587/2588	R26G/N90L/T362S/V455L	+
2589/2590	R26Q/L248R/V455L	+
2591/2592	E246N/L248R	+
2593/2594	E246N/K266V/V455L	+
2595/2596	R26Q/N90L/E246N/T362S	+
2597/2598	R26G/E246N	+
2599/2600	R26Q/L248V/K266V/T362S	+
2601/2602	T58N/N197L/E249R/K407N/V410S	+

2603/2604	R26G/N90L/E246N	+
2605/2606	R26Q/T362S/V455L	+
2607/2608	L248V/T362S/V455L	+
2609/2610	N90L/E246N/L248R	+
2611/2612	R26G/N90L/E94K/L248R/S261A/K266V/T362S/V455L	+
2613/2614	R26G/L248R	+
2615/2616	T362S	+
2617/2618	R26G/N90L/E246N/L248R	+
2619/2620	R26I/N90L/L248R	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2514 and defined as follows: "+" 2.23 to 3.99, "++" > 3.99, "+++" > 6.49.		

EXAMPLE 36

Improvements over SEQ ID NO: 2524 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0433] SEQ ID NO: 2524 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 36.1.

Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 $^{\circ}$ C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 36.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (C)-50 $^{\circ}$ C; Preincubation Time (min)-60; Reaction temperature (C)-50 $^{\circ}$ C; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-ATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15AmU*mG; Substrate conc (μ M)-10; Product -5'-6-FAM-T15AmU*mGmA-3'P.

[0434] Activity relative to SEQ ID NO: 2524 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2524 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 36.2.

Table 36.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2524)	FIOP % product Relative to SEQ ID NO: 2524
2621/2622	M30G/K266V/Q353R	+++
2623/2624	M30G/S261A/K266V/Q353R/Q468S	+++
2625/2626	K266V/Q353R/Q468S	+++
2627/2628	M30E/A189R/K266V	+++
2629/2630	K266V/Q468S	+++
2631/2632	M30G/K266V	++
2633/2634	A189R/S261A	++
2635/2636	A189R/Q353R	++
2637/2638	I38T/S81E/K318R	++
2639/2640	K266V/C307S/Q353R/Q468S	++
2641/2642	G11S/T58N/L227M/N246E	++
2643/2644	M30T/K266V/K303E	++
2645/2646	S12A/R16S/P158A/N246E/R248L/E249R	++
2647/2648	M30T/A189R/S261A/K266V/Q353R/A465E/Q468S	+
2649/2650	N246E/E249R	+
2651/2652	G9T/R16S/F62W/P157L/N246E/E249R/S362T	+
2653/2654	S79T/S81E	+
2655/2656	Y39G/S79T	+
2657/2658	Y39G	+
2659/2660	S261A/Q353R	+
2661/2662	T58N/P157L/P158A/S362T	+
2663/2664	K70A/Q353R	+
2665/2666	I38T/N197L	+
2667/2668	S81E	+
2669/2670	A189R	+
2671/2672	I38T	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2524 and defined as follows: "+" 1.09 to 1.33, "++" > 1.33, "+++" > 1.77.		

EXAMPLE 37

Improvements over SEQ ID NO: 2524 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0435] SEQ ID NO: 2524 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 37.1.

[0436] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution

was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 37.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (μL)-1; NTP (μM)-mATP-3'P; NTP conc (μM)-100; Oligonucleotide-5'-6-FAM-T15AmU*mG; Substrate conc (μM)-25; Product -5'-6-FAM-T15AmU*mGmA-3'P.

[0437] Activity relative to SEQ ID NO: 2524 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2524 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 37.2.

Table 37.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2524)	FIOP % product Relative to SEQ ID NO: 2524
2673/2674	T379L	+++
2675/2676	T379M	+++
2677/2678	K427L	+++
2679/2680	K402G	+++
2681/2682	K427M	+++
2683/2684	L327M/G406T	+++
2685/2686	W382L	+++
2687/2688	A304V/Q329R	+++
2689/2690	T379I	++
2691/2692	T484A	++
2693/2694	K506P	++
2695/2696	L340V	++
2697/2698	T379V	++
2699/2700	F504K	++
2701/2702	W382F	++
2703/2704	L403S	++
2705/2706	Q169M	++
2707/2708	L403A	++
2709/2710	S292N	++
2711/2712	K402V	++
2713/2714	K427W	++
2715/2716	L340I	+
2717/2718	L403P	+
2719/2720	P404S	+

2721/2722	K427R	+
2723/2724	Q373G	+
2725/2726	D429R	+
2727/2728	N191V/A413V	+
2729/2730	M490R	+
2731/2732	A495S	+
2733/2734	A495G	+
2735/2736	L403E	+
2737/2738	Q353H/Y459V	+
2739/2740	Y459I	+
2741/2742	A495C	+
2743/2744	K402S	+
2745/2746	G461S	+
2747/2748	P404D	+
2749/2750	Q329R	+
2751/2752	V47A/L326R	+
2753/2754	T200V	+
2755/2756	L403R	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2524 and defined as follows: "+" 1.21 to 1.43, "++" > 1.43, "+++" > 1.80.		

EXAMPLE 38

Improvements over SEQ ID NO: 2524 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0438] SEQ ID NO: 2524 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 38.1.

Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 38.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-60 °C; Preincubation Time (min)-

60; Reaction temperature (C)-60 °C; Reaction Time (min)-60; Reaction volume (μL)-1; NTP (μM)-mATP-3'P; NTP conc (μM)-100; Oligonucleotide-5'-6-FAM-T15AT*mG; Substrate conc (μM)-25; Product -5'-6-FAM-T15AT*mGmA-3'P.

[0439] Activity relative to SEQ ID NO: 2524 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2524 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 38.2.

Table 38.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2524)	FIOP % product Relative to SEQ ID NO: 2524
2675/2676	T379M	+++
2693/2694	K506P	+++
2681/2682	K427M	+++
2757/2758	S325W	+++
2673/2674	T379L	+++
2691/2692	T484A	+++
2677/2678	K427L	+++
2713/2714	K427W	+++
2759/2760	K427F	+++
2679/2680	K402G	++
2761/2762	K506T	++
2763/2764	T200N	++
2765/2766	K427C	++
2723/2724	Q373G	++
2735/2736	L403E	++
2731/2732	A495S	++
2767/2768	K508S	++
2717/2718	L403P	++
2747/2748	P404D	++
2739/2740	Y459I	++
2769/2770	K427Y	++
2745/2746	G461S	++
2771/2772	K506S	+
2773/2774	A189V	+
2743/2744	K402S	+
2775/2776	P404E	+
2777/2778	T484M	+
2709/2710	S292N	+
2779/2780	G406N	+
2781/2782	K508T	+
2783/2784	E203D	+
2785/2786	K427N	+
2719/2720	P404S	+

2733/2734	A495G	+
2787/2788	N175L	+
2789/2790	T484H	+
2695/2696	L340V	+
2791/2792	K402E	+
2793/2794	Q373C	+
2795/2796	L403G	+
2707/2708	L403A	+
2797/2798	E203M	+
2799/2800	Q179L	+
2801/2802	S293Q	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2524 and defined as follows: "+" 1.19 to 1.44, "++" > 1.44, "+++" > 1.97.		

EXAMPLE 39

Improvements over SEQ ID NO: 2638 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0440] SEQ ID NO: 2638 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 39.1.

[0441] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 39.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15AmU*mG; Substrate conc (μ M)-25; Product -5'-6-FAM-T15AmU*mGmA-3'P.
--

[0442] Activity relative to SEQ ID NO: 2638 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2638 (where

the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 39.2.

Table 39.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2638)	FIOP % product Relative to SEQ ID NO: 2638
2803/2804	S79T/A189R/C307L/V410E	+
2805/2806	M30G/T58N/S79T/A189R/C307L/R480E	+
2807/2808	S79T/C307L	+
2809/2810	G11S/M30G/S79T/A189R/R480E	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2638 and defined as follows: "+" from 1.29 to 1.94.		

EXAMPLE 40

Improvements over SEQ ID NO: 2804 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0443] SEQ ID NO: 2804 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 40.1.

[0444] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 40.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15AmG*mC; Substrate conc (μ M)-25; Product -5'-6-FAM-T15AmG*mCmA-3'P.

[0445] Activity relative to SEQ ID NO: 2804 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2804 (where

the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 40.2.

Table 40.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2804)	FIOP % product Relative to SEQ ID NO: 2804
2811/2812	A304V/L340I/K402G/L403S/K506P	+++
2813/2814	A304V/L340I/K402G/K506P	+++
2815/2816	A304V/L340V/K402G/L403S/F504K/K506P	+++
2817/2818	Q169M/A304V/K402G/L403S/K427L/K506P	+++
2819/2820	A304V/L340V/K402G/K427L/F504K/K506P	+++
2821/2822	A304V/L340I/K402G/L403A/K427L/D429R/F504K/K506P	+++
2823/2824	A304V/K402G/L403A/F504K/K506P	+++
2825/2826	L340V/K402G/K427L/D429R/K506P	+++
2827/2828	A304V/K402G/L403P/K506P	+++
2829/2830	Q169M/A304V/L340I/D429R/K506P	+++
2831/2832	Q169M/A304V/L340V/K402G/K427L	+++
2833/2834	K402G/L403A/K427M/F504K/K506P	+++
2835/2836	A304V/F504K/K506P	+++
2837/2838	A304V/L340I/K427M	+++
2839/2840	L340I/K402G/D429R/F504K/K506P	++
2841/2842	Q169M/L403A/K427L/K506P	++
2843/2844	Q169M/A304V/K427M/F504K/K506P	++
2845/2846	A304V/K402G/L403S	++
2847/2848	A304V/L403S/F504K	++
2849/2850	A304V/L340I/K402G	++
2851/2852	A304V/K402G/L403A/D429R	++
2853/2854	A304V/K402G/L403P/K427M/F504K	++
2855/2856	K402G/L403S/K427M/F504K/K506P	++
2857/2858	A304V/L340I/L403A/K427L/D429R/F504K/K506P	++
2859/2860	L403S/K427L	++
2861/2862	A304V/F504K	++
2863/2864	Q169M/A304V/L340I/F504K	++
2865/2866	Q169M/A304V/F504K	++
2867/2868	Q169M/K402G/F504K/K506P	++
2869/2870	Q169M/K402G/L403A/F504K/K506P	++
2871/2872	T379M/Q468S	++
2873/2874	K402G/K427L/F504K	++
2875/2876	K266V/L327M/Q329R/P404S/E410V	++
2877/2878	Q169M/K402G/K427L/D429R/F504K	++
2879/2880	K427L	++
2881/2882	L340V/K402G/L403A	+
2883/2884	Q169M/L340I/K427L	+
2885/2886	K402G/F504K	+
2887/2888	K402G/L403S/D429R/F504K	+
2889/2890	Q169M/L340V/K402G/F504K	+

2891/2892	L327M/Q329R	+
2893/2894	Q169M/L340I/K506P	+
2895/2896	K427M	+
2897/2898	L327M/Q329R/P404D/E410V	+
2899/2900	L340I/K402G/F504K	+
2901/2902	L340I/K506P	+
2903/2904	L327M/E410V/T484A	+
2905/2906	T379M/P404D/E410V	+
2907/2908	L327M/Q329R/T379M/P404D/G406T/E410V	+
2909/2910	Q169M/L340V/K402G/L403P/K427M/F504K/K506P	+
2911/2912	F504K/K506P	+
2913/2914	Q169M/A304V/L403S/K427L/F504K	+
2915/2916	T379L/Q468S	+
2917/2918	K506P	+
2919/2920	Q169M/L340I/K402G/L403A/K427M/D429R/F504K	+
2921/2922	Q169M/K402G/F504K	+
2923/2924	T379M/E410V	+
2925/2926	L327M	+
2927/2928	L340I/L403S/F504K	+
2929/2930	L340V	+
2931/2932	L327M/Q329R/A465E/T484A	+
2933/2934	T484A	+
2935/2936	Q169M/A304V/L340V/K427L/D429R/F504K	+
2937/2938	A304V	+
2939/2940	S292N/L327M/Q329R/Q468S	+
2941/2942	L340V/F504K	+
2943/2944	F504K	+
2945/2946	L327M/W382L/G406T/E410V/T484A	+
2947/2948	T379M/W382L/Q468S	+
2949/2950	T379L/A465E/Q468S/T484A	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2804 and defined as follows: "+" 1.20 to 2.39, "++" > 2.39, "+++" > 4.43.		

EXAMPLE 41**Improvements over SEQ ID NO: 2812 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0446] SEQ ID NO: 2812 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 41.1.

[0447] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-

mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 41.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (μL) -1; NTP (μM)-mATP-3'P; NTP conc (μM)-100; Oligonucleotide-5'-6-FAM-T15mAmU*mG; Substrate conc (μM)-25; Product -5'-6-FAM-T15mAmU*mGmA-3'P.

[0448] Activity relative to SEQ ID NO: 2812 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2812 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 41.2.

Table 41.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2812)	FIOP % product Relative to SEQ ID NO: 2812
2951/2952	M30G/Q179A/Q373G/T379M	+++
2953/2954	N246E/S325W/Q329R/T379M/G461S	+++
2955/2956	M30G/A184S/N246E/S325W/T379M/D429R/A495S	+++
2957/2958	M30G/N246E/S325W/L327M/Q329R/P404D/G461S	+++
2959/2960	M30G/Q329R/T379M	+++
2961/2962	M30G/N246E/S325W/T379M/P404D/K427L/D429R/G461S	+++
2963/2964	M30G/A184S/N246E/T379M/P404D	++
2965/2966	Q373G/T379M	++
2967/2968	M30G/Q373G	++
2969/2970	N246E/L327M/P404D/G461S/A495S	++
2971/2972	M30G/T379M/D429R/Y459I/G461S	++
2973/2974	M30G/N246E/K427L/Y459I/G461S	++
2975/2976	M30G/T379M/Y459I/G461S/F504Q	++
2977/2978	M30G/T200N/Q373G/T379M	++
2979/2980	T379M/S403L/G406N/Q468S	++
2981/2982	S403L/M441K	+
2983/2984	M30G/Q179A/T200N/Q373G/S403E	+
2985/2986	M30G/A184S/N246E/L327M/Q329R/Y459I	+
2987/2988	M30G/A184S/N246E/Y459I/G461S/A495S/T500N/F504Q	+
2989/2990	M30G/T200N/Q373G	+

2991/2992	M30G/L327M/P404D/F504S	+
2993/2994	Q353R/S403E	+
2995/2996	L327M/Q329R/T379M/F504S	+
2997/2998	M30G/Q373G/S403L	+
2999/3000	M30G	+
3001/3002	M30G/S325W/L327M	+
3003/3004	L327M/Y459I/G461S/A495S	+
3005/3006	Q373G/T379M/S403L	+
3007/3008	M30G/S325W/L327M/T379M/P404D/D429R/A495S	+
3009/3010	M30G/S403E/M441K/G460P	+
3011/3012	T200N/Q373G/T379M	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2812 and defined as follows: "+" 1.06 to 1.18 (first 50%), "++" > 1.18 (next 30%), "+++" > 1.43 (top 20%)		

EXAMPLE 42

Improvements over SEQ ID NO: 2812 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0449] SEQ ID NO: 2812 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 42.1.

[0450] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 42.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15mAmU*mG; Substrate conc (μ M)-25; Product -5'-6-FAM-T15mAmU*mGmA-3'P.

[0451] Activity relative to SEQ ID NO: 2812 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2812 (where

the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 42.2.

Table 42.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2812)	F1OP % product Relative to SEQ ID NO: 2812
3013/3014	Q370D	+++
3015/3016	E367D	+++
3017/3018	W433G	+++
3019/3020	I503T	+++
3021/3022	W433E	+++
3023/3024	W433S	+++
3025/3026	Q370S	+++
3027/3028	R264L	+++
3029/3030	W433A	+++
3031/3032	K278M	++
3033/3034	K266R	++
3035/3036	E520P	++
3037/3038	R264V	++
3039/3040	L326R	++
3041/3042	M435G	++
3043/3044	I503E	++
3045/3046	L499M	++
3047/3048	F525S	++
3049/3050	I503V	++
3051/3052	G414E	++
3053/3054	R264E	++
3055/3056	M435A	++
3057/3058	Q370G	++
3059/3060	T437R	+
3061/3062	I503A	+
3063/3064	T396R	+
3065/3066	W433R	+
3067/3068	R248K	+
3069/3070	R264T	+
3071/3072	M435E	+
3073/3074	M435S	+
3075/3076	W433M	+
3077/3078	L499R	+
3079/3080	P446G	+
3081/3082	V253I	+
3083/3084	D485E	+
3085/3086	Q370M	+
3087/3088	W433P	+
3089/3090	A444R	+
3091/3092	F525R	+
3093/3094	G231S	+

3095/3096	D198E	+
3097/3098	A444G	+
3099/3100	T437G	+
3101/3102	E233R	+
3103/3104	F525Q	+
3105/3106	R248T	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2812 and defined as follows: "+" 1.10 to 1.27, "++" > 1.27, "+++" > 1.48.		

EXAMPLE 43

Improvements over SEQ ID NO: 2812 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0452] SEQ ID NO: 2812 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 43.1.

[0453] Reactions were performed in 96-well format 200 µL BioRad PCR plates. Reactions included 10-100 µM oligonucleotide, 100-200 µM nucleotide triphosphate, 20 mM buffer, and 250 µM cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 43.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-62 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (µL)-1; NTP (µM)-mATP-3'P; NTP conc (µM)-100; Oligonucleotide-5'-6-FAM-T15AmU*mG; Substrate conc (µM)-25; Product -5'-6-FAM-T15AmU*mGmA-3'P.

[0454] Activity relative to SEQ ID NO: 2812 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2812 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 43.2.

Table 43.2

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2812)	FIOP % product Relative to SEQ ID NO: 2812
3107/3108	K266T	+++
3051/3052	G414E	+++
3083/3084	D485E	+++
3025/3026	Q370S	+++
3109/3110	A366V	+++
3093/3094	G231S	+++
3111/3112	E186D	+++
3113/3114	K368R	+++
3115/3116	Q370N	+++
3117/3118	W433V	++
3119/3120	R264A	++
3121/3122	K501R	++
3123/3124	K235R	++
3125/3126	E367A	++
3127/3128	A444H	++
3033/3034	K266R	++
3085/3086	Q370M	++
3129/3130	L188I	++
3131/3132	M435P	++
3133/3134	M435I	++
3095/3096	D198E	++
3135/3136	T437P	++
3099/3100	T437G	+
3137/3138	A515E	+
3139/3140	E243S	+
3141/3142	T437A	+
3143/3144	G442A	+
3145/3146	L287I	+
3147/3148	E243L	+
3149/3150	K297S/N440K	+
3151/3152	E243T	+
3087/3088	W433P	+
3081/3082	V253I	+
3153/3154	S439G	+
3155/3156	E243R	+
3157/3158	D485S	+
3159/3160	S439P	+
3161/3162	E233A	+
3163/3164	T437K	+
3069/3070	R264T	+
3075/3076	W433M	+
3097/3098	A444G	+
3071/3072	M435E	+
3165/3166	R248S	+

Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2812 and defined as follows: "+" 1.11 to 1.29, "++" > 1.29, "+++" > 1.48.

EXAMPLE 44

Improvements over SEQ ID NO: 2956 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0455] SEQ ID NO: 2956 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 44.1.

[0456] Reactions were performed in 96-well format 200 µL BioRad PCR plates. Reactions included 10-100 µM oligonucleotide, 100-200 µM nucleotide triphosphate, 20 mM buffer, and 250 µM cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 44.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-64 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (µL)-1; NTP (µM)-mATP-3'P; NTP conc (µM)-100; Oligonucleotide-5'-6-FAM-T15AmU*mG; Substrate conc (µM)-25; Product -5'-6-FAM-T15AmU*mGmA-3'P.

[0457] Activity relative to SEQ ID NO: 2956 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2956 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 44.2.

Table 44.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 2956)	FIOP % product Relative to SEQ ID NO: 2956
3167/3168	G140V/M142V/E153V/K427L/T484L	+++
3169/3170	M142V/E150P/P158S/H177R/K427L/K445N	+++
3171/3172	G140V/K161H/H177R/P404D/K427L/T484L	+++
3173/3174	G140V/M142V/E153V/H177R/K427L/E434N/M441V/A444S/G461S/K502G	+++

3175/3176	H177R/G365A/K427L/E434N	+++
3177/3178	K427L	++
3179/3180	G140V/M142V/Q373R/K427L/T484L	++
3181/3182	G140V/E150P/E153V/G365A/Q373R/K427L/T484L	++
3183/3184	M142V/E153V/H177R/M441V/A444S	++
3185/3186	G140V/M142V/G365A/Q373R/P404D/K427L/T484L	++
3187/3188	H177R	++
3189/3190	G140V/H177R/P404D/T484L	++
3191/3192	E150P/H177R	++
3193/3194	M142V/E150P/H177R/P404D	+
3195/3196	M142V/G461S/T484L/K502G	+
3197/3198	E153V/T484L	+
3199/3200	W325L/K427L	+
3201/3202	G140V/M142V/H177R/M441V	+
3203/3204	G140V/H177R/P404D	+
3205/3206	T484L	+
3207/3208	E153V/K161H/W325L/P404D/K427L/M441V/T484L	+
3209/3210	M142V/H177R/Q373R/M441V	+
3211/3212	G140V/P148T/K161H/H177R/P404D	+
3213/3214	G140V/M142V/H177R	+
3215/3216	M142V/H177R/Q373R	+
3217/3218	E153V	+
3219/3220	G140V/E150P/H177R/P404D/P436S/M441V/T484L/K502G	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2956 and defined as follows: "+" 1.24 to 1.76, "++" > 1.76, "+++" > 3.58.		

EXAMPLE 45

Improvements over SEQ ID NO: 3174 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0458] SEQ ID NO: 3174 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 45.1.

[0459] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 45.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (μL)-1; NTP (μM)-mATP-3'P; NTP conc (μM)-100; Oligonucleotide-5'-6-FAM-T15mAmU*mG; Substrate conc (μM)-25; Product -5'-6-FAM-T15mAmU*mGmA-3'P.

[0460] Activity relative to SEQ ID NO: 3174 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 3174 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 45.2.

Table 45.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3174)	FIOP % product Relative to SEQ ID NO: 3174
3221/3222	R264A/K266T/T396S/G414E/W433R/V441M/A515E	+++
3223/3224	D198E/R264A/K266T/G414E	+++
3225/3226	R264A/K266T/G414E/V441M/L499M/K501R/A515E/E520D	+++
3227/3228	E243S/T396S/G414E/W433V/V441M/A515E/E520D	+++
3229/3230	D198E/R264A/T396S/G414E/W433V/V441M/A515E	+++
3231/3232	D198E/E243S/T396S/G414E/S431R/W433V/V441M/L499M	+++
3233/3234	T396S/G414E/V441M/A515E	++
3235/3236	D198E/E243S/T396S/G414E/W433R/T437R/V441M/A515E	++
3237/3238	D198E/K266T/G414E/W433S/V441M/A515E	++
3239/3240	D198E/G414E/W433V/V441M/A515E/E520D	++
3241/3242	E186D/G231S/R248K/T484L	++
3243/3244	T396S/G414E/V441M	++
3245/3246	E243S/G414E/T437R/V441M/A515E	++
3247/3248	D198E/G414E/S431R/V441M/L499M/E520D	++
3249/3250	R264A/G414E	++
3251/3252	E186D/G231S/R248K/G365A/A366V/K368R/T484L	++
3253/3254	E243S/G414E/W433S/T437R/V441M	++
3255/3256	E186D/G231S/V441M/S444H/K445N	+
3257/3258	K266T/T437R/V441M/L499M/A515E/E520D	+
3259/3260	E186D/T484L/D485E	+
3261/3262	G414E/V441M/E520D	+
3263/3264	R264A/G414E/V441M	+
3265/3266	E186D/G231S/R248K/V253I/T484L	+
3267/3268	D198E/E243S/R264A/S431R/V441M	+
3269/3270	E243S/R264A/A515E/E520D	+
3271/3272	R248K/G442K/S444A/K445N/T484L	+
3273/3274	E186D/G231S/K368R/K416N/V441M/G442K/S444A/T484L/D485E	+

3275/3276	G365A/A366V/V441M/T484L/D485E	+
3277/3278	E186D/G231S/R248K/A366V/K368R/S444H	+
3279/3280	D198E/K266T/T396S/G414E/W433V/V441M/L499M/K501R	+
3281/3282	L188I/G231S/R248K/V253I/G365A/V441M/G442K/S444A/K445N/T484L	+
3283/3284	G231S/R248K/V441M/T484L	+
3285/3286	D198E/K266T/T437R/V441M/L499M	+
3287/3288	R264A/W433S/V441M	+
3289/3290	E186D/L188I/R248K/V253I/G365A/A366V/S444H/K445N	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3174 and defined as follows: "+" 1.11 to 1.54, "++" > 1.54, "+++" > 1.79.		

EXAMPLE 46

Improvements over SEQ ID NO: 3174 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0461] SEQ ID NO: 3174 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 46.1.

[0462] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 46.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-48 °C; Preincubation Time (min)-60; Reaction temperature (°C)-48 °C; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15mAmU*mG; Substrate conc (μ M)-25; Product -5'-6-FAM-T15mAmU*mGmA-3'P.
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[0463] Activity relative to SEQ ID NO: 3174 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 3174 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 46.2.

Table 46.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3174)	FIOP % product Relative to SEQ ID NO: 3174
3291/3292	E394Y	+++
3293/3294	M301	+++
3295/3296	M301T	+++
3297/3298	M301V	+++
3299/3300	E394F	+++
3301/3302	Y390C	+++
3303/3304	F194L	+++
3305/3306	A408I	+++
3307/3308	V273F	+++
3309/3310	V273L	+++
3311/3312	K274I	+++
3313/3314	V273D	+++
3315/3316	R473G	+++
3317/3318	K274G	+++
3319/3320	V273I	+++
3321/3322	R196G	+++
3323/3324	V273E/K501N	+++
3325/3326	V273Y	+++
3327/3328	V273E	+++
3329/3330	K278F	+++
3331/3332	G350W	+++
3333/3334	K266Y	+++
3335/3336	K274A	+++
3337/3338	F347I	+++
3339/3340	L312M	+++
3341/3342	V304M	+++
3343/3344	K274V	+++
3345/3346	V273G	+++
3347/3348	F269W	+++
3349/3350	F194W	++
3351/3352	K266I	++
3353/3354	K297C	++
3355/3356	Y309F	++
3357/3358	G414A	++
3359/3360	E394V	++
3361/3362	R196N	++
3363/3364	E394A	++
3365/3366	N493V	++
3367/3368	A413S	++
3369/3370	Q302S	++
3371/3372	E394M	++
3373/3374	K416G	++
3375/3376	K266V	++
3377/3378	E394G	++

3379/3380	V268C	++
3381/3382	T299L	++
3383/3384	T299M	++
3385/3386	K266T	++
3387/3388	L454F	++
3389/3390	R196L	++
3391/3392	Y309W	++
3393/3394	R477G	++
3395/3396	L298I	++
3397/3398	Y309V	++
3399/3400	K407L	++
3401/3402	K278R	++
3403/3404	L454M	++
3405/3406	A408G	++
3407/3408	K407N	++
3409/3410	K407R	++
3411/3412	V268I	++
3413/3414	Y390V	++
3415/3416	Y309L	++
3417/3418	K407M	++
3419/3420	Q353A	++
3421/3422	Y309G	++
3423/3424	Q302N	++
3425/3426	L312V	++
3427/3428	R196T	++
3429/3430	R480H	++
3431/3432	Y309M	++
3433/3434	S267A	++
3435/3436	Q468M	++
3437/3438	G411N	+
3439/3440	K278V	+
3441/3442	V268L	+
3443/3444	V304D	+
3445/3446	V273C	+
3447/3448	K266F	+
3449/3450	S267H	+
3451/3452	E190L	+
3453/3454	L359G	+
3455/3456	H263M	+
3457/3458	R196M	+
3459/3460	Q468H	+
3461/3462	A413L	+
3463/3464	A413P	+
3465/3466	G411E	+
3467/3468	Y390I	+
3469/3470	N493Y	+
3471/3472	F194C	+

3473/3474	T299N	+
3475/3476	V268T	+
3477/3478	T299A	+
3479/3480	S267Y	+
3481/3482	L298M	+
3483/3484	A408L	+
3485/3486	G350L	+
3487/3488	A413F	+
3489/3490	Y309I	+
3491/3492	A408V	+
3493/3494	P352V	+
3495/3496	R281L	+
3497/3498	R281K	+
3499/3500	L479V	+
3501/3502	L312T	+
3503/3504	V304T	+
3505/3506	E193S	+
3507/3508	W279Y	+
3509/3510	M122I/R473K	+
3511/3512	K297L	+
3513/3514	S267C	+
3515/3516	T299Y	+
3517/3518	A413V	+
3519/3520	L479F	+
3521/3522	H263R	+
3523/3524	K278M	+
3525/3526	Q468F	+
3527/3528	S267V	+
3529/3530	E410F	+
3531/3532	E193T	+
3533/3534	G315Q	+
3535/3536	G350R	+
3537/3538	R480K	+
3539/3540	A408T	+
3541/3542	F194S	+
3543/3544	Q468T	+
3545/3546	K297T	+
3547/3548	P436S	+
3549/3550	K297M	+
3551/3552	R264C	+
3553/3554	P300H	+
3555/3556	R189L	+
3557/3558	K266R	+
3559/3560	S472G	+
3561/3562	K297R	+
3563/3564	K297V	+
3565/3566	E410G	+

3567/3568	E410I	+
3569/3570	L454V	+
3571/3572	E190M	+
3573/3574	K407W	+
3575/3576	E190R	+
3577/3578	Y390L	+
3579/3580	A408M	+
3581/3582	K278H	+
3583/3584	R392V	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3174 and defined as follows: "+" 1.05 to 1.35, "++" > 1.35, "+++" > 1.84.		

EXAMPLE 47

Improvements over SEQ ID NO: 3174 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0464] SEQ ID NO: 3174 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 47.1.

[0465] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 47.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-65 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15AmU*mG; Substrate conc (μ M)-25; Product -5'-6-FAM-T15AmU*mGmA-3'P.

[0466] Activity relative to SEQ ID NO: 3174 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 3174 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 47.2.

Table 47.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3174)	FIOP % product Relative to SEQ ID NO: 3174
3315/3316	R473G	+++
3299/3300	E394F	+++
3291/3292	E394Y	+++
3385/3386	K266T	+++
3371/3372	E394M	++
3321/3322	R196G	++
3363/3364	E394A	++
3359/3360	E394V	++
3585/3586	Q468A	++
3587/3588	R477S	++
3525/3526	Q468F	++
3365/3366	N493V	++
3375/3376	K266V	+
3377/3378	E394G	+
3351/3352	K266I	+
3459/3460	Q468H	+
3543/3544	Q468T	+
3435/3436	Q468M	+
3403/3404	L454M	+
3319/3320	V273I	+
3411/3412	V268I	+
3309/3310	V273L	+
3521/3522	H263R	+
3497/3498	R281K	+
3589/3590	K416S	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3174 and defined as follows: "+" 1.20 to 1.58, "++" > 1.58, "+++" > 2.20.		

EXAMPLE 48

Improvements over SEQ ID NO: 3174 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0467] SEQ ID NO: 3174 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 48.1.

[0468] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable

aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 48.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-48 °C; Preincubation Time (min)-60; Reaction temperature (°C)-48 °C; Reaction Time (min)-60; Reaction volume (μL)-1; NTP (μM)-mATP-3'P; NTP conc (μM)-100; Oligonucleotide-5'-6-FAM-T15mC*mG*mA; Substrate conc (μM)-25; Product -5'-6-FAM-T15mC*mG*mAmA-3'P.

[0469] Activity relative to SEQ ID NO: 3174 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 3174 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 48.2.

Table 48.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 1830)	FIOP % product Relative to SEQ ID NO: 1830
3589/3590	K416S	+++
3373/3374	K416G	+++
3297/3298	M301V	+++
3301/3302	Y390C	+++
3293/3294	M301S	+++
3315/3316	R473G	+++
3295/3296	M301T	+++
3349/3350	F194W	+++
3343/3344	K274V	+++
3311/3312	K274I	+++
3325/3326	V273Y	+++
3321/3322	R196G	+++
3307/3308	V273F	++
3327/3328	V273E	++
3323/3324	V273E/K501N	++
3303/3304	F194L	++
3347/3348	F269W	++
3367/3368	A413S	++
3319/3320	V273I	++
3407/3408	K407N	++
3345/3346	V273G	++
3583/3584	R392V	++
3365/3366	N493V	++
3305/3306	A408I	++
3317/3318	K274G	++
3413/3414	Y390V	++

3361/3362	R196N	++
3385/3386	K266T	++
3391/3392	Y309W	++
3417/3418	K407M	++
3403/3404	L454M	+
3431/3432	Y309M	++
3591/3592	K407D	+
3335/3336	K274A	+
3477/3478	T299A	+
3593/3594	T299S	+
3313/3314	V273D	+
3415/3416	Y309L	+
3475/3476	V268T	+
3399/3400	K407L	+
3309/3310	V273L	+
3375/3376	K266V	+
3595/3596	D277E	+
3355/3356	Y309F	+
3337/3338	F347I	+
3453/3454	L359G	+
3561/3562	K297R	+
3481/3482	L298M	+
3569/3570	L454V	+
3291/3292	E394Y	+
3393/3394	R477G	+
3369/3370	Q302S	+
3557/3558	K266R	+
3543/3544	Q468T	+
3409/3410	K407R	+
3433/3434	S267A	+
3473/3474	T299N	+
3425/3426	L312V	+
3597/3598	K278Y	+
3457/3458	R196M	+
3441/3442	V268L	+
3499/3500	L479V	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3174 and defined as follows: "+" 1.19 to 1.43, "++" > 1.43, "+++" > 2.15.		

EXAMPLE 49**Improvements over SEQ ID NO: 3222 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0470] SEQ ID NO: 3222 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination

of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 49.1.

[0471] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 49.1-Reaction conditions	
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-50 °C; Preincubation Time (min)-60; Reaction temperature (°C)-50 °C; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15mC*mG*mA; Substrate conc (μ M)-25; Product -5'-6-FAM-T15mC*mG*mAmA-3'P.	

[0472] Activity relative to SEQ ID NO: 3222 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 3222 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 49.2.

Table 49.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3222)	FIOP % product Relative to SEQ ID NO: 3222
3599/3600	E186D/E243S/R248K/E394Y/T484L/D485E	+++
3601/3602	F194L/E515A/E520D	+++
3603/3604	D198E/R248K/E394Y/S396T/T484L/D485E/L499M	+++
3605/3606	E186D/G231S/A366V/K368R/E394Y/D485E	+++
3607/3608	D198E/E394Y/S396T/T484L/D485E/L499M	+++
3609/3610	R248K/E394Y/T484L	+++
3611/3612	R248K/G365A/A366V/K368R/E394Y/T484L/E520D	+++
3613/3614	G231S/K368R/E394Y	++
3615/3616	E186D/G231S/E243S/K368R/E394Y/D485E/L499M	++
3617/3618	E186D/F194L/R248K/S396T	++
3619/3620	E186D/E243S/R248K/A366V/K368R/E394Y	++
3621/3622	E186D/A366V/K368R/E394Y/S396T/T484L	++
3623/3624	E186D/E394Y/S396T/D485E	++
3625/3626	E394Y/S396T	++
3627/3628	E394Y/L499M	++
3629/3630	E186D/G365A/A366V/E394Y/D485E	++

3631/3632	E186D/A366V/K368R/E394Y/S396T/T484L/D485E	++
3633/3634	E243S/T484L	++
3635/3636	G365A/A366V/K368R/E394Y	+
3637/3638	E186D/R248K	+
3639/3640	G365A/K368R/E394Y/S396T/E520D	+
3641/3642	E186D/D198E/E243S/R248K/A366V/K368R/E394Y/K501R	+
3643/3644	D198E/E394Y/S396T/L499M/E515A/E520D	+
3645/3646	E243S/R248K/E394Y/S396T/T484L/D485E	+
3647/3648	F194L/D198E/E243S/A366V/K368R/L499M	+
3649/3650	G231S/G365A/K368R/E394Y/L499M/E520D	+
3651/3652	D198E/E394Y/L499M/K501R	+
3653/3654	E186D/G365A/A366V/K368R/E394Y/L499M	+
3655/3656	E186D/G365A	+
3657/3658	E243S/T484L/D485E/L499M	+
3659/3660	D198E/E243S/R248K/G365A/E394Y/K501R	+
3661/3662	E186D/E243S/T484L/E520D	+
3663/3664	E186D/E243S	+
3665/3666	D198E/G231S/E243S/R248K/D485E	+
3667/3668	E186D/G231S/R248K/D485E	+
3669/3670	G231S/T484L/D485E/L499M/K501R	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3222 and defined as follows: "+" 1.00 to 1.20, "++" > 1.20, "+++" > 1.49.		

EXAMPLE 50

Improvements over SEQ ID NO: 3670 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0473] SEQ ID NO: 3670 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 50.1.

[0474] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 50.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl₂, pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-48 °C; Preincubation Time (min)-60; Reaction temperature (°C)-48 °C; Reaction Time (min)-180; Reaction volume (μL)-1; NTP (μM)-mATP-3'P; NTP conc (μM)-100; Oligonucleotide-5'-6-FAM-T17mGmUmC; Substrate conc (μM)-50; Product -5'-6-FAM-T17mGmUmCmA-3'P.

[0475] Activity relative to SEQ ID NO: 3670 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 3670 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 50.2.

Table 50.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3670)	FIOP % product Relative to SEQ ID NO: 3670
3671/3672	K274I/K416S	+++
3673/3674	K416S	+++
3675/3676	K274A/A408I/K416S	++
3677/3678	K274A/T299N/A408I/K416S	++
3679/3680	E288V/T299N/K416S	++
3681/3682	V273I	++
3683/3684	L298M/T299N/K416S	+
3685/3686	V273I/M499L	+
3687/3688	V273I/N493V/M499L	+
3689/3690	V273I/Y309M/N493V/M499L	+
3691/3692	Y309M	+
3693/3694	N493V/M499L	+
3695/3696	Y309M/M499L	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3670 and defined as follows: "+" 1.07 to 1.39, "++" > 1.39, "+++" > 1.92.		

EXAMPLE 51

Improvements over SEQ ID NO: 3670 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0476] SEQ ID NO: 3670 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 51.1.

[0477] Reactions were performed in 96-well format 200 μL BioRad PCR plates. Reactions included 10-100 μM oligonucleotide, 100-200 μM nucleotide triphosphate, 20 mM buffer, and 250 μM cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution

was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 51.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-48 °C; Preincubation Time (min)-60; Reaction temperature (°C)-48 °C; Reaction Time (min)-180; Reaction volume (μL)-1; NTP (μM)-mATP-3'P; NTP conc (μM)-100; Oligonucleotide-5'-6-FAM-T12mAmUmA, 5'-6-FAM-T17mAmUmC, 5'-6-FAM-T22mAmUmU, 5'-6-FAM-T27mAmUmG ; Substrate conc (μM)-multiplex: 12.5 μM each of 4 oligo; Product -5'-6-FAM-T12mAmUmAmA-3'P, 5'-6-FAM-T17mAmUmCmA-3'P, 5'-6-FAM-T22mAmUmUmA-3'P, 5'-6-FAM-T27mAmUmGmA-3'P.

[0478] Activity relative to SEQ ID NO: 3670 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 3670 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 51.2.

Table 51.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3670)	FIOP % product Relative to SEQ ID NO: 3670
3697/3698	R473S	+++
3699/3700	Y308V	+++
3701/3702	Y308S	+++
3703/3704	M301S	+++
3705/3706	Y308A/I361T	+++
3707/3708	Y308G	+++
3709/3710	Y308A	+++
3711/3712	M301G	++
3713/3714	L419G	++
3715/3716	R456T	++
3717/3718	A415L	++
3719/3720	R451K	++
3721/3722	Q397F	++
3723/3724	M301Q	++
3725/3726	A415W	++
3727/3728	R456P	++
3729/3730	R473A	++
3731/3732	S267R	++
3733/3734	S472A	+
3735/3736	A313M	+
3737/3738	N493R/M499L	+

3739/3740	L307G	+
3741/3742	L419M	+
3743/3744	L307A	+
3745/3746	F475V	+
3747/3748	V452L	+
3749/3750	Y308K	+
3751/3752	R392V	+
3753/3754	D372E	+
3755/3756	D198S	+
3757/3758	S267M	+
3759/3760	N528L	+
3761/3762	L307S	+
3763/3764	Q397W	+
3765/3766	Y308H	+
3767/3768	C168S	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3670 and defined as follows: "+" 1.18 to 1.43, "++" > 1.43, "+++" > 2.07.		

EXAMPLE 52

Improvements over SEQ ID NO: 3670 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0479] SEQ ID NO: 3670 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 52.1.

[0480] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 $^{\circ}$ C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 52.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (C)-68 $^{\circ}$ C; Preincubation Time (min)-60; Reaction temperature (C)-50 $^{\circ}$ C; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15AmU*mG; Substrate conc (μ M)-50; Product -5'-6-FAM-T15AmU*mGmA-3'P.

[0481] Activity relative to SEQ ID NO: 3670 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 3670 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 52.2.

Table 52.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3670)	FIOP % product Relative to SEQ ID NO: 3670
3697/3698	R473S	+++
3769/3770	R473A	++
3771/3772	N493V/M499L	++
3773/3774	Y308L	+
3775/3776	R473M	+
3777/3778	K303H/S396A	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3670 and defined as follows: "+" 1.08 to 1.34, "++" > 1.34, "+++" > 1.78.		

EXAMPLE 53

Improvements over SEQ ID NO: 3670 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0482] SEQ ID NO: 3670 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 53.1.

[0483] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 53.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-48 °C; Preincubation Time (min)-60; Reaction temperature (°C)-48 °C; Reaction Time (min)-180; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T12mAmUmA, 5'-6-FAM-T17mAmUmC, 5'-6-FAM-T22mAmUmU, 5'-6-FAM-T27mAmUmG ; Substrate conc (μ M)-multiplex:

12.5 μ M each of 4 oligo; Product -5'-6-FAM-T12mAmUmAmA-3'P, 5'-6-FAM-T17mAmUmCmA-3'P, 5'-6-FAM-T22mAmUmUmA-3'P, 5'-6-FAM-T27mAmUmGmA-3'P.

[0484] Activity relative to SEQ ID NO: 3670 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 3670 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 53.2.

Table 53.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3670)	FIOP % product Relative to SEQ ID NO: 3670
3671/3672	K274I/K416S	+++
3675/3676	K274A/A408I/K416S	+++
3673/3674	K416S	+++
3677/3678	K274A/T299N/A408I/K416S	+++
3679/3680	E288V/T299N/K416S	++
3687/3688	V273I/N493V/M499L	++
3681/3682	V273I	++
3689/3690	V273I/Y309M/N493V/M499L	++
3685/3686	V273I/M499L	++
3693/3694	N493V/M499L	++
3779/3780	V273E/N493V	+
3781/3782	V273I/Y309M/A413S/M499L	+
3783/3784	A413S	+
3785/3786	Y309M/A413S	+
3787/3788	R281K/A413S/M499L	+
3691/3692	Y309M	+
3683/3684	L298M/T299N/K416S	+
3789/3790	A413S/N493V/M499L	+
3791/3792	A413S/M499L	+
3793/3794	V273S/Y309M/A413S/M499L	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3670 and defined as follows: "+" 1.05 to 1.18, "++" > 1.18, "+++" > 1.35.		

EXAMPLE 54

Improvements over SEQ ID NO: 3674 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0485] SEQ ID NO: 3674 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 54.1.

[0486] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 54.1-Reaction conditions	
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-48 °C; Preincubation Time (min)-60; Reaction temperature (°C)-48 °C; Reaction Time (min)-180; Reaction volume (μ L)-1; NTP (μ M)-mATP-3'P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T12mAmUmA, 5'-6-FAM-T17mAmUmC, 5'-6-FAM-T22mAmUmU, 5'-6-FAM-T27mAmUmG ; Substrate conc (μ M)-multiplex: 12.5 μ M each of 4 oligo; Product -5'-6-FAM-T12mAmUmAmA-3'P, 5'-6-FAM-T17mAmUmCmA-3'P, 5'-6-FAM-T22mAmUmUmA-3'P, 5'-6-FAM-T27mAmUmGmA-3'P.	

[0487] Activity relative to SEQ ID NO: 3674 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 3674 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 54.2.

Table 54.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3674)	FIOP % product Relative to SEQ ID NO: 3674
3795/3796	F194L/R196G/Y390C	+++
3797/3798	Y390C/E394F/R480K	+++
3799/3800	R196G/Y390C/E394F	+++
3801/3802	F194L/R196G/Y390C/R480K	+++
3803/3804	F194L/R196G/Y390C/E394F/R480K	+++
3805/3806	F194L/R196G/Y390C/L454M/R480K	+++
3807/3808	Y390C/E394F	++
3809/3810	R196G/Y390C/E394F/R480K	++
3811/3812	F194L/Y390C	++
3813/3814	F194L/R196G/Y390C/E394F/G460V/R480K	++
3815/3816	R196G/Y390C/E394F/L454M/R480K	++
3817/3818	R196G/Y390C	++
3819/3820	Y390C	++
3821/3822	Y390C/E394F/L454M/R480K	++
3823/3824	R196G/Y390C/E394F/L454M	++
3825/3826	Y390C/E394F/L454M	++

3827/3828	Y390C/R480K	++
3829/3830	Y390C/L454M	+
3831/3832	R196G/Y390C/L454M	+
3833/3834	R196G/E394F	+
3835/3836	K297R/R473G/N493V	+
3837/3838	F194L/E394F	+
3839/3840	R196G/E394F/R480K	+
3841/3842	E394F/R480K	+
3843/3844	E394F	+
3845/3846	F194L/R196G/L454M	+
3847/3848	F194L/E394F/L454M/R480K	+
3849/3850	K297R/T470S/R473G	+
3851/3852	F194L/R196G/E394F/L454M/R480K	+
3853/3854	R196G/E394F/L454M/R480K	+
3855/3856	R196G/E394F/L454M	+
3857/3858	F194L/E394F/L454M	+
3859/3860	R196G/L454M	+
3861/3862	R196G	+
3863/3864	L454M	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3674 and defined as follows: "+" 1.22 to 2.98, "++" > 2.98, "+++" > 3.79.		

EXAMPLE 55**Improvements over SEQ ID NO: 3796 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0488] SEQ ID NO: 3796 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 55.1.

[0489] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 10-100 μ M oligonucleotide, 100-200 μ M nucleotide triphosphate, 20 mM buffer, and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 55.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl₂, pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-48 °C; Preincubation Time (min)-

60; Reaction temperature (C)-48 °C; Reaction Time (min)-180; Reaction volume (μL)-1; NTP (μM)-mATP-3'P; NTP conc (μM)-200; Oligonucleotide-5'-6-FAM-T12mAmUmA, 5'-6-FAM-T17mAmUmC, 5'-6-FAM-T22mAmUmU, 5'-6-FAM-T27mAmUmG, 5'-6-FAM-T57mUmUmC, 5'-6-FAM-T32mAmCmC, 5'-6-FAM-T37mAmGmC, 5'-6-FAM-T42mAmAmC, 5'-6-FAM-T47mGmUmC, 5'-6-FAM-T52mCmUmC ; Substrate conc (μM)-multiplex: 10 μM each of 10 oligo; Product -5'-6-FAM-T12mAmUmAmA-3'P, 5'-6-FAM-T17mAmUmCmA-3'P, 5'-6-FAM-T22mAmUmUmA-3'P, 5'-6-FAM-T27mAmUmGmA-3'P, 5'-6-FAM-T57mUmUmCmA-3'P, 5'-6-FAM-T32mAmCmCmA-3'P, 5'-6-FAM-T37mAmGmCmA-3'P, 5'-6-FAM-T42mAmAmCmA-3'P, 5'-6-FAM-T47mGmUmCmA-3'P, 5'-6-FAM-T52mCmUmCmA-3'P.

[0490] Activity relative to SEQ ID NO: 3796 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 3796 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 55.2.

Table 55.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3796)	FIOP % product Relative to SEQ ID NO: 3796
3865/3866	D198P/R451K/N493V	+++
3867/3868	D198P/S267Q/R314A/R451K	+++
3869/3870	S267R/R451K	+++
3871/3872	R451K	+++
3873/3874	D198P/S267Q/D409L/R451K	+++
3875/3876	R451K/H494V	++
3877/3878	V328T/R451K	++
3879/3880	R314A/R451K	++
3881/3882	S267R/R314A/V328T/R451K/H494R/M499L	++
3883/3884	D198P	++
3885/3886	R451K/N493V/M499L	++
3887/3888	S267Q/R451K/H494V/M499L	++
3889/3890	R314A/V328T/R451K/M499L	++
3891/3892	I208V/Y308V	+
3893/3894	V328T/D409L/R451K	+
3895/3896	D198P/S267Q/R314A/F475V	+
3897/3898	D198P/S267R/A313L/R451K/F475V/H494V/M499L	+
3899/3900	S267R	+
3901/3902	D198P/S267Q/F475V	+
3903/3904	D409L	+
3905/3906	I208V/Y308A/S461N	+
3907/3908	Y308A	+
3909/3910	F475V	+
3911/3912	R451K/N493V/H494V	+
3913/3914	D409L/F475V/H494V	+

3915/3916	Y308V	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3796 and defined as follows: "+" 1.12 to 1.33, "++" > 1.33, "+++" > 1.62.		

EXAMPLE 56 Activity improvement over SEQ ID NO: 3870

Activity improvement of shake-flask purified TdT variants with 3'phosphate-blocked nucleotides and modified RNA oligonucleotide substrates.

[0491] TdT variants of SEQ ID NO: 3870, 3918, and 3920 were produced in shake flask and purified as described in Example 3.

[0492] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 250 μ M oligonucleotide, 300 μ M nucleotide triphosphate, 4 μ M TdT, 5 μ M IPP, 20 mM triethanolamine (pH 7.8), and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 56.1-Reaction conditions
Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; IPP SEQ ID NO: 3942 (μ M)- 0.5; Preincubation Temp (C) – none; Preincubation Time (min)- none; Reaction temperature (C)- 50 °C; Reaction Time (min)- 120; Reaction volume (μ L)- 1; TdT concentration (μ M)- 4; NTP- mATP; NTP conc (μ M)-300; Oligonucleotide-10x multiplex [5'-6-FAM-T12mAmUmA, 5'-6-FAM-T17mAmUmC, 5'-6-FAM-T22mAmUmU, 5'-6-FAM-T27mAmUmG, 5'-6-FAM-T57mUmUmC, 5'-6-FAM-T32mAmCmC, 5'-6-FAM-T37mAmGmC, 5'-6-FAM-T42mAmAmC, 5'-6-FAM-T47mGmUmC, 5'-6-FAM-T52mCmUmC], 5'-6-FAM-T15mAmU(2'dF)G, 5'-6-FAM-T15mAmG(MOE)C; Oligonucleotide conc (μ M)-250 or 25 each oligo for 10x multiplex; Product- 10x multiplex [5'-6-FAM-T12mAmUmAmA-3'P, 5'-6-FAM-T17mAmUmCmA-3'P, 5'-6-FAM-T22mAmUmUmA-3'P, 5'-6-FAM-T27mAmUmGmA-3'P, 5'-6-FAM-T57mUmUmCmA-3'P, 5'-6-FAM-T32mAmCmCmA-3'P, 5'-6-FAM-T37mAmGmCmA-3'P, 5'-6-FAM-T42mAmAmCmA-3'P], 5'-6-FAM-T15mAmU(2'dF)GmA-3'P, 5'-6-FAM-T15mAmG(MOE)CmA-3'P.

[0493] Activity relative to SEQ ID NO: 3870 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 3870. The results are shown in Tables 56.2, 56.3, and 56.4.

Table 56.2 - Products- 10x multiplex [5'-6-FAM-T12mAmUmAmA-3'P, 5'-6-FAM-T17mAmUmCmA-3'P, 5'-6-FAM-T22mAmUmUmA-3'P, 5'-6-FAM-T27mAmUmGmA-3'P, 5'-6-FAM-T57mUmUmCmA-3'P, 5'-6-FAM-T32mAmCmCmA-3'P, 5'-6-FAM-T37mAmGmCmA-3'P, 5'-6-FAM-T42mAmAmCmA-3'P], 5'-6-FAM-T15mAmU(2'dF)GmA-3'P, 5'-6-FAM-T15mAmG(MOE)CmA-3'P.

FAM-T57mUmUmCmA-3'P, 5'-6-FAM-T32mAmCmCmA-3'P, 5'-6-FAM-T37mAmGmCmA-3'P, 5'-6-FAM-T42mAmAmCmA-3'P]		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3870)	FIOP % product Relative to SEQ ID NO: 3870
3917/3918	M301W	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3870 and defined as follows: "+" from 3.25 to 4.37.		

Table 56.3 Product 5'-6-FAM-T15mAmU(2'dF)GmA-3'P		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3870)	FIOP % product Relative to SEQ ID NO: 3870
3917/3918	M301W	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3870 and defined as follows: "+" from 3.25 to 4.37.		

Table 56.4 Product 5'-6-FAM-T15mAmG(MOE)CmA-3'P		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3870)	FIOP % product Relative to SEQ ID NO: 3870
3917/3918	M301W	+
3919/3920	M301G	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3870 and defined as follows: "+" from 3.25 to 4.37.		

EXAMPLE 57

Gene Acquisition and Expression of Wild-type Alkaline Phosphatases

[0494] Synthetic genes encoding an N-terminal 6-histidine tagged version of multiple wild-type (WT) alkaline phosphatases were cloned into the pCK110900 vector system (See e.g., US Pat. No. 9,714,437, which is hereby incorporated by reference in its entirety) and subsequently expressed in an *E. coli* strain derived from W3110.

[0495] Cells transformed with the alkaline phosphatase expression constructs were grown at shake-flask scale using IPTG induction as described in Example 3. Cells were then lysed, purified, and dialyzed into storage buffer (20 mM Tris-HCl, pH 7.4, 100 mM KCl, 0.1 mM EDTA, and 50% glycerol). After overnight dialysis, protein samples were removed, and enzyme concentrations were measured by absorption at 280nm using a NanoDrop™ 1000 spectrophotometer. Soluble protein concentrations are summarized in Table 57.1 below, showing a fold improvement in soluble protein production following shake-flask purification relative to the alkaline phosphatase from *Thermotoga neapolitana* (SEQ ID NO: 3930).

Table 57.1 Soluble Enzyme Production of Variants Relative to SEQ ID NO: 3922		
SEQ ID NO: (nt/aa)	Source organism of AP gene sequence	FIOP Soluble Enzyme Production (Relative to SEQ ID NO: 3930)
3923/3924	<i>Pyrococcus furiosus</i>	+
3921/3922	<i>Thermotoga maritima</i>	+

3925/3926	<i>Thermotoga sp. 50_64</i>	++
3927/3928	<i>Pseudothermotoga lettingae</i>	+
3929/3930	<i>Thermotoga neapolitana</i>	+
3931/3932	<i>Thermoflexibacter ruber</i>	+++
3933/3934	<i>Bacillus licheniformis</i>	++
Levels of increased soluble enzyme production were relative to the reference polypeptide of SEQ ID NO: 3930 and defined as follows: "+" 1.00 to 1.30, "++" > 1.30, "+++" > 1.80.		

EXAMPLE 58

Gene Acquisition and Expression of Wild-type Inorganic Pyrophosphatases

[0496] Synthetic genes encoding a C-terminal 6-histidine tagged version of multiple wild-type (WT) inorganic pyrophosphatase enzymes were cloned into the pCK110900 vector system (See e.g., US Pat. No. 9,714,437, which is hereby incorporated by reference in its entirety) and subsequently expressed in an *E. coli* strain derived from W3110.

[0497] Cells transformed with the inorganic pyrophosphatase expression constructs were grown at shake-flask scale using IPTG induction as described in Example 3. Cells were then lysed, purified, and dialyzed into storage buffer (20 mM Tris-HCl, pH 7.4, 100 mM KCl, 0.1 mM EDTA, and 50% glycerol). After overnight dialysis, protein samples were removed, and enzyme concentrations were measured by absorption at 280nm using a NanoDrop™ 1000 spectrophotometer. Soluble protein concentrations are summarized in Table 58.1 below, showing a fold improvement in soluble protein production following shake-flask purification relative to the inorganic pyrophosphatase from *Thermocrinis ruber* (SEQ ID NO: 3936).

Table 58.1 Soluble Enzyme Production of Variants Relative to SEQ ID NO: 3936		
SEQ ID NO: (nt/aa)	Source organism of IPP gene sequence	FIOP Soluble Enzyme Production (Relative to SEQ ID NO: 3936)
3935/3936	<i>Thermocrinis ruber</i>	+
3937/3938	<i>Aquifex pyrophilus</i>	+
3939/3940	<i>Thermus oshimai</i>	+
3941/3942	<i>Sulfolobus sp. A20</i>	+++
3943/3944	<i>Geobacillus zalihae</i>	++
3945/3946	<i>Bacillus thermozeamaize</i>	++
3947/3948	<i>Bacillus smithii</i>	+
Levels of increased soluble enzyme production were determined relative to the reference polypeptide of SEQ ID NO: 3936 and defined as follows: "+" 1.00 to 4.80, "++" > 4.80, "+++" > 6.10.		

EXAMPLE 59

3'Phosphate deblocking activity of shake-flask purified wild-type alkaline phosphatases

[0498] Wild-type (WT) alkaline phosphatases (APs) SEQ ID NO: 3924, 3922, 3926, 3928, 3930, 3932, and 3934 (as described in Example 56) were assayed for 3'dephosphorylation activity of oligonucleotide 5'-6-FAM-T15mGmAmC-3'P.

[0499] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 25 μ M 3'blocked oligonucleotide, 1.2 μ M AP, 20 mM triethanolamine (pH 7.8), and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for AP, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) AP solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 59.1-Reaction conditions	
Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Preincubation Temp (C) – none; Preincubation Time (min)- none; Reaction temperature (C)- 37 °C; Reaction Time (min)- 60; Reaction volume (μ L)- 1; AP concentration (μ M)- 1.2; Oligonucleotide-5'-6-FAM-T15mGmAmC-3'P; Oligonucleotide conc (μ M)- 25; Product- 5'-6-FAM-T15mGmAmC.	

[0500] Percent dephosphorylation was calculated as the product peak area divided by the sum of the total peak area of the electropherogram. The results are shown in Table 59.2.

Table 59.2 Dephosphorylation activity of alkaline phosphatases with a 3' phosphorylated oligonucleotide	
SEQ ID NO: (nt/aa)	Conversion to de-phosphorylated oligonucleotide
3923/3924	+
3921/3922	+
3925/3926	+
3927/3928	++
3929/3930	+++
3931/3932	++
3933/3934	+
Percent dephosphorylation is defined as follows: "+" 97.11 to 98.29, "++" > 98.29, "+++" > 98.39.	

EXAMPLE 60

Suppression of by-products arising from TdT reactions by shake-flask purified wild-type inorganic pyrophosphatases.

[0501] Wild-type (WT) inorganic pyrophosphatases SEQ ID NO: 3936, 3938, 3940, 3942, 3944, 3946, and 3948 (as described in Example 59) were assayed for suppression of by-products generated in a reaction containing TdT SEQ ID NO: 3674.

[0502] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 80 μ M oligonucleotide, 100 μ M 3'phosphate blocked NTP, 10 μ M TdT, 0.5 μ M IPP, 20 mM triethanolamine (pH 7.8), and 250 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for IPP, were pre-mixed in a single solution and were aliquoted into each well of the

96-well plates (ii) IPP solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 60.1-Reaction conditions
Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Preincubation Temp (C) – none; Preincubation Time (min)- none; Reaction temperature (C)- 50 °C; Reaction Time (min)- 120; Reaction volume (μL)- 1; IPP concentration (μM)- 0.5; TdT concentration (μM)- 10; NTP (μM)-3'P-mATP; NTP conc (μM)- 100; Oligonucleotide-5'-6-FAM-T15mGmAmC 5'-6-FAM-T15mGmAmC-3'P; Oligonucleotide conc (μM)- 80; Product- 5'-6-FAM-T15mGmAmC-3'P; By-Product-unknown.

[0503] The relative suppression of by-products relative to a control without IPP was calculated as the percent by-product of the variant compared with the percent by-product observed by the control reaction with no IPP present. The results are shown in Table 60.2.

Table 60.2 Suppression of TdT reaction by-products by IPP	
SEQ ID NO: (nt/aa)	Relative suppression of by-products
3935/3936	+
3937/3938	++
3939/3940	+++
3941/3942	+
3943/3944	++
3945/3946	+
3947/3948	+
Levels of suppression of by-products are defined as follows: "+" 4.01 to 4.32, "++" > 4.32, "+++" > 4.41.	

EXAMPLE 61 Activity improvement over SEQ ID NO: 660

Activity improvement of shake-flask purified TdT variants with 3'phosphate-blocked nucleotides and modified RNA oligonucleotide substrates.

[0504] TdT variants of SEQ ID NO: 660, 1654, 2254, 2812, 3674 and 3674 were produced in shake flask and purified as described in Example 3.

[0505] Reactions were performed in 96-well format 200 μL BioRad PCR plates. Reactions included 25 μM oligonucleotide, 200 μM nucleotide triphosphate, 10 μM TdT, 0.5 μM IPP, 20 mM triethanolamine (pH 7.8), and 250 μM cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution and were aliquoted into each well of the 96-well plates (ii) TdT solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated

temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 61.1-Reaction conditions	
Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; IPP SEQ ID NO: 3944 (μM)- 0.5; Preincubation Temp (C) – none; Preincubation Time (min)- none; Reaction temperature (C)- 40 °C; Reaction Time (min)- 999 min; Reaction volume (μL)- 1; TdT concentration (μM)- 10; NTP- 3'P-mA, 3'P-(2'dF)A, 3'P-(2'dF)U, 3'P-mC, 3'P-mG, 3'P-mU; NTP conc (μM)-200; Oligonucleotide- 5'-6-FAM- T15AT*mG, 5'-6-FAM-T22*(2'dF)A(2'dF)GmA, 5'-6-FAM-T15AmU*mG, 5'-6-FAM- T22(2'dF)C(2'dF)G(2'dF)A, 5'-6-FAM-T27(2'dF)GmA(2'dF)U, 5'-6-FAM-T15mGmA*G, 5'-6-FAM- T11mCmGmA, 5'-6-FAM-T15mUmGmA, 5'-6-FAM-T11mC*mA*mG, 5'-6-FAM-T15mAmU*mG, 5'-6-FAM-T15mA(2'dF)UmC, 5'-6-FAM-T15mCmUmG, 5'-6-FAM-T27(2'dF)C*(2'dF)G*(2'dF)A, 5'- 6-FAM-T11mU*(2'dF)A*(2'dF)A, 5'-6-FAM-T17mAmUmC, 5'-6-FAM-T48mG*mA*mC, 5'-6- FAM-T15mAmCmU, 5'-6-FAM-T15mAmG(MOE)C, 5'-6-FAM-T17*(2'dF)A*(2'dF)A(2'dF)G, 5'-6- FAM-T15mAmU(2'dF)G, 5'-6-FAM-T15mAmU(2'dF)U, 5'-6-FAM-T15mAmU(2'dF)C; Oligonucleotide conc (μM)-25; Product- 5'-6-FAM-T15AT*mGmA-3'P, 5'-6-FAM- T22*(2'dF)A(2'dF)GmA(2'dF)A-3'P, 5'-6-FAM-T15AmU*mGmA-3'P, 5'-6-FAM- T22(2'dF)C(2'dF)G(2'dF)A(2'dF)A-3'P, 5'-6-FAM-T27(2'dF)GmA(2'dF)U(2'dF)A-3'P, 5'-6-FAM- T15mGmA*G(2'dF)A-3'P, 5'-6-FAM-T11mCmGmA(2'dF)A-3'P, 5'-6-FAM-T15mUmGmA*G-3'P, 5'-6-FAM-T11mC*mA*mG(2'dF)A-3'P, 5'-6-FAM-T15mAmU*mGmA-3'P, 5'-6-FAM- T11mC*mA*mGmA-3'P, 5'-6-FAM-T15mA(2'dF)UmCmA-3'P, 5'-6-FAM-T15mCmUmGmA-3'P, 5'- 6-FAM-T27(2'dF)C*(2'dF)G*(2'dF)A(2'dF)A-3'P, 5'-6-FAM-T11mU*(2'dF)A*(2'dF)A(2'dF)A-3'P, 5'-6-FAM-T17mAmUmCmA-3'P, 5'-6-FAM-T15mGmA*G(2'dF)U-3'P, 5'-6-FAM- T48mG*mA*mCmA-3'P, 5'-6-FAM-T15mAmCmU(2'dF)A-3'P, 5'-6-FAM-T15mGmA*GmA-3'P, 5'- 6-FAM-T15mAmCmU(2'dF)U-3'P, 5'-6-FAM-T15mAmCmUmC-3'P, 5'-6-FAM-T15mAmCmUmG- 3'P, 5'-6-FAM-T15mAmCmUmA-3'P, 5'-6-FAM-T15mAmG(MOE)CmA-3'P, 5'-6-FAM- T17*(2'dF)A*(2'dF)A(2'dF)G(2'dF)A-3'P, 5'-6-FAM-T15mGmA*GmA-3'P, 5'-6-FAM- T15mAmU(2'dF)GmA-3'P, 5'-6-FAM-T15mAmU(2'dF)UmA-3'P, 5'-6-FAM-T15mGmA*GmA-3'P, 5'-6-FAM-T15mAmCmU*mU-3'P, 5'-6-FAM-T15mGmA*GmA-3'P, 5'-6-FAM- T15mGmA*GmA-3'P, 5'-6-FAM-T15mAmCmU*mG-3'P, 5'-6-FAM-T15mAmU(2'dF)CmA-3'P.	

[0506] SEQ ID NO: 660, 1654, 2254, 2812, 3674 and 3674, shown in Table 61.2, were evaluated in the experiments described in Tables 61.3-61.5.

Table 61.2 – Sequences of variants used in tables 61.3-61.6	
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 660)

1653/1654	G92R/D94E/T101E/T108K/M137A/V141E/I155E/C201A/C213S/L264R/D314R/W333A/T344V/D392R/R394E/E395W/R397Q/R406G/I408A/H425D/S442G/S446P/R461G/E476R/R481W/H485D/S525F
2253/2254	G92R/D94E/T101E/T108K/M137A/V141E/I155E/T160M/I163V/Q165P/C201A/T203E/M205R/C213S/V219R/R258W/K263H/L264R/D314R/W333A/T344V/F353Q/D392R/R394E/E395W/R397Q/R406G/I408A/L411G/H413A/F414G/H425D/E441M/S442G/S446P/D460G/R461G/G468Q/E476R/R481W/H485D/K488N/S525F
2811/2812	R26Q/I38T/S79T/S81E/N90L/G92R/D94E/T101E/T108K/M137A/V141E/I155E/T160M/I163V/Q165P/A189R/C201A/T203E/M205R/C213S/V219R/E246N/L248R/R258W/K263H/L264R/A304V/C307L/D314R/K318R/W333A/L340I/T344V/F353Q/T362S/D392R/R394E/E395W/S396T/R397Q/F398W/K402G/L403S/R406G/I408A/A410E/L411G/H413A/F414G/H425D/E441M/S442G/S446P/V455L/D460G/R461G/L466M/G468Q/E476R/R481W/H485D/K488N/K506P/S525F
3673/3674	R26Q/M30G/I38T/S79T/S81E/N90L/G92R/D94E/T101E/T108K/M137A/G140V/V141E/M142V/E153V/I155E/T160M/I163V/Q165P/H177R/A184S/A189R/C201A/T203E/M205R/C213S/V219R/G231S/L248R/R258W/K263H/L264A/K266T/A304V/C307L/D314R/K318R/S325W/W333A/L340I/T344V/F353Q/T362S/T379M/D392R/R394E/E395W/R397Q/F398W/K402G/L403S/R406G/I408A/A410E/L411G/H413A/F414E/K416S/H425D/K427L/D429R/W433R/E434N/E441M/S442G/A444S/S446P/V455L/D460G/R461S/L466M/G468Q/E476R/R481W/T484L/H485E/K488N/A495S/L499M/K501R/K502G/K506P/A515E/S525F
3917/3918	R26Q/M30G/I38T/S79T/S81E/N90L/G92R/D94E/T101E/T108K/M137A/G140V/V141E/M142V/E153V/I155E/T160M/I163V/Q165P/H177R/A184S/A189R/F194L/R196G/C201A/T203E/M205R/C213S/V219R/G231S/L248R/R258W/K263H/L264A/K266T/S267R/M301W/A304V/C307L/D314R/K318R/S325W/W333A/L340I/T344V/F353Q/T362S/T379M/Y390C/D392R/R394E/E395W/R397Q/F398W/K402G/L403S/R406G/I408A/A410E/L411G/H413A/F414E/K416S/H425D/K427L/D429R/W433R/E434N/E441M/S442G/A444S/S446P/R451K/V455L/D460G/R461S/L466M/G468Q/E476R/R481W/T484L/H485E/K488N/A495S/L499M/K501R/K502G/K506P/A515E/S525F

[0507] Activity relative to SEQ ID NO: 660 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 660. The results are shown in Table 61.3.

Table 61.3 - Product 5'-6-FAM-T15AT*mGmA-3'P	
SEQ ID NO: (nt/aa)	FIOP Product % conversion Relative to SEQ ID NO: 660
1653/1654	+
2253/2254	++
2811/2812	+++
3673/3674	+
3917/3918	++
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 660 and defined as follows: "+" 1.00 to 12.00, "++" > 12.00, "+++" > 19.00.	

[0508] Activity relative to SEQ ID NO: 1654 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 1654. The results are shown in Table 61.4.

Table 61.4 - Products ^a 5'-6-FAM-T22*(2'dF)A(2'dF)GmA(2'dF)A-3'P, ^b 5'-6-FAM-T15AmU*mGmA-3'P, ^c 5'-6-FAM-T22(2'dF)C(2'dF)G(2'dF)A(2'dF)A-3'P, ^d 5'-6-FAM-

T27(2'dF)GmA(2'dF)U(2'dF)A-3'P, ^a 5'-6-FAM-T15mGmAmC(2'dF)A-3'P, ^f 5'-6-FAM-T11mCmGmA(2'dF)A-3'P, ^g 5'-6-FAM-T15mUmGmAmA-3'P, ^h 5'-6-FAM-T11mC*mA*mG(2'dF)A-3'P, ⁱ 5'-6-FAM-T15mAmU*mGmA-3'P, ^j 5'-6-FAM-T11mC*mA*mGmA-3'P, ^k 5'-6-FAM-T15mA(2'dF)UmCmA-3'P.											
FIOP Product % conversion Relative to SEQ ID NO: 1654											
SEQ ID NO: (nt/aa)	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>	<i>h</i>	<i>i</i>	<i>j</i>	<i>k</i>
2253/2254	+	+	+	+	+	+	+	+	+	+	+
2811/2812	+	+	+	+	+	+	+	+	++	++	++
3673/3674	+	+	+	+	+	+	++	++	++	+++	+++
3917/3918	+	+	+	+	+	+	++	++	++	+++	+++
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 1654 and defined as follows: "+" from 1.00 to 100.00; "++" 100.01-200.00; "+++" 200.01-287.00".											

[0509] Activity relative to SEQ ID NO: 2254 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2254. The results are shown in Table 61.5.

Table 61.5 - Products ^a 5'-6-FAM-T15mCmUmGmA-3'P, ^b 5'-6-FAM-T27(2'dF)C*(2'dF)G*(2'dF)A(2'dF)A-3'P, ^c 5'-6-FAM-T11mU*fA*fA(2'dF)A-3'P, ^d 5'-6-FAM-T17mAmUmCmA-3'P, ^e 5'-6-FAM-T15mGmAmC(2'dF)U-3'P, ^f 5'-6-FAM-T48mG*mA*mCmA-3'P, ^g 5'-6-FAM-T15mAmCmU(2'dF)A-3'P, ^h 5'-6-FAM-T15mGmAmCmA-3'P, ⁱ 5'-6-FAM-T15mAmCmU(2'dF)U-3'P, ^j 5'-6-FAM-T15mAmCmUmC-3'P, ^k 5'-6-FAM-T15mAmCmUmG-3'P, ^l 5'-6-FAM-T15mAmCmUmA-3'P, ^m 5'-6-FAM-T15mAmG(MOE)CmA-3'P.													
FIOP Product % conversion Relative to SEQ ID NO: 2254													
SEQ ID NO: (nt/aa)	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>	<i>h</i>	<i>i</i>	<i>j</i>	<i>k</i>	<i>l</i>	<i>m</i>
2811/2812	+	+	+	+	+	+	+	+	+	+	+	+	+
3673/3674	+	+	+	+	+	+	++	+	++	++	+++	+++	++
3917/3918	+	+	+	+	++	+	++	++	++	++	++	++	+++
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2254 and defined as follows: "+" from 1.00 to 50.00; "++" 50.01-100.00; "+++" 100.01-307.01".													

[0510] Activity relative to SEQ ID NO: 2812 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 2812. The results are shown in Table 61.6.

Table 61.6 - Products ^a 5'-6-FAM-T17*fA*fAfG(2'dF)A-3'P, ^b 5'-6-FAM-T15mGmAmCmC-3'P, ^c 5'-6-FAM-T15mAmU(2'dF)GmA-3'P, ^d 5'-6-FAM-T15mAmU(2'dF)UmA-3'P, ^e 5'-6-FAM-T15mGmAmCmG-3'P.					
FIOP Product % conversion Relative to SEQ ID NO: 2812					
SEQ ID NO: (nt/aa)	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>
3673/3674	+	+	+	+	++
3917/3918	+	+	++	++	+++

Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 2812 and defined as follows: "+" from 1.00 to 20.00; "++" 20.01-50.00; "+++" 50.01-824.00".

[0511] Activity relative to SEQ ID NO: 3674 (Activity FIOP) was calculated as the percent product of the variant compared with the percent product observed by the reaction with SEQ ID NO: 3674. The results are shown in Table 61.7.

Table 61.7 - Products ^a 5'-6-FAM-T15mAmCmU*mU-3'P, ^b 5'-6-FAM-T15mGmAmC*mU-3'P, ^c 5'-6-FAM-T15mGmAmC*mG-3'P, ^d 5'-6-FAM-T15mAmCmU*mG-3'P, ^e 5'-6-FAM-T15mAmU(2'dF)CmA-3'P.					
FIOP Product % conversion Relative to SEQ ID NO: 3674					
SEQ ID NO: (nt/aa)	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>
3917/3918	+	+++	+++	++	++
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3674 and defined as follows: "+" from 1.00 to 25.00; "++" 25.01-60.00; "+++" 60.01-79.00".					

EXAMPLE 62

Iterative Enzymatic Extension of RNA Oligomer using engineered TdT variant SEQ ID NO: 3918

[0512] TdT enzyme variant SEQ ID NO: 3918 was produced in shake flask and purified as described in Example 3. Inorganic pyrophosphatase SEQ ID NO: 3944 was produced and purified, as described in Example 58. Alkaline phosphatase SEQ ID NO: 3934 was produced and purified, as described in Example 57.

Solution Phase Extension

[0513] Fluorescently labeled RNA oligomer 5'-BiosG-T3(iFluorT)T9mAmUmA was reacted with TdT and mATP-3'P in a 1.1 mL Axygen 96 DeepWell plate. The reaction included 4 μ M oligonucleotide, 1 μ M inorganic pyrophosphatase, 8 μ M TdT, 72 μ M nucleotide triphosphate, and 250 μ M CoCl₂ in 20 mM TEA-HCl at pH 7.8. The reaction was set up as follows: (i) all reaction components except for oligonucleotide were mixed in a 1.6 mL Eppendorf tube at a volume of 196 μ L, (ii) a 4- μ L aliquot of a 200 μ M oligonucleotide stock was added to the Eppendorf tube, mixed well, then transferred to one well of an Axygen 96 DeepWell plate. The plate was then incubated at 40 °C for 18 h with 400 rpm agitation. A 4- μ L aliquot was removed from the reaction plate, diluted to 1.25 nM final oligonucleotide concentration, then analyzed by capillary electrophoresis according to Example 4.

[0514] Next, 80 μ L of Dynabeads MyOne Streptavidin C1 magnetic beads were washed with binding and wash buffer according to the Dynabeads protocol. After isolation, the beads were redispersed in 196 μ L 2X binding and wash buffer and then combined with the 196 μ L of crude extension reaction product containing 5'-BiosG-T3(iFluorT)T9mAmUmAmA-3'P in a 2 mL Eppendorf tube. The tube was placed on a slowly rotating tube rack in a 30 °C incubator for 45 min. Afterwards, the beads were isolated and

washed three times with 200 μ L binding and wash buffer followed by washing three times with 200 μ L 20 mM TEA-HCl (pH 7.8).

Immobilized Oligo Deprotection

[0515] Isolated beads bound to the biotinylated oligonucleotide were redispersed in 200 μ L deprotection buffer containing 1 μ M alkaline phosphatase, 250 μ M CoCl₂, 20 mM TEA-HCl, and 6 v/v % formamide at pH 7.8. The reaction mixture was mixed well then transferred to one well of an Axygen 1.1 mL 96 DeepWell plate and incubated at 50 °C for 0.5 h with 650 rpm agitation.

[0516] Using the above procedure for the deprotection step, four sequential addition and deprotection steps were performed starting from biotinylated oligo 5'-BiosG-T3(iFluorT)T9mAmUmA : 5'-BiosG-T3(iFluorT)T9mAmUmAmA-3'P was reacted with alkaline phosphatase to give 5'-BiosG-T3(iFluorT)T9mAmUmAmA; 5'-BiosG-T3(iFluorT)T9mAmUmAmAmG-3'P was reacted with alkaline phosphatase to give 5'-BiosG-T3(iFluorT)T9mAmUmAmAmG; 5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA-3'P was reacted with alkaline phosphatase to give 5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA; and 5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmAfA-3'P was reacted with alkaline phosphatase to give 5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmAfA.

[0517] Afterward, a 5- μ L aliquot was transferred to one well of a BioRad 96 well PCR plate which was placed on a magnetic plate holder to isolate the beads. The supernatant was removed, and the beads redispersed in 20 μ L 95:5 formamide:water containing 10 mM EDTA. The mixture was then incubated at 90 °C for 2.5 min in a thermal cycler. The beads were then immediately isolated on a magnetic plate holder, and a 4- μ L aliquot of the supernatant solution was diluted 50-fold in deionized water, which was subsequently analyzed by capillary electrophoresis according to Example 4.

[0518] The beads in the remaining 195 μ L crude reaction product were isolated, then washed with three times with 200 μ L of a solution containing 1 g/L bovine serum albumin, 1 mM EDTA, and 20 mM TEA-HCl at pH 7.8. The beads were then isolated and redispersed in 60 μ L of a 0.1 % sodium dodecyl sulfate (SDS) solution in nuclease free water. The mixture was mixed well, transferred to one well of a BioRad 96 well PCR plate, and incubated at 99 °C for 5 min in a thermal cycler. Immediately afterward the beads were isolated, and the supernatant was collected and diluted to 120 μ L with deionized water.

[0519] A fresh 80- μ L aliquot of Dynabeads MyOne Streptavidin C1 were washed with binding and wash buffer according to the Dynabeads protocol and then redispersed in 120 μ L 2X binding and wash buffer in a 1.6 mL Eppendorf tube. The dispersed beads were then combined with the 120 μ L of free biotinylated oligonucleotide and placed on a slowly rotating tube holder in a 30 °C incubator for 30 min. The beads were then isolated and washed three times with 200 μ L 20 mM TEA-HCl (pH 7.8).

Table 62.1 Dephosphorylation of Immobilized Oligonucleotide-3'P

Starting Oligonucleotide	Product Oligonucleotide	Conversion (%)
5'-BiosG-T3(iFluorT)T9mAmUmAmA-3'P	5'-BiosG-T3(iFluorT)T9mAmUmAmA	100

5'-BiosG-T3(iFluorT)T9mAmUmAmAmG-3'P	5'-BiosG-T3(iFluorT)T9mAmUmAmAmG	99
5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA-3'P	5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA	95
5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmAfA-3'P	5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmAfA	99
Conversion of nucleotide was determined as percent of the product peak divided by the sum of the total fluorescence in the electropherogram.		

Immobilized Oligo Extension

[0520] The beads were isolated and then redispersed in 200 μ L of reaction buffer containing 16 μ M TdT, 1 μ M inorganic pyrophosphatase, 72 μ M nucleotide, 1 mM CoCl₂, 20 mM TEA-HCl, 6 % v/v formamide, and 0.1 % v/v PEG3350 at pH 7.8. After mixing well, the suspension was transferred to one well of an Axygen 1.1 mL 96 DeepWell plate and incubated at 50 °C for 3.5 h with 650 rpm agitation.

[0521] Using the above procedure for the addition step, four sequential addition and deprotection steps were performed starting from biotinylated oligo 5'-BiosG-T3(iFluorT)T9mAmUmA: 5'-BiosG-T3(iFluorT)T9mAmUmAmA was reacted with TdT and mGTP-3'P to give 5'-BiosG-T3(iFluorT)T9mAmUmAmAmG-3'P. 5'-BiosG-T3(iFluorT)T9mAmUmAmAmG was reacted with TdT and mATP-3'P to give 5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA. 5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA was reacted with TdT and fATP-3'P to give 5'-BiosG-T3(iFluorT)T9mAmUmAmAmGfA-3'P.

[0522] Afterward, a 5- μ L aliquot was transferred to one well of a BioRad 96 well PCR plate which was placed on a magnetic plate holder to isolate the beads. The supernatant was removed, and the beads redispersed in 20 μ L 95:5 formamide:water containing 10 mM EDTA. The mixture was then incubated at 90 °C for 2.5 min in a thermal cycler. The beads were then immediately isolated on a magnetic plate holder, and 4- μ L aliquot of the supernatant solution was diluted 50-fold in deionized water, which was analyzed by capillary electrophoresis according to Example 4.

[0523] The beads in the remaining 195 μ L crude reaction product were isolated and washed three times 200 μ L solution containing 1 g/L bovine serum albumin, 1 mM EDTA, and 20 mM TEA-HCl at pH 7.8. Then, the beads were isolated and washed three times with 200 μ L solution containing 1 g/L bovine serum albumin and 20 mM TEA-HCl at pH 7.8.

Table 62.2 TdT Catalyzed Extension of Oligonucleotide using Nucleotide-3'P		
Starting Oligonucleotide	Product Oligonucleotide	Conversion (%)
5'-BiosG-T3(iFluorT)T9mAmUmA	5'-BiosG-T3(iFluorT)T9mAmUmAmA-3'P	86
5'-BiosG-T3(iFluorT)T9mAmUmAmA	5'-BiosG-T3(iFluorT)T9mAmUmAmAmG-3'P	97
5'-BiosG-T3(iFluorT)T9mAmUmAmAmG	5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA-3'P	91

Table 62.2 TdT Catalyzed Extension of Oligonucleotide using Nucleotide-3'P		
Starting Oligonucleotide	Product Oligonucleotide	Conversion (%)
5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmA	5'-BiosG-T3(iFluorT)T9mAmUmAmAmGmAfA-3'P	100
Conversion of nucleotide was determined as percent of the product peak divided by the sum of the total fluorescence in the electropherogram.		

EXAMPLE 63

Improvements over SEQ ID NO: 3870 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0524] SEQ ID NO: 3870 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 63.1.

[0525] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μM oligonucleotide, 100-600 μM nucleotide triphosphate, 20-100 mM buffer, and 250-600 μM cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 63.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (C)-45; Preincubation Time (min)-60; Reaction temperature (C)-45; Reaction Time (min)-120; Reaction volume (μL)-1; NTP (μM) -mCTP-3'P ; NTP conc (μM)-200; Oligonucleotide-5'-6-FAM-T11mC*mA*mG, 5'-6-FAM-T57mUmU(2'dF)C, 5'-6-FAM-T17*mA*mGmA, 5'-6-FAM-T22*mGmA, 5'-6-FAM-T27mAmAmA, 5'-6-FAM-T32mAmAmA(2'dF)G, 5'-6-FAM-T37mG*mA*mC, 5'-6-FAM-T42*mA*mCmU, 5'-6-FAM-T47*mCmUmU, 5'-6-FAM-T52mUmUmU; Substrate conc (μM)-10 μM each of ten oligo; Product -5'-6-FAM-T11mC*mA*mGmC-3'P , 5'-6-FAM-T57mUmU(2'dF)CmC-3'P , 5'-6-FAM-T17*mA*mGmA-C-3'P , 5'-6-FAM-T22*mGmA-C-3'P , 5'-6-FAM-T27mAmAmA-C-3'P , 5'-6-FAM-T32mAmAmA(2'dF)GmC-3'P , 5'-6-FAM-T37mG*mA*mCmC-3'P , 5'-6-FAM-T42*mA*mCmUmC-3'P , 5'-6-FAM-T47*mCmUmUmC-3'P , 5'-6-FAM-T52mUmUmUmC-3'P

[0526] Activity relative to SEQ ID NO: 3870 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 3870 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 63.2.

Table 63.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3870)	FIOP Product Peak Area Relative to SEQ ID NO: 3870
5475/5476	T200K	+++
4047/4048	F204I	+++
4049/4050	G406L	+++
4051/4052	G406S	+++
4053/4054	E342Q	+++
4055/4056	P404G	++
4057/4058	T295V	++
4059/4060	Q179M	++
4061/4062	Y192M	++
4063/4064	R171G	++
4065/4066	Q179P	++
4067/4068	M490R	++
4069/4070	Q169T	+
4071/4072	S403T	+
4073/4074	M490E	+
4075/4076	Y192T	+
4077/4078	F204V	+
4079/4080	S495C	+
4081/4082	T295S	+
4083/4084	S403L	+
4085/4086	M490L	+
4087/4088	L522I	+
4089/4090	D199G	+
4091/4092	T295G	+
4093/4094	P165A	+
4095/4096	N197E	+
4097/4098	I187R	+
4099/4100	V210M	+
4101/4102	Y521V	+
4103/4104	N197R	+
4105/4106	N493K	+
4107/4108	M490S	+
4109/4110	N175I	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3870 and defined as follows: "+" 1.01 to 1.04, "++" 1.04 to 1.07, "+++" 1.07 to 1.12		

EXAMPLE 64**Improvements over SEQ ID NO: 3870 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0527] SEQ ID NO: 3870 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 64.1.

[0528] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 64.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-68; Preincubation Time (min)-60; Reaction temperature (C)-50; Reaction Time (min)-60; Reaction volume (μ L)-1; NTP (μ M)-fATP-3'P ; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15mAmU*mG; Substrate conc (μ M)-50; Product - 5'-6-FAM-T15mAmU*mG(2'dF)A-3'P.

[0529] Activity relative to SEQ ID NO: 3870 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 3870 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 64.2.

Table 64.2

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3870)	FIOP Product Peak Area Relative to SEQ ID NO: 3870
4047/4048	F204I	++
4049/4050	G406L	++
4051/4052	G406S	+
4053/4054	E342Q	+
4055/4056	P404G	+++
4059/4060	Q179M	+
4071/4072	S403T	+

4081/4082	T295S	++
4083/4084	S403L	++
4087/4088	L522I	+
4089/4090	D199G	+
4103/4104	N197R	++
4105/4106	N493K	++
4111/4112	N175M	+++
4113/4114	Q179K	+++
4115/4116	N191M	+++
4117/4118	V341C	+++
4119/4120	E203F	+++
4121/4122	T295N	+++
4123/4124	L484S	+++
4125/4126	D183C	++
4127/4128	K508N	++
4129/4130	T173S	++
4131/4132	N175L	++
4133/4134	P165C	++
4135/4136	N197C	++
4137/4138	G406R	++
4139/4140	N191Q	++
4141/4142	R429V	++
4143/4144	S509R	++
4145/4146	R267S	++
4147/4148	S403W	++
4149/4150	L491V	++
4151/4152	N197T	++
4153/4154	T295A	++
4155/4156	W325L	++
4157/4158	A507G	++
4159/4160	H494T	+
4161/4162	I340S	+
4163/4164	E203G	+
4165/4166	W481Q	+
4167/4168	K195R	+
4169/4170	D183W	+
4171/4172	P165R	+
4173/4174	T200M	+
4175/4176	A319E	+
4177/4178	E203M	+
4179/4180	N175V	+
4181/4182	Y192A	+
4183/4184	Q179S	+
4185/4186	W325G	+
4187/4188	Y259F	+
4189/4190	N176V	+
4191/4192	Q179T	+

4193/4194	R480A	+
4195/4196	R291M	+
4197/4198	S495R	+
4199/4200	G406T	+
4201/4202	L387I	+
4203/4204	S403K	+
4205/4206	P404M	+
4207/4208	E257S	+
4209/4210	S403R	+
4211/4212	D199R	+
4213/4214	A483C	+
4215/4216	S495M	+
4217/4218	M490V	+
4219/4220	N191V	+
4221/4222	E257P	+
4223/4224	W398V	+
4225/4226	H494A	+
4227/4228	L374V	+
4229/4230	R429F	+
4231/4232	Q169M	+
4233/4234	R429H	+
4235/4236	D199W	+
4237/4238	S403V	+
4239/4240	D199S	+
4241/4242	S293C	+
4243/4244	E399S	+
4245/4246	Q179V	+
4247/4248	N197F	+
4249/4250	R480Q	+
4251/4252	N191F	+
4253/4254	S495N	+
4255/4256	M490A	+
4257/4258	E203V	+
4259/4260	P165T	+
4261/4262	D199V	+
4263/4264	S293T	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3870 and defined as follows: "+" 1.10 to 1.50, "++" 1.50 to 1.97, "+++", 1.97 to 4.12.		

EXAMPLE 65**Improvements over SEQ ID NO: 3918 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0530] SEQ ID NO: 3918 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (e.g., saturation mutagenesis and recombination

of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 65.1.

[0531] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 65.1-Reaction conditions	
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (C)-48; Preincubation Time (min)-60; Reaction temperature (C)-48; Reaction Time (min)-120; Reaction volume (μ L)-1; NTP (μ M)-*mGTP-3'P ; NTP conc (μ M)-200; Oligonucleotide-5'-6-FAM-T11mC*mA*mG, 5'-6-FAM-T57mUmU(2'dF)C, 5'-6-FAM-T17*mA*mGmA, 5'-6-FAM-T22*mGmA*AmA, 5'-6-FAM-T27mAmAmA, 5'-6-FAM-T32mAmAmA(2'dF)G, 5'-6-FAM-T37mG*mA*mC, 5'-6-FAM-T42*mA*mCmU, 5'-6-FAM-T47*mCmUmU, 5'-6-FAM-T52mUmUmU; Substrate conc (μ M)-10 μ M each of ten oligo; Product -5'-6-FAM-T11mC*mA*mG*mG-3'P , 5'-6-FAM-T57mUmU(2'dF)C*mG-3'P , 5'-6-FAM-T17*mA*mGmA*mG-3'P , 5'-6-FAM-T22*mGmA*AmA*mG-3'P , 5'-6-FAM-T27mAmAmA*mG-3'P , 5'-6-FAM-T32mAmAmA(2'dF)G*mG-3'P , 5'-6-FAM-T37mG*mA*mC*mG-3'P , 5'-6-FAM-T42*mA*mCmU*mG-3'P , 5'-6-FAM-T47*mCmUmU*mG-3'P , 5'-6-FAM-T52mUmUmU*mG-3'P .	

[0532] Activity relative to SEQ ID NO: 3918 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 3918 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 65.2.

Table 65.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 3918)	FIOP Product Peak Area Relative to SEQ ID NO: 3918
4265/4266	H7-/D400W/Y459R/F504Q	+++
4267/4268	W481R	+++
4269/4270	D400W/Y459V/M499R/T500S/F504Q	+++
4271/4272	F335V/W481R/L484R	+++
4273/4274	W481R/E512R	+++
4275/4276	W395N/L484R	+++

4277/4278	D400W/Y459V	+++
4279/4280	E203R/Y459V	+++
4281/4282	F335V/G402R/W481R/L484R	+++
4283/4284	L484R	+++
4285/4286	Y459R	+++
4287/4288	D400W/Y459R/T500S/R501P	+++
4289/4290	G402K/W481D/N493T	++
4291/4292	W395T/W481R	++
4293/4294	E203R/Y459R/T500S	++
4295/4296	V218L	++
4297/4298	G402R/L484R/N493T	++
4299/4300	D400W/Y459V/R501P/F504G	++
4301/4302	F335V/G402K/W481R/L484E	++
4303/4304	L484E	++
4305/4306	F335V/G402K/E512R	++
4307/4308	E203R/Y459R/M499R/F504G	++
4309/4310	F335V/W481R/N493T	++
4311/4312	W395N/G402K	++
4313/4314	Y459V	++
4315/4316	N493T	++
4317/4318	M499P	++
4319/4320	D400W	++
4321/4322	F335V/G402K/W481R/L484R	++
4323/4324	W481D	++
4325/4326	G402K/W481R/L484R	++
4327/4328	W395T	++
4329/4330	Y459V/R501P	++
4331/4332	W395N/W481R/E512R	++
4333/4334	E367S	++
4335/4336	F504G	+
4337/4338	R501P	+
4339/4340	D400W/F504Q	+
4341/4342	E367R	+
4343/4344	T173I/E367S/Y459V/T500S	+
4345/4346	H3Q	+
4347/4348	G402K	+
4349/4350	F504Q	+
4351/4352	F335V	+
4353/4354	F335V/W481R/E512R	+
4355/4356	M499R	+
4357/4358	E367S/Y459V/T500S	+
4359/4360	E203R	+
4361/4362	W395T/G402K/W481R/L484R	+
4363/4364	E203R/D400W/Y459R/R501P	+
4365/4366	G402R	+
4367/4368	Q373G	+
4369/4370	E485Q	+

4371/4372	W395N	+
4373/4374	G402K/W481D	+
4375/4376	D400W/R501P	+
4377/4378	Q376S	+
4379/4380	T500S	+
4381/4382	E203R/E367S/Y459V/T500S/R501P	+
4383/4384	P458R	+
4385/4386	F335V/W481D/L484R	+
4387/4388	G460M	+
4389/4390	D336Q	+
4391/4392	E367T	+
4393/4394	G460R	+
4395/4396	P215Q	+
4397/4398	C390A	+
4399/4400	Q370E	+
4401/4402	K384S	+
4403/4404	D338Q	+
4405/4406	E512R	+
4407/4408	G402S	+
4409/4410	K294D	+
4411/4412	G402K/W481D/L484R/E512R	+
4413/4414	E515P	+
4415/4416	G402K/E512R	+
4417/4418	A339R	+
4419/4420	S164G	+
4421/4422	W395E	+
4423/4424	E512G	+
4425/4426	D338E	+
4427/4428	R501N	+
4429/4430	W395A	+
4431/4432	N380G	+
4433/4434	H516T	+
4435/4436	E203R/E367S/Y459R	+
4437/4438	S184A	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 3918 and defined as follows: "+" 1.08 to 1.20, "++" 1.20 to 1.32, "+++" 1.32 to 1.65.		

EXAMPLE 66**Improvements over SEQ ID NO: 4266 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0533] SEQ ID NO: 4266 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 66.1.

[0534] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μM oligonucleotide, 100-600 μM nucleotide triphosphate, 20-100 mM buffer, and 250-600 μM cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 66.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (C)-48; Preincubation Time (min)-60; Reaction temperature (C)-50; Reaction Time (min)-120; Reaction volume (μL)-1; NTP (μM)-*mGTP-3P; NTP conc (μM)-200; Oligonucleotide-5'-6-FAM-T12mC*mA*mG, 5'-6-FAM-T57mUmU(2'dF)C, 5'-6-FAM-T17*mA*mGmA, 5'-6-FAM-T22*mGmA, 5'-6-FAM-T27mAmAmA, 5'-6-FAM-T32mAmAmA(2'dF)G, 5'-6-FAM-T37mG*mA*mC, 5'-6-FAM-T42*mA*mCmU, 5'-6-FAM-T47*mCmUmU, 5'-6-FAM-T52mUmUmU; Substrate conc (μM)-15 μM each of ten oligo; Product -5'-6-FAM-T12mC*mA*mG*mG-3P, 5'-6-FAM-T57mUmU(2'dF)C*mG-3P, 5'-6-FAM-T17*mA*mGmA*mG-3P, 5'-6-FAM-T22*mGmA*mG-3P, 5'-6-FAM-T27mAmAmA*mG-3P, 5'-6-FAM-T32mAmAmA(2'dF)G*mG-3P, 5'-6-FAM-T37mG*mA*mC*mG-3P, 5'-6-FAM-T42*mA*mCmU*mG-3P, 5'-6-FAM-T47mGmUmCmA-3P, 5'-6-FAM-T52mUmUmU*mG-3P.

[0535] Activity relative to SEQ ID NO: 4266 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 4266 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 66.2.

Table 66.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 4266)	FIOP Product Peak Area Relative to SEQ ID NO: 4266
4439/4440	L311R	+++
4441/4442	F346Q	+++
4443/4444	R472S/M498P/T499S/Q503G	+++
4445/4446	R472G/M498P/T499S/Q503G	+++
4447/4448	S471N	++
4449/4450	Q301G	++
4451/4452	Y308Q	++
4453/4454	P299S	++
4455/4456	P299E	++
4457/4458	V343L	++

4459/4460	Q301S/Q503G	+
4461/4462	L418V/Q503G	+
4463/4464	E192T	+
4465/4466	P299T	+
4467/4468	P299K/M498P/T499S/Q503G	+
4469/4470	E192G	+
4471/4472	K296G	+
4473/4474	E192V	+
4475/4476	R280H	+
4477/4478	M473Q	+
4479/4480	L297F	+
4481/4482	K406S	+
4483/4484	Y308R	+
4485/4486	G349Q	+
4487/4488	L392R/M498P/T499S/Q503G	+
4489/4490	E192Y	+
4491/4492	Y308T	+
4493/4494	V343C	+
4495/4496	K296P	+
4497/4498	L359I/M498P/T499S/Q503G	+
4499/4500	M465E	+
4501/4502	D411E	+
4503/4504	R280G/M498P/T499S/Q503G	+
4505/4506	F346W	+
4507/4508	A407S	+
4509/4510	R188K	+
4511/4512	E192S/M498P/T499S/Q503G	+
4513/4514	D491R	+
4515/4516	G410Q	+
4517/4518	L358G	+
4519/4520	Y308K	+
4521/4522	G410A	+
4523/4524	Y308H/Q503G	+
4525/4526	L418G/M498P/T499S/Q503G	+
4527/4528	E192Y/M498P/T499S/Q503G	+
4529/4530	D491G	+
4531/4532	V272P	+
4533/4534	I419V/Q503G	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 4266 and defined as follows: ""+"" 1.06 to 1.21, ""++"" 1.21 to 1.52, ""+++"" 1.52 to 3.08 "		

EXAMPLE 67

Improvements over SEQ ID NO: 4266 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0536] SEQ ID NO: 4266 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 67.1.

[0537] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μM oligonucleotide, 100-600 μM nucleotide triphosphate, 20-100 mM buffer, and 250-600 μM cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 67.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (C)-48; Preincubation Time (min)-60; Reaction temperature (C)-48; Reaction Time (min)-120; Reaction volume (μL) -1; NTP (μM)-*mGTP-3P; NTP conc (μM)-200; Oligonucleotide-5'-6-FAM-T12mC*mA*mG, 5'-6-FAM-T57mUmU(2'dF)C, 5'-6-FAM-T17*mA*mGmA, 5'-6-FAM-T22*mGmA*AmA, 5'-6-FAM-T27mAmAmA, 5'-6-FAM-T32mAmAmA(2'dF)G, 5'-6-FAM-T37mG*mA*mC, 5'-6-FAM-T42*mA*mCmU, 5'-6-FAM-T47*mCmUmU, 5'-6-FAM-T52mUmUmU; Substrate conc (μM)-10 μM each of ten oligo; Product -5'-6-FAM-T12mC*mA*mG*mG-3P, 5'-6-FAM-T57mUmU(2'dF)C*mG-3P, 5'-6-FAM-T17*mA*mGmA*mG-3P, 5'-6-FAM-T22*mGmA*AmA*mG-3P, 5'-6-FAM-T27mAmAmA*mG-3P, 5'-6-FAM-T32mAmAmA(2'dF)G*mG-3P, 5'-6-FAM-T37mG*mA*mC*mG-3P, 5'-6-FAM-T42*mA*mCmU*mG-3P, 5'-6-FAM-T47*mCmUmU*mG-3P, 5'-6-FAM-T52mUmUmU*mG-3P.

[0538] Activity relative to SEQ ID NO: 4266 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 4266 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 67.2.

Table 67.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 4266)	FIOP Product Peak Area Relative to SEQ ID NO: 4266
4535/4536	E202R	+++
4537/4538	M498P/T499S/Q503G	+++

4539/4540	S163Q/F203I/E366S	+++
4541/4542	S163Q/Q328L/P363G/W480D	++
4543/4544	S163Q/P363G/W480D/E485Q	++
4545/4546	N190M/W480D/E485Q	++
4547/4548	S362T/P363G	++
4549/4550	F203I/S362T/E366S	++
4551/4552	F203I/M498P/T499S/Q503G	++
4553/4554	N190M/E202R/F203I/P363G/E366S/W480D/L483R/E485Q	++
4555/4556	S163Q/N190M	+
4557/4558	N190M/E202R/F203I/W480D	+
4559/4560	Q328L/R428V	+
4561/4562	Q328A/E485Q	+
4563/4564	E202R/F203I/E485Q	+
4565/4566	F203I/Q328A/R428V	+
4567/4568	T172S/N174M/Q178K/V340C	+
4569/4570	L95M/R428V/W480D	+
4571/4572	F203I/Q328A/W480D/E485Q	+
4573/4574	W480D/L483R	+
4575/4576	Q328L/W480D/L483R	+
4577/4578	S362T/P363R/E366S/R428V/W480D/L483R/M498P/T499S/Q503G	+
4579/4580	F203I/Q328A/P363G/R428V/L483R	+
4581/4582	E202R/F203I/Q328A/S362T/P363G/E366S/R428V/W480D/E485Q/M498P/T499S/Q503G	+
4583/4584	Q178K	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 4266 and defined as follows: "+" 1.09 to 1.15 , "++" 1.15 to 1.19 , "+++" 1.19 to 1.29 "		

EXAMPLE 68

Improvements over SEQ ID NO: 4558 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0539] SEQ ID NO: 4558 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 68.1.

[0540] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 68.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (C)-48; Preincubation Time (min)-60; Reaction temperature (C)-48; Reaction Time (min)-120; Reaction volume (μL) -1; NTP (μM)-mATP-3P; NTP conc (μM)-25; Oligonucleotide-5'-6-FAM-T17*(2'dF)A*(2'dF)A(2'dF)G, 5'-6-FAM-T32mAmAmA(2'dF)G, 5'-6-FAM-T47*mA(2'dF)A(2'dF)A, 5'-6-FAM-T57mUmU(2'dF)C; Substrate conc (μM)-12.5 μM each of four oligo; Product -5'-6-FAM-T17*(2'dF)A*(2'dF)A(2'dF)GmA-3P, 5'-6-FAM-T32mAmAmA(2'dF)GmA-3P, 5'-6-FAM-T47*mA(2'dF)A(2'dF)AmA-3P, 5'-6-FAM-T57mUmU(2'dF)CmA-3P.

[0541] Activity relative to SEQ ID NO: 4558 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 4558 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 68.2.

Table 68.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 4558)	FIOP Product Peak Area Relative to SEQ ID NO: 4558
4585/4586	T172S/Q178K/G401K	+++
4587/4588	T172S/N174M/Q178R/G401K/P403G	+++
4589/4590	W394T	+++
4591/4592	V340C/W394T	+++
4593/4594	G405L/L483R	+++
4595/4596	S402L/P403G	++
4597/4598	T172S/N174M/Q178R/G401K/P403G/K507R	++
4599/4600	Q178K/G401K/P403G	++
4601/4602	G401K	++
4603/4604	N174M/Q178R	++
4605/4606	V94I/A365F/K367R	++
4607/4608	L483R	++
4609/4610	G401K/S402L/P403G/K507N	++
4611/4612	T172S/Q178K	++
4613/4614	C389A/W394E	+
4615/4616	W324L/N379T/G405L/L483R	+
4617/4618	T172S/N174M/Q178K/S402L/S508R	+
4619/4620	Q178K/S402L/P403G	+
4621/4622	T172S/N174M/G401K/P403G/K507R	+
4623/4624	V340C	+
4625/4626	A365F/K367R/R428C	+
4627/4628	A365H/C389A/W394E	+
4629/4630	Q375G/K376H	+

4631/4632	A365F	+
4633/4634	Q375G/L380V/L483R	+
4635/4636	K376H/L483R	+
4637/4638	Q375G/N379T/L483R	+
4639/4640	K367R	+
4641/4642	Q375G/L380V	+
4643/4644	N379T/L483R	+
4645/4646	Q375G/L380V/G400P/L483R	+
4647/4648	A318E/Q375G/L380V	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 4558 and defined as follows: "+" 1.03 to 1.19 , "++" 1.19 to 1.33 , "+++" 1.33 to 1.56 "		

EXAMPLE 69

Improvements over SEQ ID NO: 4442 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0542] SEQ ID NO: 4442 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 69.1.

[0543] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 69.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-48; Preincubation Time (min)-60; Reaction temperature (°C)-48; Reaction Time (min)-120; Reaction volume (μ L) -1; NTP (μ M)-mATP-3P; NTP conc (μ M)-200; Oligonucleotide-5'-6-FAM-T17*(2'dF)A*(2'dF)A(2'dF)G, 5'-6-FAM-T32mAmAmA(2'dF)G, 5'-6-FAM-T47*mA(2'dF)A(2'dF)A, 5'-6-FAM-T57mUmU(2'dF)C; Substrate conc (μ M)-12.5 μ M each of four oligo; Product -5'-6-FAM-T17*(2'dF)A*(2'dF)A(2'dF)GmA-3P, 5'-6-FAM-T32mAmAmA(2'dF)GmA-3P, 5'-6-FAM-T47*mA(2'dF)A(2'dF)AmA-3P, 5'-6-FAM-T57mUmU(2'dF)CmA-3P.
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[0544] Activity relative to SEQ ID NO: 4442 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 4442 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 69.2.

Table 69.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 4442)	FIOP Product Peak Area Relative to SEQ ID NO: 4442
4649/4650	K296P/P299T	+++
4651/4652	E192T/S402L/K507R	+++
4653/4654	S402L	+++
4655/4656	E192T/R280H	+++
4657/4658	E185G/N190M	+++
4659/4660	M281L	++
4661/4662	E189R/N190M	++
4663/4664	W394M	++
4665/4666	R313A	++
4667/4668	A312Q	++
4669/4670	D182N	++
4671/4672	W480L	++
4673/4674	N174M/K296P/P299E	++
4675/4676	R313I	++
4677/4678	E189H/N190M	++
4679/4680	D182R	++
4681/4682	A318V	++
4683/4684	S402L/K507R	++
4685/4686	S460A	++
4687/4688	L306A	++
4689/4690	Y308Q	++
4691/4692	A318P	+
4693/4694	V327A	+
4695/4696	A318R	+
4697/4698	W257K	+
4699/4700	Y307N	+
4701/4702	L305F	+
4703/4704	Q259R	+
4705/4706	D182A	+
4707/4708	L306R	+
4709/4710	V451L	+
4711/4712	W394L	+
4713/4714	H493K	+
4715/4716	R313L	+
4717/4718	A318T	+
4719/4720	Q259C	+
4721/4722	S260A	+

4723/4724	W394Y	+
4725/4726	A318N	+
4727/4728	A312R	+
4729/4730	S494T	+
4731/4732	R476L/W480D	+
4733/4734	S494R	+
4735/4736	A318Y	+
4737/4738	E522T	+
4739/4740	N190M	+
4741/4742	A318L	+
4743/4744	E189A/N190M	+
4745/4746	A312H	+
4747/4748	A414C	+
4749/4750	L306M	+
4751/4752	A312K	+
4753/4754	D182C	+
4755/4756	T316F	+
4757/4758	W381F	+
4759/4760	S404A	+
4761/4762	A414P	+
4763/4764	G405L	+
4765/4766	L374M	+
4767/4768	E189V/N190M	+
4769/4770	S395G	+
4771/4772	N190M/L193F	+
4773/4774	V289I	+
4775/4776	A318M	+
4777/4778	W257R	+
4779/4780	W480D	+
4781/4782	A312V	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 4442 and defined as follows: ""+"" 1.01 to 1.09, ""++"" 1.09 to 1.21, ""+++"" 1.21 to 1.62 "		

EXAMPLE 70**Improvements over SEQ ID NO: 4442 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0545] SEQ ID NO: 4442 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 70.1.

[0546] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT,

were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 70.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-65; Preincubation Time (min)-60; Reaction temperature (C)-50; Reaction Time (min)-60; Reaction volume (μL) -1; NTP (μM)-fATP-3P; NTP conc (μM)-100; Oligonucleotide-5'-6-FAM-T15mAmU*mG; Substrate conc (μM)-50; Product - 5'-6-FAM-T15mAmU*mG(2'dF)A-3P.

[0547] Activity relative to SEQ ID NO: 4442 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 4442 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 70.2.

Table 70.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 4442)	FIOP Product Peak Area Relative to SEQ ID NO: 4442
4671/4672	W480L	+
4687/4688	L306A	+++
4707/4708	L306R	+
4721/4722	S260A	+
4731/4732	R476L/W480D	+
4739/4740	N190M	+
4771/4772	N190M/L193F	+
4779/4780	W480D	+++
4783/4784	W480E	+++
4785/4786	R476W/W480D	+++
4787/4788	W480D/Y481W	++
4789/4790	L306T	++
4791/4792	A414E	++
4793/4794	Q259K	++
4795/4796	S494Q	+
4797/4798	W480M	+
4799/4800	F461M	+
4801/4802	L306C	+
4803/4804	R432H	+
4805/4806	W381M	+
4807/4808	M281C	+

4809/4810	R455A	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 4442 and defined as follows: ""+"" 1.10 to 1.19 , ""++"" 1.19 to 1.26 , ""+++"" 1.26 to 1.33 "		

EXAMPLE 71

Improvements over SEQ ID NO: 4654 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0548] SEQ ID NO: 4654 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 71.1.

[0549] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 71.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-65; Preincubation Time (min)-60; Reaction temperature (C)-50; Reaction Time (min)-60; Reaction volume (μ L) -1; NTP (μ M)-mATP-3P; NTP conc (μ M)-100; Oligonucleotide-5'-6-FAM-T15mAmU*mG; Substrate conc (μ M)-50; Product -5'-6-FAM-T15mAmU*mGmA-3P.

[0550] Activity relative to SEQ ID NO: 4654 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 4654 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 71.2.

Table 71.2

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 4654)	FIOP Product Peak Area Relative to SEQ ID NO: 4654
4811/4812	Q375G/G405L/K406S	+++
4813/4814	W480D/L483R	+++
4815/4816	N190M/L380V/G405L	++

4817/4818	W394T/W480D	++
4819/4820	Q301S/W394T/W480D	++
4821/4822	N190M/G405L/K406S	++
4823/4824	Q375G	++
4825/4826	V272A/A318E/W480D/L483R	++
4827/4828	N190M	++
4829/4830	V272A/Q301S/E393K/W394T/W480D	+
4831/4832	Q375G/G405L	+
4833/4834	W394T	+
4835/4836	N190M/Q375G	+
4837/4838	N190M/Q375G/L380V	+
4839/4840	Q375G/L380V	+
4841/4842	N190M/D197N/Y308Q	+
4843/4844	N190M/Y308Q/L380V/G405L	+
4845/4846	L380V	+
4847/4848	A318E	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 4654 and defined as follows: "+" 1.06 to 1.28, "++" 1.28 to 1.46, "+++" 1.46 to 1.67"		

EXAMPLE 72

Improvements over SEQ ID NO: 4850 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0551] SEQ ID NO: 4814 was selected as the parent TdT enzyme and the gene was re-cloned into pCK900 to re-introduce a missing histidine in the *N*-terminal poly-histidine sequence, generating SEQ ID NO: 4850. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 72.1.

[0552] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 72.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-48; Preincubation Time (min)-60; Reaction temperature (°C)-48; Reaction Time (min)-60; Reaction volume (μ L) -0.1; NTP (μ M)-

*mUTP-3P; NTP conc (μM)-200; Oligonucleotide-5'-6-FAM-T11mC*mA*mG, 5'-6-FAM-T17*mA*mGmA, 5'-6-FAM-T37mG*mA*mC, 5'-6-FAM-T42*mA*mCmU; Substrate conc (μM)-25 μM each of four oligo; Product -5'-6-FAM-T11mC*mA*mG*mU-3P, 5'-6-FAM-T17*mA*mGmA*mU-3P, 5'-6-FAM-T37mG*mA*mC*mU-3P, 5'-6-FAM-T42*mA*mCmU*mU-3P.

[0553] Activity relative to SEQ ID NO: 4850 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 4850 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 72.2.

Table 72.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 4850)	FIOP Product Peak Area Relative to SEQ ID NO: 4850
4851/4852	L298F/P300E/W395L	+++
4853/4854	L298F/P300E/V328A/W395L	+++
4855/4856	E193V/L307T	+++
4857/4858	K297P/P300S	+++
4859/4860	K297P/L298F/P300T/R392S	+++
4861/4862	L298F/P300S/V328A	+++
4863/4864	K297P/P300E/V328A	+++
4865/4866	L298F/P300E/R392S/W395L	++
4867/4868	P300E/R392S/W395L	++
4869/4870	A319P	++
4871/4872	L298F/P300S/R392S/W395L/D492E	+
4873/4874	E190R/D322N	+
4875/4876	L298F/P300E/D481W	+
4877/4878	D492E	+
4879/4880	D322N	+
4881/4882	P300T	+
4883/4884	L298F/P300T/L360V	+
4885/4886	P300E	+
4887/4888	E193V	+
4889/4890	S261A/D322N/L421V	+
4891/4892	L298F/P300S/R392S/W395L	+
4893/4894	L421V	+
4895/4896	R392S	+
4897/4898	R189K/E193V/A207V/L307T/Q353R	+
4899/4900	R189K	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 4850 and defined as follows: "+" 1.17 to 1.45, "++" 1.45 to 1.70, "+++" 1.70 to 1.89 "		

EXAMPLE 73

Improvements over SEQ ID NO: 4856 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0554] SEQ ID NO: 4856 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 73.1.

[0555] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 73.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (C)-48; Preincubation Time (min)-60; Reaction temperature (C)-48; Reaction Time (min)-120; Reaction volume (μ L) -0.1; NTP (μ M)-mATP-3P; NTP conc (μ M)-200; Oligonucleotide-5'-6-FAM-T11mU*(2'dF)A*(2'dF)A, 5'-6-FAM-T17*(2'dF)A*(2'dF)A(2'dF)G, 5'-6-FAM-T27(2'dF)GmA(2'dF)U, 5'-6-FAM-T47*mA(2'dF)A(2'dF)A; Substrate conc (μ M)-12.5 μ M each of four oligo; Product -5'-6-FAM-T11mU*(2'dF)A*(2'dF)AmA-3P, 5'-6-FAM-T17*(2'dF)A*(2'dF)A(2'dF)GmA-3P, 5'-6-FAM-T27(2'dF)GmA(2'dF)UmA-3P, 5'-6-FAM-T47*mA(2'dF)A(2'dF)AmA-3P.

[0556] Activity relative to SEQ ID NO: 4856 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 4856 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 73.2.

Table 73.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 4856)	FIOP Product Peak Area Relative to SEQ ID NO: 4856
4901/4902	E394V	+++
4903/4904	G354S	+++
4905/4906	R392K	+++
4907/4908	Q353S	+++
4909/4910	E394A	+++

4911/4912	Q302A/T307L	+++
4913/4914	Q353R	+++
4915/4916	A413G	++
4917/4918	L419G	++
4919/4920	M490E	++
4921/4922	L393V	++
4923/4924	Q302S/T307L	++
4925/4926	T317C	++
4927/4928	W395Y	++
4929/4930	R477Q	++
4931/4932	R392H	++
4933/4934	S495R	++
4935/4936	M490Q	++
4937/4938	L419V	+
4939/4940	S10N/A413S	+
4941/4942	Q397S	+
4943/4944	P436S/E512D	+
4945/4946	R477S	+
4947/4948	G402V	+
4949/4950	G460S	+
4951/4952	P364Q	+
4953/4954	S495Q	+
4955/4956	G460A	+
4957/4958	D412M	+
4959/4960	R392G	+
4961/4962	E486V	+
4963/4964	G460P	+
4965/4966	V268T	+
4967/4968	S362V	+
4969/4970	Q260R	+
4971/4972	D412G	+
4973/4974	G402T	+
4975/4976	P404Q	+
4977/4978	M490N	+
4979/4980	G518S	+
4981/4982	G402L	+
4983/4984	M490T	+
4985/4986	M490S	+
4987/4988	G402P	+
4989/4990	Q397G	+
4991/4992	G402K	+
4993/4994	M490V	+
4995/4996	P404L	+
4997/4998	L393R	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 4856 and defined as follows: "+" 1.02 to 1.22 , "++" 1.22 to 1.44 , "+++" 1.44 to 1.94		

EXAMPLE 74**Improvements over SEQ ID NO: 4904 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0557] SEQ ID NO: 4904 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 74.1.

[0558] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 74.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (C)-48; Preincubation Time (min)-60; Reaction temperature (C)-48; Reaction Time (min)-120; Reaction volume (μ L) -1; NTP (μ M)-fUTP-3P; NTP conc (μ M)-200; Oligonucleotide-5'-6-FAM-T11mAmAmA(2'dF)U, 5'-6-FAM-T18mA(2'dF)GmA(2'dF)G, 5'-6-FAM-T26mU(2'dF)GmU(2'dF)C, 5'-6-FAM-T47*mA(2'dF)A(2'dF)A; Substrate conc (μ M)-37.5 μ M each of four oligo; Product -5'-6-FAM-T11mAmAmA(2'dF)U(2'dF)U-3P, 5'-6-FAM-T18mA(2'dF)GmA(2'dF)G(2'dF)U-3P, 5'-6-FAM-T26mU(2'dF)GmU(2'dF)C(2'dF)U-3P, 5'-6-FAM-T47*mA(2'dF)A(2'dF)A(2'dF)U-3P.
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[0559] Activity relative to SEQ ID NO: 4904 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 4904 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 74.2.

Table 74.2

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 4904)	FIOP Product Peak Area Relative to SEQ ID NO: 4904
4999/5000	K297P/L298F/Y308N/R392K/W395Y	+++
5001/5002	L194F/P300T/Q302A/A413G	+++
5003/5004	P300E/T317C	+++
5005/5006	W395Y	++
5007/5008	P300E	+

5009/5010	L298F/R392K/F525A	+
5011/5012	L194F/P300E/Q302S/D481M	+
5013/5014	E190R	+
5015/5016	P300T/M490E	+
5017/5018	E190R/P300T/R477Q/M490E	+
5019/5020	E190R/L287V/P300T/Q302A	+
Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 4904 and defined as follows: "+" 1.01 to 1.15, "++" 1.15 to 1.32, "+++" 1.32 to 1.52		

EXAMPLE 75

Improvements over SEQ ID NO: 5002 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0560] SEQ ID NO: 5002 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 75.1.

[0561] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 75.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (C)-48; Preincubation Time (min)-60; Reaction temperature (C)-48; Reaction Time (min)-120; Reaction volume (μ L) -1; NTP (μ M)-mATP-3P; NTP conc (μ M)-300; Oligonucleotide-5'-6-FAM-T18mA(2'dF)GmA(2'dF)G, 5'-6-FAM-T26mU(2'dF)GmU(2'dF)C, 5'-6-FAM-T46mA(2'dF)GmU(2'dF)G, 5'-6-FAM-T51mU*(2'dF)A*(2'dF)A(2'dF)G; Substrate conc (μ M)-62.5 μ M each of four oligo; Product -5'-6-FAM-T18mA(2'dF)GmA(2'dF)GmA-3P, 5'-6-FAM-T26mU(2'dF)GmU(2'dF)CmA-3P, 5'-6-FAM-T46mA(2'dF)GmU(2'dF)GmA-3P, 5'-6-FAM-T51mU*(2'dF)A*(2'dF)A(2'dF)GmA-3P.
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[0562] Activity relative to SEQ ID NO: 5002 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 5002

(where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 75.2.

Table 75.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 5002)	F1OP Product Peak Area Relative to SEQ ID NO: 5002
5021/5022	L298F/R392K/E394V/M490E	+++
5023/5024	R392K/E394V	+++
5025/5026	L393V/E394V/R433H/R477V/M490E	+++
5027/5028	R392K/L393V/E394V/R433H	+++
5029/5030	R392K/E394V/R477V/S495Q	+++
5031/5032	L298F/A302S/R392K/L393V/E394A/R433H	+++
5033/5034	R392K/E394V/S495Q	+++
5035/5036	E394V/M490E	+++
5037/5038	L298F/L393V/E394V/W395Y/R433H/R477V	+++
5039/5040	E394V/R477V	+++
5041/5042	L298F/E394V/R433H	+++
5043/5044	R392H/E394A/R433H/R477L	+++
5045/5046	L298F/R392K/L393V/E394V	+++
5047/5048	R392H/L393V/E394V/M490E	++
5049/5050	R392K/L393V/E394V/R477V/S495Q	++
5051/5052	F194W	++
5053/5054	I324M	++
5055/5056	Y309R	++
5057/5058	E190R/F194L	++
5059/5060	I208V	++
5061/5062	S472T	++
5063/5064	K274V	++
5065/5066	E526L	++
5067/5068	F194L	++
5069/5070	R392H/L393V/E394A/R477L	+
5071/5072	E526Y	+
5073/5074	L298F/R392K/L393V/E394A/R477L	+
5075/5076	K274G	+
5077/5078	K274W	+
5079/5080	R392H/E394V/R433H/S495Q	+
5081/5082	I208S	+
5083/5084	F194L/D198Q	+
5085/5086	M466V	+
5087/5088	F194L/D198V	+
5089/5090	G411L/G413A	+
5091/5092	L359C	+
5093/5094	F194L/D198G	+
5095/5096	E523T	+
5097/5098	A313S	+
5099/5100	V273S	+

5101/5102	L298F/A302S/R392K/E394A	+
5103/5104	V273Q	+
5105/5106	V273M/Q347F/S354G	+
5107/5108	R281Q	+
5109/5110	I361L	+
5111/5112	I324T	+
5113/5114	L360V	+
5115/5116	R473S	+
5117/5118	L359V	+
5119/5120	A463V	+
5121/5122	L202T	+
5123/5124	A408G/G413A	+
5125/5126	R477L	+
5127/5128	R392K/E394A/W395Y	+
5129/5130	R281G	+
5131/5132	L298I/T300P/A302Q	+
5133/5134	G460S/F525A	+
5135/5136	L298F/L393V/E394V/R477L/S495Q	+
5137/5138	F194L/D198P	+
5139/5140	V290A	+
5141/5142	L393V/E394A	+
5143/5144	R314L	+
5145/5146	A408L/G413A	+
5147/5148	P352T	+
5149/5150	Y308N/G402V/G460A	+
5151/5152	E190T/F194L	+
5153/5154	S405T	+
5155/5156	E190L/F194L	+
5157/5158	A463P	+
5159/5160	F194Y	+
5161/5162	G413A	+
5163/5164	T300P/A302Q/K303V	+
5165/5166	D492R	+
5167/5168	G11D/E523R	+
5169/5170	L467A	+
5171/5172	I208G	+
5173/5174	A264S	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 5002 and defined as follows: ""+"" 1.02 to 1.37 , ""++"" 1.37 to 1.75 , ""+++"" 1.75 to 2.53 "		

EXAMPLE 76**Improvements over SEQ ID NO: 5028 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0563] SEQ ID NO: 5028 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination

of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 76.1.

[0564] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 76.1-Reaction conditions	
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-20 mM TEA, 0.25 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (C)-48; Preincubation Time (min)-60; Reaction temperature (C)-48; Reaction Time (min)-120; Reaction volume (μ L) -0.1; NTP (μ M)-fUTP-3P; NTP conc (μ M)-300; Oligonucleotide-5'-6-FAM-T11mCmCmU(2'dF)A, 5'-6-FAM-T31mU(2'dF)CmA(2'dF)U, 5'-6-FAM-T41mA(2'dF)U(2'dF)C(2'dF)C, 5'-6-FAM-T51mU*(2'dF)A*(2'dF)A(2'dF)G; Substrate conc (μ M)-62.5 μ M each of four oligo; Product -5'-6-FAM-T11mCmCmU(2'dF)A(2'dF)U-3P, 5'-6-FAM-T31mU(2'dF)CmA(2'dF)U(2'dF)U-3P, 5'-6-FAM-T41mA(2'dF)U(2'dF)C(2'dF)C(2'dF)U-3P, 5'-6-FAM-T51mU*(2'dF)A*(2'dF)A(2'dF)G(2'dF)U-3P.	

[0565] Activity relative to SEQ ID NO: 5028 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 5028 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 76.2.

Table 76.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 5028)	FIOP Product Peak Area Relative to SEQ ID NO: 5028
5175/5176	K274W	+++
5177/5178	F194L/I208V/G411L	+++
5179/5180	F194L/Y309R	+++
5181/5182	V273S/K274W	+++
5183/5184	F194L/D198V	++
5185/5186	F194W	++
5187/5188	K274V/L359V/E526Y	++
5189/5190	K274V	++
5191/5192	K274G/R281Q/E526Y	++
5193/5194	D198V/I208S	++
5195/5196	Y309R/A313S/G411L	++

5197/5198	V82I/F194L/D198G/A313S	++
5199/5200	M466V/E526Y	++
5201/5202	Y309R	++
5203/5204	F194L/D198V/G411L	++
5205/5206	G411L	++
5207/5208	F194L/D198Q	+
5209/5210	K274M/E523T	+
5211/5212	F194W/A313S	+
5213/5214	D198Q/I208S/A313S/G411L	+
5215/5216	F194L	+
5217/5218	F194W/G411L	+
5219/5220	I324M/E526Y	+
5221/5222	F194W/D198V/A313S	+
5223/5224	M466V	+
5225/5226	F194L/D198V/Y309R	+
5227/5228	E523T	+
5229/5230	E526Y	+
5231/5232	D198Q/I208V/Y309R/G411L	+
5233/5234	D198Q	+
5235/5236	F194L/D198Q/I208V/A313S	+
5237/5238	V273Q/K274G	+
5239/5240	F194W/D198Q	+
5241/5242	F194W/D198V	+
5243/5244	D198G	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 5028 and defined as follows: "+" 1.02 to 1.17, ""++"" 1.17 to 1.28, ""+++"" 1.28 to 1.56 "		

EXAMPLE 77

Improvements over SEQ ID NO: 5192 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0566] SEQ ID NO: 5192 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 77.1.

[0567] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time,

then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 77.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-100 mM TEA, 0.6 mM CoCl₂, pH 7.8; Lysate concentration (vol%)-0.25; Preincubation Temp (°C)-48; Preincubation Time (min)-60; Reaction temperature (°C)-48; Reaction Time (min)-60; Reaction volume (μL) -0.1; NTP (μM)-mUTP-3P; NTP conc (μM)-480; Oligonucleotide-5'-6-FAM-T16*(2'dF)C*mA(2'dF)A(2'dF)A, 5'-6-FAM-T21mU(2'dF)CmU(2'dF)C, 5'-6-FAM-T31mAmAmA(2'dF)G, 5'-6-FAM-T46mA(2'dF)GmU(2'dF)G; Substrate conc (μM)-100 μM each of four oligo; Product -5'-6-FAM-T16*(2'dF)C*mA(2'dF)A(2'dF)AmU-3P, 5'-6-FAM-T21mU(2'dF)CmU(2'dF)CmU-3P, 5'-6-FAM-T31mAmAmA(2'dF)GmU-3P, 5'-6-FAM-T46mA(2'dF)GmU(2'dF)GmU-3P.

[0568] Activity relative to SEQ ID NO: 5192 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products in the multiplexed assay divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 5192 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 77.2.

Table 77.2

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 5192)	FIOP Product Peak Area Relative to SEQ ID NO: 5192
5245/5246	V394Y	+++
5247/5248	V394I	+++
5249/5250	T200S	+++
5251/5252	R177L	+++
5253/5254	Q179L	+++
5255/5256	L326Q	+++
5257/5258	K430R	++
5259/5260	K508S	++
5261/5262	L403V	++
5263/5264	V394F	++
5265/5266	E520I/Y526E	++
5267/5268	T200A	++
5269/5270	T200R	++
5271/5272	T295H	++
5273/5274	D198L	++
5275/5276	T200K	++
5277/5278	V193R	++
5279/5280	K383H	++
5281/5282	T200V	++
5283/5284	L326T	++
5285/5286	K508A	++

5287/5288	V394Q	++
5289/5290	Q329K	++
5291/5292	L96S/T295N	+
5293/5294	V394L	+
5295/5296	T295K	+
5297/5298	D199R	+
5299/5300	K383R	+
5301/5302	V394H	+
5303/5304	T200Y	+
5305/5306	E485L	+
5307/5308	V193F	+
5309/5310	D409S	+
5311/5312	V193S	+
5313/5314	L403R	+
5315/5316	T295G	+
5317/5318	N176H	+
5319/5320	T300A/V394F	+
5321/5322	I187V	+
5323/5324	K195L	+
5325/5326	D199S	+
5327/5328	L326M/N380R	+
5329/5330	E203A	+
5331/5332	E203C	+
5333/5334	V304I	+
5335/5336	E203L	+
5337/5338	T200E	+
5339/5340	Q376R	+
5341/5342	R177T	+
5343/5344	V193C	+
5345/5346	T200Q	+
5347/5348	Q329L	+
5349/5350	V394S	+
5351/5352	K377R	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 5192 and defined as follows: ""+"" 1.02 to 1.14 , ""++"" 1.14 to 1.23 , ""+++"" 1.23 to 1.47 "		

EXAMPLE 78**Improvements over SEQ ID NO: 5192 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors**HTP Screening for Improved TdT Variants

[0569] SEQ ID NO: 5192 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 78.1.

[0570] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 78.1-Reaction conditions	
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-100 mM TEA, 0.6 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-65; Preincubation Time (min)-60; Reaction temperature (C)-50; Reaction Time (min)-60; Reaction volume (μ L) -1; NTP (μ M)-fATP-3P; NTP conc (μ M)-200; Oligonucleotide-5'-6-FAM-T15mAmU*mG; Substrate conc (μ M)-100; Product - 5'-6-FAM-T15mAmU*mG(2'dF)A-3P.	

[0571] Activity relative to SEQ ID NO: 5192 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 5192 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 78.2.

Table 78.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 5192)	FIOP Product Peak Area Relative to SEQ ID NO: 5192
5353/5354	L326R	+++
5291/5292	L96S/T295N	+++
5327/5328	L326M/N380R	+++
5355/5356	E203G	+++
5357/5358	S184H	+++
5359/5360	Q169E	++
5361/5362	N197F	++
5299/5300	K383R	++
5363/5364	Q179G	++
5347/5348	Q329L	+
5365/5366	K383S	+
5367/5368	S292L	+
5265/5266	E520I/Y526E	+
5369/5370	D199G	+
5371/5372	T295L	+
5261/5262	L403V	+
5373/5374	W325M	+

5293/5294	V394L	+
5345/5346	T200Q	+
5375/5376	Q373G	+
5377/5378	V394W	+
5287/5288	V394Q	+
5279/5280	K383H	+
5285/5286	K508A	+
5379/5380	D198S	+
5381/5382	S184G	+
5383/5384	R177W	+
5349/5350	V394S	+
5385/5386	S292M	+
5351/5352	K377R	+
5255/5256	L326Q	+
5387/5388	S292Q	+
5389/5390	S184P	+
5391/5392	S292R	+
5393/5394	N197M/T307A	+
5395/5396	K377N	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 5192 and defined as follows: "+" 1.08 to 2.16 , "++" 2.16 to 3.64 , "+++" 3.64 to 7.07 "		

EXAMPLE 79

Improvements over SEQ ID NO: 5246 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0572] SEQ ID NO: 5246 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 79.1.

[0573] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μ M oligonucleotide, 100-600 μ M nucleotide triphosphate, 20-100 mM buffer, and 250-600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 79.1-Reaction conditions
Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-100 mM TEA, 0.6 mM CoCl ₂ , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-58.5; Preincubation Time (min)-60;

Reaction temperature (C)-50; Reaction Time (min)-60; Reaction volume (μL) -1; NTP (μM)-fATP-3'P; NTP conc (μM)-480; Oligonucleotide-5'-6-FAM-T15mAmUmCmU, T15mAmUmCmU; Substrate conc (μM)-100 μM FAM-labelled,400 μM unlabelled; Product (detected)-5'-6-FAM-T15mAmUmCmU(2'dF)A-3'P.

[0574] Activity relative to SEQ ID NO: 5246 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 5246 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 79.2.

Table 79.2		
SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 5246)	FIOP Product Peak Area Relative to SEQ ID NO: 5246
5397/5398	E233R	+++
5399/5400	E485L	+++
5401/5402	R459M	++
5403/5404	L381Y	++
5405/5406	G365H	++
5407/5408	L381H	++
5409/5410	E367S	+
5411/5412	N197Q	+
5413/5414	G365R	+
5415/5416	E233L	+
5417/5418	R180K	+
5419/5420	M441E	+
5421/5422	D336C	+
5423/5424	R459W	+
5425/5426	A252R	+
5427/5428	P364A	+
5429/5430	K384A	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 5246 and defined as follows: "+" "+" 1.02 to 1.28 , "++" "+" 1.28 to 1.52 , "+++" "+" 1.52 to 1.99 "		

EXAMPLE 80

Improvements over SEQ ID NO: 5246 in the extension of oligonucleotide acceptor molecules with nucleotide triphosphate donors

HTP Screening for Improved TdT Variants

[0575] SEQ ID NO: 5246 was selected as the parent TdT enzyme. Libraries of engineered genes were produced from the parent gene using various techniques (*e.g.*, saturation mutagenesis and recombination of previously identified beneficial mutations). The polypeptides encoded by each gene were produced in HTP and prepared as described in Table 80.1.

[0576] Reactions were performed in 96-well or 384-well format BioRad PCR plates. Reactions included 10-500 μM oligonucleotide, 100-600 μM nucleotide triphosphate, 20-100 mM buffer, and 250-600 μM cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the reaction plates (ii) TdT lysate solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 80.1-Reaction conditions

Lysis buffer-TEA (50 mM, pH 7.5), 0.1 g/L lysozyme; Reaction buffer-100 mM TEA, 0.6 mM CoCl_2 , pH 7.8; Lysate concentration (vol%)-25; Preincubation Temp (C)-58.5; Preincubation Time (min)-60; Reaction temperature (C)-50; Reaction Time (min)-60; Reaction volume (μL) -1; NTP (μM)-fATP-3'P; NTP conc (μM)-200; Oligonucleotide-5'-6-FAM-T15mAmU*mG, FAM-T15mAmU*mG; Substrate conc (μM)-50 μM FAM-labelled, 50 μM unlabelled; Product (detected)-5'-6-FAM-T15mAmU*mG(2'dF)A-3'P.

[0577] Activity relative to SEQ ID NO: 5246 (Activity FIOP) was calculated as the percent product of the variant, defined as the sum of the area of products divided by the sum of the total peak area, compared with the percent product observed by the reaction with SEQ ID NO: 5246 (where the percent product may be set as the average of replicates or else the highest single sample as appropriate). The results are shown in Table 80.2.

Table 80.2

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 5246)	FIOP Product Peak Area Relative to SEQ ID NO: 5246
5431/5432	D198L/T200K/E203G/T295N	+++
5433/5434	R177W/T200K/T295N/L326M	+++
5435/5436	T200K/N380R	+++
5437/5438	S184H/T200K/T295N/L326M	++
5439/5440	T200K/L326M	++
5441/5442	D198L/T200K/E203G	++
5443/5444	R177W/T200K/E203G/T295N	++
5445/5446	D198L/T200K	++
5447/5448	E190M/T200K/E203G/T295N/N380R	++
5449/5450	S184H/D198L/T200K/E203G/T295N	++
5451/5452	T295N	+
5453/5454	E203G	+
5455/5456	E190M/T200K/T295N	+
5457/5458	R177W/D198L/T200K	+
5459/5460	D198L	+
5461/5462	R177W/T200K/E203G	+

5463/5464	E190M/D198L/T200K/E203G	+
5465/5466	E203G/N380R	+
"Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 5246 and defined as follows: "+" 1.22 to 1.55 , "++" 1.55 to 1.75 , "+++" 1.75 to 1.90 "		

EXAMPLE 81

Expression of inorganic pyrophosphatase-TdT fusion proteins

[0578] Synthetic genes encoding an *N*-terminal and *C*-terminal hexahistidine tagged version of two wild-type (WT) inorganic pyrophosphatases (SEQ ID NO: 3942 and 3944) were fused to a truncated TdT variant generating four IPP-TdT fusion constructs (SEQ ID NO: 5468, 5470, 5472, and 5474). The TdT was derived from SEQ ID NO: 5028 by deleting the first 156 amino acids. A GSGGTG linker was introduced between IPP and TdT. The fused proteins were constructed using well-established techniques (e.g Gibson assembly cloning). Fused proteins were subsequently expressed in an *E. coli* strain derived from W3110.

[0579] Cells transformed with the fusion protein expression constructs were grown at shake-flask scale using IPTG induction as described in Example 3. Cells were then lysed, purified, and dialyzed into storage buffer (20 mM Tris-HCl, pH 7.4, 100 mM KCl, 0.1 mM EDTA, and 50% glycerol). After overnight dialysis, protein samples were removed, and enzyme concentrations were measured by absorption at 280nm using a NanoDrop™ 1000 spectrophotometer. Soluble protein concentrations are summarized in Table 81.1 below, showing relative purified protein product levels following shake-flask purification relative to SEQ ID NO: 5468.

Table 81.1		
SEQ ID NO: (nt/aa)	Description of IPP-TdT fusion variant	Levels of Soluble Enzyme Production (Relative to SEQ ID NO: 5468)
5467/5468	<i>N</i> -terminal 6xhis tagged SEQ ID NO: 3942 fused with truncated SEQ ID NO: 5028	+
5469/5470	<i>N</i> -terminal 6xhis tagged SEQ ID NO: 3944 fused with truncated SEQ ID NO: 5028	++
5471/5472	<i>C</i> -terminal 6xhis tagged SEQ ID NO: 3942 fused with truncated SEQ ID NO: 5028	+
5473/5474	<i>C</i> -terminal 6xhis tagged SEQ ID NO: 3944 fused with truncated SEQ ID NO: 5028	++
Levels of increased soluble enzyme production were relative to the reference polypeptide of SEQ ID NO: 5468 and defined as follows: 1.31 to 1.41 , "++" 1.41 to 1.49 , "+++" 1.49 to 1.48 "		

EXAMPLE 82

Suppression of by-products by IPP-TdT fusion proteins

[0580] TdT-IPP fusion proteins (SEQ ID NO: 5468, 5470, 5472, and 5474) described in Example 81 were assayed for activity and suppression of by-products and compared to reactions containing TdT SEQ

ID NO: 5028 with no inorganic pyrophosphatase (control) and to reactions with TdT SEQ ID NO: 5028 with inorganic pyrophosphatases SEQ ID NO: 3942 or 3944.

[0581] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 400 μ M oligonucleotide, 480 μ M 3'phosphate blocked NTP, 10 μ M TdT or IPP-TdT fusion, 0 or 0.5 μ M IPP, 100 mM triethanolamine (pH 7.8), and 600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT or IPP-TdT fusion and IPP, were pre-mixed in a single solution, and were aliquoted into each well of the 96-well plates (ii) IPP was then added if present, (iii) TdT or IPP-TdT fusion was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 $^{\circ}$ C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 82.1

Reaction buffer-100 mM TEA, 0.6 mM CoCl₂, pH 7.8; Reaction temperature (C)- 50; Reaction Time (min)- 60; Reaction volume (μ L)- 1; IPP concentration (μ M)- 0 or 0.05; TdT or IPP-TdT fusion concentration (μ M)- 10; NTP (μ M)-3'P-mATP; NTP conc (μ M)- 480; Oligonucleotide- 5'-6-FAM-T31mAmAmA(2'dF)G, or 5'-6-FAM-T16mC*mA*mGmA; Oligonucleotide conc (μ M)- 400; Product- 5'-6-FAM-T31mAmAmA(2'dF)GmA-3'P, or 5'-6-FAM-T16mC*mA*mGmA-3'P; By-Product- unknown.

[0582] The ratio of product to by-product relative to a control with TdT SEQ ID NO: 5028 without IPP was calculated as the area of percent area product/percent area by-product ratio of the reaction compared with the percent area product/percent area by-product ratio observed by the control reaction with no IPP present. The results are shown in Table 82.2.

Table 82.2 Suppression of TdT reaction by-products by IPP

SEQ ID NO: (nt/aa)		Percent area product/percent area by-product compared relative to no IPP control	
TdT or IPP-TdT fusion	IPP	Reaction with 5'-6-FAM-T31mAmAmA(2'dF)G	Reaction with 5'-6-FAM-T16mC*mA*mGmA
5027/5028	none	+	+
5027/5028	3941/3942	+	+
5027/5028	3943/3944	++	+
5467/5468	none	++	++
5469/5470	none	++	++
5471/5472	none	++	+
5473/5474	none	++	+++
Levels of the percent area product/percent area by-product were determined relative to the reference polypeptide of SEQ ID NO: 5028 (control reaction without IPP) and defined as follows: "+" 2.44 to 6.12, "++" 6.12 to 7.99, "+++" 7.99 to 7.51"			

EXAMPLE 83**Activity Improvements Over Multiple Rounds of Evolution**

Activity improvement of shake-flask purified TdT variants with 3'phosphate-blocked nucleotides and modified RNA oligonucleotide substrates.

[0583] TdT variants of SEQ ID NO: 36, 660, 1596, 2956, 3674, 3918, 4850 and 5246 were produced in shake flask and purified as described in Example 3.

[0584] Reactions were performed in 96-well format 200 μ L BioRad PCR plates. Reactions included 400 μ M oligonucleotide, 800 μ M nucleotide triphosphate, 10 μ M TdT, 5 μ M IPP, 100 mM triethanolamine (pH 7.8), and 600 μ M cobalt (II) chloride. The reactions were set up as follows: (i) all reaction components, except for TdT, were pre-mixed in a single solution, and was aliquoted into each well of the 96-well plates (ii) TdT solution was then added into the wells to initiate the reaction. The reaction plate was heat-sealed with a peelable aluminum seal and incubated in a thermocycler at the indicated temperature and reaction time, then held at 4 °C until the reaction was quenched. Reactions were quenched and processed for CE analysis as described in Example 4.

Table 83.1

Reaction buffer-100 mM TEA, 0.6 mM CoCl₂, pH 7.8; IPP SEQ ID NO: 3944 (μM)- 5; Reaction temperature (C)- 50; Reaction Time (min)- 60 min; Reaction volume (μL)- 1; TdT concentration (μM)- 10; NTP- as listed in Table 83.2; NTP conc (μM)-800; Oligonucleotide- as listed in table 83.2; Oligonucleotide conc (μM)-400; Products- 5'-6-FAM-T11AmC*mA*mGmA-3'P; 5'-6-FAM-T16mC*mA*mGmAmA-3'P; 5'-6-FAM-T21*mA*mGmAmAmA-3'P; 5'-6-FAM-T26*mGmAmAmAmA-3'P; 5'-6-FAM-T31mAmAmA(2'dF)GmA-3'P; 5'-6-FAM-T36mAmA(2'dF)GmAmA-3'P; 5'-6-FAM-T41(2'dF)GmA(2'dF)GmUmA-3'P; 5'-6-FAM-T46mA(2'dF)GmU(2'dF)GmA-3'P; 5'-6-FAM-T51(2'dF)GmU(2'dF)GmU mU-3'P; 5'-6-FAM-T56(2'dF)GmU(2'dF)CmUmU-3'P; 5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmU-3'P; 5'-6-FAM-T16mA(2'dF)GmA(2'dF)GmU-3'P; 5'-6-FAM-T21mU(2'dF)CmU(2'dF)CmU-3'P; 5'-6-FAM-T26mU(2'dF)GmU(2'dF)CmU-3'P; 5'-6-FAM-T31mU(2'dF)CmAmUmU-3'P; 5'-6-FAM-T41mAmUmCmU(2'dF)A-3'P; 5'-6-FAM-T46mUmCmUmU(2'dF)A-3'P; 5'-6-FAM-T51mCmUmUmA(2'dF)A-3'P; 5'-6-FAM-T10mAmAmA(MOE)A(2'dF)A-3'P; 5'-6-FAM-T12mAmG(MOE)C(2'dF)A-3'P; 5'-6-FAM-T26mCmCmU*mUmC-3'P; 5'-6-FAM-T26mCmCmU*mU*mG-3'P; 5'-6-FAM-T26mCmCmU*mU*mU-3'P; 5'-6-FAM-T26mCmCmU*mU(2'dF)G-3'P; 5'-6-FAM-T26mCmCmU*mU(2'dF)U-3'P; 5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmAmC-3'P; 5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA*mG-3'P; 5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA*mU-3'P; 5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA(2'dF)G-3'P; 5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA(2'dF)U-3'P.
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Percent conversion was calculated as the percent product of the variant, defined as the sum of the area of products divided by the sum of the total peak area. The results are shown in Table 83.2.

Table 83.2									
Reaction substrates		Percent conversion to product by SEQ ID NO: (aa) (see Table 83.1 for list of products detected)							
Oligonucleotide Description	Nucleo- tide	36	660	1596	2956	3674	3918	4850	5246
5'-6-FAM-T11AmC*mA*mG	mATP-3'P	~	~	~	++	+++	+++	+++	+++
5'-6-FAM-T16mC*mA*mGmA	mATP-3'P	~	~	~	~	+	++	++	+++
5'-6-FAM-T21*mA*mGmA	mATP-3'P	~	~	~	~	~	++	++	+++
5'-6-FAM-T26*mGmA	mATP-3'P	~	~	~	~	~	++	+++	+++
5'-6-FAM-T31mAmA(2'dF)G	mATP-3'P	~	~	~	+	+	+	+	+++
5'-6-FAM-T36mAmA(2'dF)GmA	mATP-3'P	~	~	~	~	+	+++	+++	+++
5'-6-FAM-T41(2'dF)GmA(2'dF)GmU	mATP-3'P	~	~	~	~	++	+++	+++	+++
5'-6-FAM-T46mA(2'dF)GmU(2'dF)G	mATP-3'P	~	~	~	~	~	~	~	+++
5'-6-FAM-T51(2'dF)GmU(2'dF)GmU	mUTP-3'P	~	~	~	~	++	+++	+++	+++
5'-6-FAM-T56(2'dF)GmU(2'dF)CmU	mUTP-3'P	~	~	~	~	+	++	+++	+++
5'-6-FAM-T10(2'dF)CmU(2'dF)CmA	mUTP-3'P	~	~	~	+	++	++	+++	+++
5'-6-FAM-T21mU(2'dF)CmU(2'dF)C	mUTP-3'P	~	~	~	~	~	+	+	+++
5'-6-FAM-T26mU(2'dF)GmU(2'dF)C	mUTP-3'P	~	~	~	~	~	~	+	+++
5'-6-FAM-T31mU(2'dF)CmA	mUTP-3'P	~	~	~	~	+++	+++	+++	+++
5'-6-FAM-T36(2'dF)CmA	mUTP-3'P	~	~	~	~	+	+++	+++	+++
5'-6-FAM-T41mAmUmCmU	fATP-3'P	~	~	~	~	+	+++	+++	+++
5'-6-FAM-T46mUmCmUmU	fATP-3'P	~	~	~	~	+	+++	+++	+++
5'-6-FAM-T51mCmUmUmA	fATP-3'P	~	~	~	+	+	+++	+++	+++

5'-6-FAM-T10mAmAmA(MOE)A	fATP-3'P	~	~	~	+	+	++	+++	+++
5'-6-FAM-T12mAmG(MOE)C	fATP-3'P	~	~	~	~	~	++	+++	+++
5'-6-FAM-T26mCmCmU*mU	mCTP-3'P	~	~	~	+	+	+++	+++	+++
5'-6-FAM-T26mCmCmU*mU	*mGTP-3'P	~	~	~	~	~	++	+++	+++
5'-6-FAM-T26mCmCmU*mU	*mUTP-3'P	~	~	~	~	~	+++	+++	+++
5'-6-FAM-T26mCmCmU*mU	mGTP-3'P	~	~	~	~	++	+++	+++	+++
5'-6-FAM-T26mCmCmU*mU	mUTP-3'P	~	~	~	~	++	+++	+++	+++
5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA	mCTP-3'P	~	~	~	+++	+++	++	+++	+++
5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA	*mGTP-3'P	~	~	~	~	+	+	++	++
5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA	*mUTP-3'P	~	~	~	+	+	+	++	+++
5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA	mGTP-3'P	~	~	~	+++	+++	++	+++	+++
5'-6-FAM-T21*(2'dF)A*(2'dF)A(2'dF)GmA	mUTP-3'P	~	~	~	+++	+++	++	+++	+++

Levels of activity were determined by the % conversion to product and defined as follows: "~" from 0 - 1, "+" from 1-10, "++" from 10-25, "+++ from 25-64.

EXAMPLE 84

High performance liquid chromatography (HPLC) analysis of nucleotides

[0585] A 5 to 20 μL volume of crude reaction product is diluted to a final total nucleotide concentration of 250 μM using 75 % MeOH in milli-Q water as diluent. The reaction container is then vortexed and centrifuged at 4000 rpm for 5 min at 4 °C. 100 μL is transferred from the supernatant to a 96 well round bottom plate and then 10 μL is injected onto an Ultimate 3000 HPLC system using a PAL autosampler according to the method outlined in table 84.1.

Table 84.1. Achiral HPLC Parameters (Ion Pairing Gradient)	
Instrument	Ultimate 3000 HPLC System with a PAL autosampler
Column	Agilent Zorbax RR StableBond Aq, 150 x 3.0 mm x 3.5 μm
Guard Column	Agilent Zorbax StableBond Aq, 5.0 x 3.0 mm, 1.8 μm
Mobile Phases	A: 50 mM sodium phosphate, 2 mM tetrabutylammonium bisulfate, pH 7.0

Table 84.1. Achiral HPLC Parameters (Ion Pairing Gradient)	
	B: Acetonitrile C: Water
LC Gradient	- 0 – 4.0 min: 9.0 % B, 48.5 – 16.2 % C - 4.0 – 4.5 min: 9.0 % B, 16.2 – 48.5 % C - 4.5 – 5.5 min: 9.0 % B, 48.5 % C
Flow rate	1.2 mL/min
Run time	5.5 min
Column temperature	30 °C
Injection volume	10 µL
UV Detector	254 nm

EXAMPLE 85

General Enzyme Immobilization Procedure on Affinity Resin

[0586] The desired His-tagged protein variant was first produced in shake flask and purified as described in Example 3. The storage buffer from an aliquot of the desired protein stock was then exchanged for TEA·HCl (20 mM, pH 7.8) by diluting 10-fold with TEA·HCl followed by concentration through a Sartorius VivaSpin 6 (10,000 MWCO) spin filter at 4000 rpm and 4 °C. The reduced volume sample is then diluted another 2.5 fold with TEA·HCl (20 mM, pH 7.8) to restore the original volume.

[0587] Between 2 and 20 mg affinity resin was weighed out, either wet or dry, into a 2.0 mL Eppendorf tube. For larger preparations, 0.5 to 1.0 g affinity resin was weighed out into a 15 mL conical tube. The volume of protein stock containing the desired wt. % of protein vs resin mass was transferred to the tube containing resin. Using TEA·HCl (20 mM, pH 7.8) as diluent, the volume was diluted to 150 µL in each 2.0 mL Eppendorf tube, or 6.0 mL in each 15 mL conical tube.

[0588] The samples were then incubated at 4 °C for 24 to 48 h using either 500 rpm agitation or on a tube rotator operating at 10 rpm. Before subjecting to activity assays, the supernatant was removed, and the resin was washed three times. For smaller preparations in 2.0 mL Eppendorf tubes, 150 µL wash volumes of TEA·HCl (20 mM, pH 7.8) were used, allowing the mixture to agitate at room temperature and 500 rpm for 10 minutes during each wash. Between each wash the tubes were briefly centrifuged to collect resin at the bottom. In the case of a larger preparation in a 15 mL conical tube, the resin was collected by vacuum filtration and then washed three times with 100 mL of TEA·HCl (25 mM, pH 7.5), allowing the resin to mix well on a rotator for 10 minutes at room temperature during each wash.

EXAMPLE 86

Immobilization of Purified TdT on Affinity Resin

[0589] The TdT enzyme variant of SEQ ID NO: 3918 was produced in shake flask and purified as described in Example 3. The storage buffer was then exchanged for TEA·HCl (20 mM, pH 7.8) as described in Example 85, resulting in a final protein concentration of 7.2 g/L as measured by UV-Vis absorbance at 280 nm (A280) on a nanodrop spectrophotometer.

[0590] Affinity resins were purchased from ChiralVision, Purolite, or EnginZyme pre-functionalized with a chelating agent and pre-loaded with a metal for binding his-tagged proteins by the supplier. The metals used can include but are not limited to Ni(II), Co(II), Cu(II), Fe(II), Fe(III), Zn(II) with chelators that include but are not limited to iminodiacetate (IDA), nitrilotriacetate (NTA), and ethylenediaminetetracetate (EDTA).

[0591] Resins were weighed out in 4.0 to 6.5 mg quantities in 2.0 mL Eppendorf tubes and charged with enzyme from the 7.2 g/L TdT stock solution, and immobilization was carried out as described in Example 85 with a 24 h incubation. The tubes containing ChiralVision resins were charged with the appropriate volume of TdT stock such that the mass of protein in the sample was 3.0 % of the wet mass of resin. The same procedure was repeated for the Purolite Chromalite resins, but with a target of 2.5 wt. % vs the wet mass of resin. The same procedure was repeated for the EnginZyme resins but with a target of 2.5 wt. % vs dry mass of resin.

[0592] The samples were briefly centrifuged to collect the resin in the bottom of the Eppendorf tube and 60 μ L was transferred from the supernatant to a Greiner 96 well clear bottom half-well plate. The A280 was measured on a plate-reader and compared to a negative control which contained TdT stock that had been incubated under the same conditions with no resin present. The difference in A280 was then used to calculate the percent of immobilization of the protein in solution:

$$\text{Percent Immobilization} = \left(1 - \frac{A_{280}}{A_{280_{neg. ctrl.}}} \right) \times 100$$

Table 86.1 Immobilization of Purified TdT on Affinity Resin

Resin	Metal	Percent Immobilization of Added Protein
ChiralVision IB-HIS-1	Ni(II)	++
ChiralVision IB-HIS-2	Ni(II)	++
ChiralVision IB-HIS-2	Co(II)	++
ChiralVision IB-HIS-3	Ni(II)	+
ChiralVision IB-HIS-4	Ni(II)	+
ChiralVision IB-HIS-5	Ni(II)	+++
ChiralVision IB-HIS-6	Ni(II)	+
ChiralVision IB-HIS-7	Ni(II)	+
ChiralVision IB-HIS-8	Ni(II)	++
ChiralVision IB-HIS-9	Ni(II)	++
ChiralVision IB-HIS-10	Ni(II)	++
ChiralVision IB-HIS-11	Ni(II)	++
ChiralVision IB-HIS-12	Ni(II)	++
ChiralVision IB-HIS-13	Ni(II)	+++
ChiralVision IB-HIS-14	Ni(II)	+
ChiralVision IB-HIS-15	Ni(II)	+
ChiralVision IB-HIS-16	Ni(II)	+
Purolite Chromalite	Co(II)	++
Purolite Chromalite	Cu(II)	++
Purolite Chromalite	Fe(II)	++
Purolite Chromalite	Ni(II)	++
Purolite Chromalite	Zn(II)	++
EnginZyme EziG ¹ Opal	Fe(III)	+++
EnginZyme EziG ² Choral	Fe(III)	+++

EnginZyme EziG ³ Amber	Fe(III)	+++
Levels of immobilization are determined by ratio of A280 vs negative control and defined as follows: "+" 0 to 50%, "++" 50 to 80%, "+++" 80 to 100%		

EXAMPLE 87**Co-Immobilization of IPP and TdT on Affinity Resin and Enzymatic Single Extension of RNA Oligomer**

[0593] The TdT enzyme variant of SEQ ID NO: 5028 was produced in shake flask and purified as described in Example 3.

[0594] The inorganic pyrophosphatase (IPP) of SEQ ID NO: 3944 was produced and purified, as described in Example 58.

[0595] For both the TdT and IPP, the storage buffer in 0.5 mL of stock was exchanged for TEA HCl (20 mM, pH 7.8) as described in Example 85, yielding solutions of IPP and TdT in 2.0 and 9.6 g/L concentrations, respectively.

[0596] ChiralVision IB-HIS-2 resin, loaded with CoCl₂, was weighed out in 4.5 to 9.0 mg quantities into 2.0 mL Eppendorf tubes and enzyme immobilization was carried out according to Example 85 using mixtures of the IPP and TdT stock solutions with a 24 h incubation. Each resin sample contained 1.0, 3.0, or 6.0 wt. % TdT vs wet weight of resin in combination with 0, 0.2, 0.8, or 1.6 wt. % IPP vs wet weight of resin.

[0597] The samples were removed from the rotator and briefly centrifuged to collect the resin the bottom of each tube. After removing the supernatant, each resin sample was washed three times with TEA HCl (20 mM, pH 7.8) according to Example 85.

[0598] To test the activity of the immobilized enzymes, a 1.6 mL reaction stock solution was prepared containing: mATP-3'P (30 μM), 5'-6-FAM-T15mAmUfG (0.2 μM), T15mAmUfG (18.8 μM), CoCl₂ (0.25 mM), TEA·HCl (20 mM, pH 7.8). The reaction stock was then added to each resin sample (20 μL stock per mg wet weight of resin). The reaction was then incubated at 50 °C, 500 rpm. After 1h, a 2 μL aliquot was removed from the supernatant of each reaction mixture and transferred into 98 μL 1 mM EDTA quench solution. Another 2 μL aliquot was removed from the quench solution and processed for CE analysis as described in Example 4. The conversion of 5'-6-FAM-T15mAmUfG to 5'-6-FAM-T15mAmUfGmA-3'P was measured as the % fluorescence of product vs total fluorescence in the CE electropherogram.

Table 87.1 Single RNA Extension Using Co-immobilized IPP SEQ ID NO: 3944 and TdT SEQ ID NO: 5002		
Wt. % TdT	Wt. % IPP	% 5'-6-FAM-T15mAmUfGmA-3'P
1	0	+
1	0.2	+
1	0.8	++
1	1.6	++
3	0	++
3	0.2	+++

Table 87.1 Single RNA Extension Using Co-immobilized IPP SEQ ID NO: 3944 and TdT SEQ ID NO: 5002		
Wt. % TdT	Wt. % IPP	% 5'-6-FAM-T15mAmUfGmA-3'P
3	0.8	+++
3	1.6	+++
6	0	+++
6	0.2	+++
6	0.8	+++
6	1.6	+++
Conversion of oligonucleotide 5'-6-FAM-T15mAmUf to 5'-6-FAM-T15mAmUfGmA-3'P was determined as percent of total fluorescence in the CE trace and defined as follows: "+" 0 to 50%, "++" 50 to 80%, "+++" 80 to 100%		

EXAMPLE 88

Immobilization of Alkaline Phosphatase on Affinity Resin and Enzymatic 3'-Dephosphorylation of RNA Oligomer

[0599] The alkaline phosphatase (AP) of SEQ ID NO: 3932 was produced in shake flask and purified as described in Example 3. The storage buffer in a 0.5 mL aliquot was exchanged for TEA HCl (20 mM, pH 7.8) as described in Example 85, giving a solution containing 7.1 g/L protein as measured by A280 on a nanodrop spectrophotometer.

[0600] Affinity resins from ChiralVision were weighed out in 2.5 to 3.0 mg quantities in Eppendorf tubes and charged with the appropriate amount of AP stock solution such that the amount of protein was 2.5 wt.% of the wet mass of resin in each tube. The immobilization was then carried out with a 24 h incubation according to Example 85.

[0601] The samples were then diluted to 150 μ L using TEA HCl (20 mM, pH 7.8) as diluent and incubated at 5 °C for 24 h with 500 rpm agitation. The A280 of the resulting supernatant solution was compared to that of a solution of AP that had been incubated under the same conditions but without resin present. The percent immobilization was then calculated as: $\left(1 - \frac{A280}{A280_{neg. ctrl.}}\right) \times 100$

Table 88.1 Immobilization of Alkaline Phosphatase SEQ ID NO: 3932 on Affinity Resin		
Resin	Metal	Percent Immobilization of Added Protein
ChiralVision IB-HIS-2	Ni(II)	++
ChiralVision IB-HIS-7	Ni(II)	+
ChiralVision IB-HIS-8	Ni(II)	+
ChiralVision IB-HIS-12	Ni(II)	++
Levels of immobilization are determined by ratio of A280 vs negative control and defined as follows: "+" 0 to 50%, "++" > 50%		

[0602] To measure the 3'-dephosphorylation activity of the immobilized AP, a 1.8 mL reaction stock was prepared containing: 5'-6-FAM-T15mAmG*mGmA-3'P (0.125 μ M), T15mAmG*mGmA-3'P (99.9 μ M), CoCl₂ (0.25 mM), and TEA HCl (20 mM, pH 7.8). 150 μ L of reaction buffer was then transferred to each

sample of resin and then briefly centrifuged to collect the resin in the bottom of the container. The reactions were then incubated at 50 °C with 500 rpm agitation.

[0603] After 0.5 h, a 2 μ L aliquot was removed from the supernatant of each reaction mixture and transferred into 98 μ L 1 mM EDTA quench solution. A subsequent 2 μ L aliquot was removed from the quench solution and processed for CE analysis as described in Example 4. The conversion of 5'-6-FAM-T15mAmG*mGmA-3'P to 5'-6-FAM-T15mAmG*mGmA was measured as the % fluorescence of product vs total fluorescence in the CE electropherogram.

Table 88.2 Dephosphorylation of 5'-6-FAM-T15mAmG*mGmA-3'P with Immobilized AP		
Resin	Oligo/AP Molar Ratio	% Conversion of Substrate
ChiralVision IB-HIS-2	14	+
ChiralVision IB-HIS-7	28	+
ChiralVision IB-HIS-8	18	+
ChiralVision IB-HIS-12	21	+
Levels of conversion of 5'-6-FAM-T15mAmG*mGmA-3'P to 5'-6-FAM-T15mAmG*mGmA determined as percent of total fluorescence in the CE trace and defined as follows: "+" > 90%		

EXAMPLE 89

Immobilization of Alkaline Phosphatase on Affinity Resin and Enzymatic Dephosphorylation of ATP

[0604] The alkaline phosphatase (AP) of SEQ ID NO: 3932 was produced in shake flask and purified as described in Example 3. The storage buffer in a 0.5 mL aliquot was exchanged for TEA HCl (20 mM, pH 7.8) as described in Example 85, giving a solution containing 12.2 g/L protein as measured by A280 on a nanodrop spectrophotometer.

[0605] Affinity resins from ChiralVision were weighed out in 4.0 to 5.5 mg quantities in Eppendorf tubes and charged with the appropriate amount of AP stock solution such that the amount of protein was 5.0 wt.% of the wet mass of resin in each tube. The immobilization was then carried out with a 24 h incubation according to Example 85.

[0606] Each resin sample was washed according to the general procedure outline in Example 85. To measure the dephosphorylation activity of the immobilized AP, a reaction stock was prepared containing: ATP (1 mM), CoCl₂ (0.25 mM), and TEA HCl (20 mM, pH 7.8). The reaction stock was transferred to each resin sample (200 μ L stock per mg resin). The reaction mixtures briefly centrifuged to collect the resin in the bottom of the container and then incubated at 50 °C with 500 rpm agitation.

[0607] After 1 h, a 5 μ L aliquot was removed from each reaction supernatant and transferred to 115 μ L 75% MeOH/milli-Q water quench solution in a 96 well round bottom plate. The plate was sealed, vortexed, and centrifuged at 4000 rpm, 4 °C, for 5 min. 100 μ L was then transferred out of each well into fresh wells on a separate 96 well round bottom plate and analyzed by HPLC according to Example 84. The percent conversion of ATP was measured as the area under curve (AUC) attributed to ATP vs the total AUC for the entire chromatogram.

Table 89.1 Reaction of Immobilized Alkaline Phosphatase SEQ ID NO: 3932 with ATP

Resin	ATP/AP Molar Ratio	Percent Conversion of ATP
ChiralVision IB-HIS-1	54	+
ChiralVision IB-HIS-2	60	++
ChiralVision IB-HIS-7	73	++
ChiralVision IB-HIS-8	60	++
ChiralVision IB-HIS-12	55	++
ChiralVision IB-HIS-16	959	++

Levels of conversion ATP determined as percent of total AUC in chromatogram and defined as "+" 0 to 80%, "++" > 80%.

EXAMPLE 90**Immobilization of Alkaline Phosphatase on Epoxide Functionalized Resin**

[0608] The alkaline phosphatase (AP) of SEQ ID NO: 3932 was produced in shake flask and purified as described in Example 3. An aliquot of the purified AP in storage buffer was diluted into MOPS buffer (20 mM, pH 7.0) to give two stocks, with concentrations of 1.0 and 0.1 g/L.

[0609] Epoxide functionalized resins purchased from ChiralVision, IB-COV-2 and IB-COV-7, were weighed out in 19 to 26 mg quantities in 2.0 mL Eppendorf tubes and briefly centrifuged to collect the resin in the bottom. The 1.0 and 0.1 g/L stocks of AP in MOPS (20 mM, pH 7.0) were then added to the resin samples such that two samples received 2.5 wt. % protein vs wet weight of resin, and the other two received 0.25 wt. % protein vs wet weight of resin. These samples were incubated 48 h at 4°C, with no agitation for the 0 to 24 h and then 10 rpm rotation for 24 to 48 h.

[0610] To measure the percent of AP that was immobilized, the supernatant was tested for residual enzymatic activity and compared to a sample of AP that had been subjected to the same immobilization conditions but with no resin present. First, 20 µL aliquots were transferred from the supernatant of each sample and diluted to 200 µL with MOPS (20 mM, pH 7.0) buffer. Three controls were prepared by serially diluting a 0.1 g/L AP stock into 200 µL MOPS (20 mM, pH 7.0) at 5, 20, and 40-fold dilutions.

[0611] In a separate 15 mL conical tube, 5.0 mL reaction buffer was prepared containing: *p*-nitrophenylphosphate (4 mM), CoCl₂ (0.5 mM), and TEA HCl (40 mM, pH 7.8). Then, in a 96 well clear flat-bottom plate, 100 µL from the 10x dilution of each sample supernatant and each control was combined with 100 µL from the *p*-nitrophenylphosphate reaction buffer. The reaction was monitored at 405 nm in a plate reader at 25 °C for 10 min, acquiring data every 10 seconds. The ratio of the reaction rates to the negative control rate gave the percent of AP immobilized: $\left(1 - \frac{Rate}{Rate_{neg. ctrl.}}\right) \times 100$

Table 90.1 Covalent Immobilization of SEQ ID NO: 3932

Resin	Wt. % Added Protein vs Resin Wet Weight	Percent Immobilization of Protein
ChiralVision IB-COV-2	0.25%	++
ChiralVision IB-COV-2	2.50%	+++
ChiralVision IB-COV-7	0.25%	++

Table 90.1 Covalent Immobilization of SEQ ID NO: 3932		
Resin	Wt. % Added Protein vs Resin Wet Weight	Percent Immobilization of Protein
ChiralVision IB-COV-7	2.50%	+
Levels of immobilization on resin determined by relative activity in supernatant vs free enzyme when reacted with <i>p</i> -nitrophenyl phosphate, defined as: “+” 0 to 60%, “++” 60 to 90%, “+++” 90 to 100%.		

EXAMPLE 91

Immobilization of TdT-IPP Fusion Construct on Affinity Resin

[0612] The TdT-IPP fusion constructs of SEQ ID NO: 5470, SEQ ID NO: 5468, SEQ ID NO: 5472, and SEQ ID NO: 5474 were produced in shake flask and purified as described in Example 3. The enzyme stocks in storage buffer were used directly without exchanging the buffer.

[0613] Immobilization in 2.0 mL Eppendorf tubes was carried out according to Example 85 with 7.5 to 10 mg ChiralVision IB-HIS-2 resin (pre-loaded with CoCl₂) and 2.5 wt. % protein vs wet weight resin. A 48-hour incubation period at 4 °C was used.

[0614] After immobilization, the A280 of the resulting supernatant solution was compared to that of a solution of each enzyme that had been incubated under the same conditions but without resin present. The percent immobilization was then calculated as: $\left(1 - \frac{A_{280}}{A_{280_{neg. ctrl.}}}\right) \times 100$

Table 91.1 Immobilization of Purified TdT-IPP Fusion Construct on Affinity Resin			
Resin	Metal	Enzyme	Percent Immobilization of Added Protein
ChiralVision IB-HIS-2	Co(II)	SEQ ID NO: 5470	++
ChiralVision IB-HIS-2	Co(II)	SEQ ID NO: 5468	+
ChiralVision IB-HIS-2	Co(II)	SEQ ID NO: 5472	++
ChiralVision IB-HIS-2	Co(II)	SEQ ID NO: 5474	+
Levels of immobilization are determined by ratio of A280 vs negative control and defined as follows: “+” 0 to 50%, “++” > 50%			

EXAMPLE 92

Iterative Enzymatic Extension of RNA Oligomer

[0615] The TdT enzyme variant of SEQ ID NO: 5246 was produced in shake flask and purified as described in Example 3.

[0616] The inorganic pyrophosphatase (IPP) of SEQ ID NO: 3944 was produced and purified, as described in Example 58.

[0617] The alkaline phosphatase (AP) of SEQ ID NO: 3932 was produced and purified, as described in Example 57.

[0618] TdT and IPP were co-immobilized on ChiralVision IB-HIS-2 resin (0.5 g), pre-loaded with CoCl₂, at 2.5 and 0.2 wt. % vs wet weight of resin, respectively, according to Example 85.

[0619] AP was immobilized on a separate batch of Co(II)-loaded ChiralVision IB-HIS-2 resin (0.5 g) at 2.5 wt. % protein vs wet weight of resin, according to Example 85.

[0620] First Extension: 5'-6-FAM-T10(2'dF)CmU(2'dF)CmA + mATP-3'P to 5'-6-FAM-T10(2'dF)CmU(2'dF)CmA-3'P

[0621] To a 2.0 mL Eppendorf tube was added 23 mg IB-HIS-2 resin, charged with 2.5 wt. % TdT (SEQ ID NO: 5246) and 0.2 wt. % IPP (SEQ ID NO: 3944). The tube was briefly centrifuged to collect the resin in the bottom. In a separate Eppendorf tube, 450 μ L reaction master mix was prepared containing: mATP-3'P (150 μ M), 5'-6-FAM-T10(2'dF)CmU(2'dF)CmA (100 μ M), CoCl₂ (0.25 mM), MOPS (20 mM, pH 8.0). The reaction was then incubated at 50 °C for 1.5 h at 500 rpm.

[0622] After 1.5 h, the supernatant was transferred into a separate Eppendorf tube containing a fresh 23 mg of IB-HIS-2 resin, charged with 2.5 wt. % TdT and 0.2 wt. % IPP. The reaction was incubated for a further 1.5 h at 50 °C with 500 rpm agitation.

[0623] Afterward, a 2 μ L aliquot was removed for CE analysis according to Example 4, while the reaction sample was allowed to cool in a 4 °C fridge for 10 minutes. Then, the supernatant was separated from the resin and placed in a 1.5 mL conical Eppendorf tube and centrifuged for 3 min at 14,000 rpm to pellet residual resin particulate. The supernatant was then transferred into a new 2.0 mL Eppendorf tube and the general AP catalyzed dephosphorylation procedure outlined below was carried out.

[0624] Second Extension: 5'-6-FAM-T10(2'dF)CmU(2'dF)CmA + fGTP-3'P to 5'-6-FAM-T10(2'dF)CmU(2'dF)CmA(2'dF)G-3'P

[0625] To a 2.0 mL Eppendorf tube was added 15 mg IB-HIS-2 resin charged with 2.5 wt. % TdT (SEQ ID NO: 5246) and 0.2 wt. % IPP (SEQ ID NO: 3944). In a separate 2.0 mL Eppendorf tube, 100 μ L from the de-phosphorylate product of the first extension was diluted with 1.5 μ L fGTP-3'P (20 mM), 5 μ L CoCl₂ (10 mM), 20 μ L TEA·HCl (200 mM, pH 7.8), and 174 μ L milli-Q water. The reaction solution was then transferred onto the resin and incubated at 50 °C for 1.5 h with 500 rpm agitation.

[0626] Afterward, a 2 μ L aliquot was removed for CE analysis according to Example 4, while the reaction sample was allowed to cool in a 4 °C fridge for 10 minutes. Then, the supernatant was separated from the resin and placed in a 1.5 mL conical Eppendorf tube and centrifuged for 3 min at 14,000 rpm to pellet residual resin particulate. The supernatant was then transferred into a new 2.0 mL Eppendorf tube and the general AP catalyzed dephosphorylation procedure outlined below was carried out.

[0627] Third Extension: 5'-6-FAM-T10(2'dF)CmU(2'dF)CmA(2'dF)G + mUTP-3'P to 5'-6-FAM-T10(2'dF)CmU(2'dF)CmA(2'dF)GmU-3'P

[0628] To a 2.0 mL Eppendorf tube was added 15 mg IB-HIS-2 resin charged with 2.5 wt. % TdT (SEQ ID NO: 5246) and 0.2 wt. % IPP (SEQ ID NO: 3944). In a separate tube, 100 μ L from the de-phosphorylated product of the second extension was diluted with 7.5 μ L mUTP-3'P (2 mM), 5 μ L TEA·HCl (20 mM, pH 7.8), and 38 μ L milli-Q water. The reaction solution was then transferred to the tube containing resin and incubated at 50 °C with 500 rpm agitation.

[0629] After 1.5 h, the supernatant was separated from the resin and transferred to an Eppendorf tube containing a fresh 15 mg of IB-HIS-2 resin charged with 2.5 wt. % TdT and 0.2 wt. % IPP. To this

mixture was also added an additional 1.9 μL CoCl_2 (10 mM). The reaction was incubated at 50 °C with 500 rpm agitation for an additional 1.5 h.

[0630] Afterward, a 2 μL aliquot was removed for CE analysis according to Example 4, while the reaction sample was allowed to cool in a 4 °C fridge for 10 minutes. Then, the supernatant was separated from the resin and placed in a 1.5 mL conical Eppendorf tube and centrifuged for 3 min at 14,000 rpm to pellet residual resin particulate. The supernatant was then transferred into a new 2.0 mL Eppendorf tube and the general AP catalyzed dephosphorylation procedure outlined below was carried out.

[0631] Fourth Extension: 5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU + fGTP-3'P to 5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)G-3'P

[0632] To a 2.0 mL Eppendorf tube was added 8 mg IB-HIS-2 resin charged with 2.5 wt. % TdT (SEQ ID NO: 5246) and 0.2 wt. % IPP (SEQ ID NO: 3944). In a separate tube, 75 μL from the de-phosphorylated product of the third extension was diluted with 4.0 μL fGTP-3'P (2 mM). The reaction solution was then transferred to the tube containing resin and incubated at 50 °C with 500 rpm for 1.5 h.

[0633] Afterward, a 2 μL aliquot was removed for CE analysis according to Example 4, while the reaction sample was allowed to cool in a 4 °C fridge for 10 minutes. Then, the supernatant was separated from the resin and placed in a 1.5 mL conical Eppendorf tube and centrifuged for 3 min at 14,000 rpm to pellet residual resin particulate. The supernatant was then transferred into a new 2.0 mL Eppendorf tube and the general AP catalyzed dephosphorylation procedure outlined below was carried out.

[0634] Fifth Extension: 5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)G + mUTP-3'P to 5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)G-3'P

[0635] To a 2.0 mL Eppendorf tube was added 8 mg IB-HIS-2 resin charged with 2.5 wt. % TdT (SEQ ID NO: 5246) and 0.2 wt. % SEQ ID NO: 3944. In a separate tube, 75 μL from the de-phosphorylated product of the fourth extension was diluted with 4.0 μL mUTP-3'P (2 mM). The reaction solution was then transferred to the tube containing resin and incubated at 50 °C with 500 rpm for 1.5 h.

[0636] Afterward, another 8 mg IB-HIS-2 resin charged with 2.5 wt. % TdT (SEQ ID NO: 5246) and 0.2 wt. % SEQ ID NO: 3944 weighed out into a separate 2.0 mL Eppendorf tube. The reaction mixture was separated from the used resin and added to the tube containing fresh resin along with 1.0 μL CoCl_2 (10 mM). The reaction was then incubated for 1.5 h at 50 °C with 500 rpm agitation.

[0637] Afterward, another 8 mg IB-HIS-2 resin charged with 2.5 wt. % TdT (SEQ ID NO: 5246) and 0.2 wt. % SEQ ID NO: 3944 was weighed out into a separate 2.0 mL Eppendorf tube. The reaction mixture was separated from the used resin and added to the tube containing fresh resin along with 4.0 μL mUTP-3'P (2 mM). The reaction was then incubated for another 1.5 h at 50 °C with 500 rpm agitation.

[0638] Afterward, another 8 mg IB-HIS-2 resin charged with 2.5 wt. % SEQ ID NO: 5246 and 0.2 wt. % SEQ ID NO: 3944 was weighed out into a separate 2.0 mL Eppendorf tube. The reaction mixture was separated from the used resin and added to the tube containing fresh resin and incubated for another 1.5 h at 50 °C with 500 rpm agitation.

[0639] Afterward, a 2 μ L aliquot was removed for CE analysis according to Example 4, while the reaction sample was allowed to cool in a 4 °C fridge for 10 minutes. Then, the supernatant was separated from the resin and placed in a 1.5 mL conical Eppendorf tube and centrifuged for 3 min at 14,000 rpm to pellet residual resin particulate.

[0640] Sixth Extension: 5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU + fCQP => 5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU(2'dF)C-3'P

[0641] To a 2.0 mL Eppendorf tube was added 8 mg IB-HIS-2 resin charged with 2.5 wt. % TdT and 0.2 wt. % IPP. In a separate tube, 75 μ L from the de-phosphorylated product of the fifth extension was diluted with 4.0 μ L fCQP (2 mM). The reaction solution was then transferred to the tube containing resin and incubated at 50 °C with 500 rpm for 1.5 h.

[0642] Afterward, a 2 μ L aliquot was removed for CE analysis according to Example 4, while the reaction sample was allowed to cool in a 4 °C fridge for 10 minutes. Then, the supernatant was separated from the resin and placed in a 1.5 mL conical Eppendorf tube and centrifuged for 3 min at 14,000 rpm to pellet residual resin particulate. The supernatant was then transferred into a new 2.0 mL Eppendorf tube and the general AP catalyzed dephosphorylation procedure outlined below was carried out.

Table 92.1 Extension of Oligonucleotides Catalyzed by Immobilized TdT		
Starting Oligonucleotide	Product Oligonucleotide	Conversion (%)
5'-6-FAM-T10(2'dF)CmU(2'dF)CmA	5'-6-FAM-T10(2'dF)CmU(2'dF)CmA-3'P	+
5'-6-FAM-T10(2'dF)CmU(2'dF)CmA	5'-6-FAM-T10(2'dF)CmU(2'dF)CmA(2'dF)G-3'P	++
5'-6-FAM-T10(2'dF)CmU(2'dF)CmA(2'dF)G	5'-6-FAM-T10(2'dF)CmU(2'dF)CmA(2'dF)GmU-3'P	++
5'-6-FAM-T10(2'dF)CmU(2'dF)CmA(2'dF)GmU	5'-6-FAM-T10(2'dF)CmU(2'dF)CmA(2'dF)GmU(2'dF)G-3'P	++
5'-6-FAM-T10(2'dF)CmU(2'dF)CmA(2'dF)GmU(2'dF)G	5'-6-FAM-T10(2'dF)CmU(2'dF)CmA(2'dF)GmU(2'dF)GmU-3'P	+
5'-6-FAM-T10(2'dF)CmU(2'dF)CmA(2'dF)GmU(2'dF)GmU	5'-6-FAM-T10(2'dF)CmU(2'dF)CmA(2'dF)GmU(2'dF)GmU(2'dF)C-3'P	++
Conversion of nucleotide was determined as percent of total fluorescence in the CE trace and defined as follows: "+" 80 to 90%, "++" > 90%.		

[0643] General AP Catalyzed 3'-Dephosphorylation of RNA Oligomer

[0644] The supernatant was removed and added to a 2.0 mL Eppendorf tube containing IB-HIS-2 resin charged with 2.5 wt. % AP (SEQ ID NO: 3932), 0.05 mg resin per μ L reaction volume was used. The sample was then incubated at 50 °C for 20 minutes.

[0645] Afterward, a 2 μ L aliquot was removed for CE analysis according to Example 4. Meanwhile, the supernatant was separated from the resin, and transferred into a fresh Eppendorf tube. An equal volume of 25:24:1 chloroform:phenol:isoamyl alcohol was then added and the sample sealed and vortexed. After briefly centrifuging, the top aqueous layer was collected and transferred to a fresh sample tube. Then, an equal volume of 49:1 chloroform:isoamyl alcohol was added and the sample vortexed. After briefly centrifuging, the bottom organic layer was removed and discarded. The 49:1 chloroform:isoamyl alcohol extraction procedure was then repeated two more times. After the third time, the aqueous top layer was removed and transferred to a fresh 2.0 mL Eppendorf tube.

[0646] The aqueous layer containing de-phosphorylated oligo product was then placed in a vacuufuge for 15 minutes to remove residual organic solvents. The total volume lost was typically around 50 μ L and was accounted for when adding reagents during subsequent extensions.

Table 92.2 Dephosphorylation of 3'-phosphorylated Oligonucleotides by Immobilized Alkaline Phosphatase		
Starting Oligonucleotide	Product Oligonucleotide	Conversion (%)
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA-3'P	5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA	+
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)G-3'P	5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)G	+
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU-3'P	5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU	+
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)G-3'P	5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)G	+
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU-3'P	5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU	+
5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU(2'dF)C-3'P	5'-6-FAM-T10(2'dF)CmU(2'dF)CmAmA(2'dF)GmU(2'dF)GmU(2'dF)C	+
Conversion of nucleotide was determined as percent of total fluorescence in the CE trace and defined as follows: "+", > 95%.		

[0647] While the invention has been described with reference to the specific embodiments, various changes can be made and equivalents can be substituted to adapt to a particular situation, material, composition of matter, process, process step or steps, thereby achieving benefits of the invention without departing from the scope of what is claimed.

[0648] For all purposes in the United States of America, each and every publication and patent document cited in this disclosure is incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and

patent documents is not intended as an indication that any such document is pertinent prior art, nor does it constitute an admission as to its contents or date.

CLAIMS

What is claimed is:

1. An engineered terminal deoxynucleotidyl transferase comprising a polypeptide sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a reference sequence selected from SEQ ID NO: 2, 8, 12, 16, 24, 36, 54, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192, 5246, or a functional fragment thereof, and at least one amino acid residue difference relative to the reference sequence.
2. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises residue differences relative to the reference sequence of SEQ ID NO: 2 at a set of amino acid positions selected from:
80/121/185/315;
80/121/185/190/289/290/293/313/342/499;
80/121/185/190/289/290/293/313/315/336/342/359/391/414/470/474/499/522/523;
80/121/185/190/244/289/290/293/313/315/317/336/342/359/391/470/474/499/522/523;
80/121/174/185/190/196/244/266/273/284/288/289/290/293/313/315/317/324/336/342/352/359/391/394/397/401/419/428/431/462/470/474/499/522/523;
80/106/121/185/190/205/289/290/293/313/315/336/342/359/391/470/474/499/522/523;
80/106/121/185/190/205/289/290/293/313/342/470/474/499/523;
80/106/121/185/190/244/289/290/293/307/342/359/470/474/499;
80/121/131/185/190/205/244/289/290/293/313/315/336/342/359/391/414/470/474/499/522/523;
80/121/131/185/190/205/244/289/290/293/313/336/342/359/391/414/470/474/499/522/523;
80/121/131/185/190/289/290/293/313/342/470/474/499/522/523;
80/121/174/179/185/190/236/244/288/289/290/293/313/315/317/336/342/359/363/391/394/408/426/462/470/474/499/522/523;
80/121/174/185/186/190/236/244/273/284/288/289/290/293/313/315/317/336/342/352/359/391/394/395/419/428/431/462/470/474/499/522/523;
80/121/174/185/190/193/196/244/273/284/288/289/290/293/297/313/315/317/324/336/342/352/359/376/380/391/394/401/415/419/428/431/435/441/462/470/474/499/522/523;
80/121/174/185/190/193/244/273/284/288/289/290/293/297/313/315/317/336/342/352/359/391/394/415/419/428/431/462/470/474/499/522/523;
80/121/174/185/190/236/244/273/282/284/288/289/290/293/313/315/317/336/342/352/359/391/394/395/419/428/431/462/470/474/499/522/523;
80/121/174/185/190/244/273/284/288/289/290/293/313/315/317/336/342/352/359/391/394/419/428/431/462/470/474/499/522/523;
80/121/174/185/190/244/273/284/288/289/290/293/313/315/317/336/342/359/391/394/428/431/462/470/4

74/499/522/523;
 80/121/174/185/190/244/284/288/289/290/293/313/315/317/336/342/352/359/391/394/419/428/431/462/470/474/499/522/523;
 80/121/174/185/190/244/284/288/289/290/293/313/315/317/336/342/352/359/391/394/428/431/462/470/474/499/522/523;
 80/121/174/185/190/244/284/288/289/290/293/313/315/317/336/342/359/391/394/428/431/462/470/474/499/522/523;
 80/121/185/190/196/244/289/290/293/313/315/317/336/342/359/391/470/474/499/522/523;
 80/121/185/190/201/289/290/293/313/342/470/474/499/522/523;
 80/121/185/190/244/273/289/290/293/313/315/317/336/342/352/359/391/419/435/470/474/499/522/523;
 80/121/185/190/244/289/290/293/300/313/315/317/336/342/359/391/470/474/499/522/523;
 80/121/185/190/244/289/290/293/313/315/317/336/342/359/380/391/401/419/470/474/499/522/523;
 80/121/185/190/244/289/290/293/313/315/317/336/342/359/391/392/470/474/499/522/523;
 80/121/185/190/244/289/290/293/313/315/317/336/342/359/391/395/470/474/499/522/523;
 80/121/185/190/244/289/290/293/313/336/342/359/391/414/470/474/499/522/523;
 80/121/185/190/289/290/293/313/336/342/359/391/470/474/499/522/523;
 80/121/190/289/290;
 80/185/236/289/293;
 121/185/190/213/289/290/293; and
 185/289/290/293.

3. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 36 at amino acid positions selected from:

174/244/273/284/288/315/317/336/352/359/391/394/419/428/431/462/470/474/522/523;
 174/244/284/288/315/317/336/359/391/394/428/431/462/470/474/522/523;
 244/315/317/336/359/391/470/474/522/523; and
 336/359/391/470/474/522/523; and/or any combination thereof.

4. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 8 at amino acid positions selected from:

129/196, 173, 183, 186, 193, 195, 196, 263, 266, 268, 281, 282, 297, 300, 303, 316, 318, 320, 324, 343,
 360, 392, 395, 397, 411, 415, 417, 421, 454, 456, 477, 481, and 492, and/or any combinations thereof.

5. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 16 at amino acid positions selected from: 186, 186/236/318, 186/236/395, 186/282/318, 193/196, 193/196/266/324/376/380, 193/196/297, 193/196/297/324/376/380/401/415/435/441, 193/196/324, 193/196/324/397/401/441, 193/196/376/380, 193/297/324/376/435, 193/297/324/380, 193/297/415, 193/435, 196, 196/266, 196/266/324/397/401, 196/297/324/435, 236/282, 236/282/395, 236/318/481, 266/297/380/397/401, 282, 282/318, 282/481, 297/380/401/441, 297/435, 318/395, 376/401/441, 415, and 435/441, and/or any combinations thereof.

6. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 24 at amino acid positions selected from: 12, 13, 14, 17, 18, 20, 21, 22, 23, 24, 26, 27, 29, 30, 31, 33, 34, 35, 37, 41, 53, 57, 58, 61, 92, 94, 97, 101, 102, 103, 104, 105, 106, 107, 108, 124, 126, 133, 135, 137, 138, 139, 140, 141, 142, 144, 145, 147, 149, 150, 152, 153, 154, 155, 156, 156/294, 159, 160, 161, 162, 163, 7/135, 12, 13, 14, 15, 16, 17/131, 18, 20, 23, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 44, 45, 46, 57, 65, 77, 85, 89, 93, 94, 97, 101, 102, 103, 105, 106, 108, 109, 110, 119, 123, 124, 126, 130, 131, 132, 133, 134, 135, 137, 138, 139, 149, 150, 153, and 156, and/or any combinations thereof.

7. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 268 at amino acid positions selected from: 14/53/300, 14/53/419, 106/300/415/419/456, 140, and 300/395/419, and/or any combinations thereof.

8. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 648 at amino acid positions selected from: 12/14/34, 12/14/34/37/94/140/141/145, 12/14/34/37/106/140/142/150/152/153, 12/14/34/37/141/142, 12/14/34/37/142/145, 12/14/34/37/142/161, 12/14/34/37/150, 12/14/34/37/150/153, 12/14/34/140/142, 12/14/34/140/150, 12/14/34/142/150/153, 12/14/92/94, 12/14/94/150/152, 12/14/106/107/141/142, 12/14/106/108/140/141/145/150, 12/14/106/108/152, 12/14/141/142, 12/14/150/152/153, 12/14/153, 12/34/92/140, 12/34/150/152, 12/37/94/141/150/152/153, 12/37/140/141/150/162, 12/161, 14, 14/31/34/37/140/141/145/161/162, 14/34/37, 14/34/37/145, 14/34/37/152, 14/34/94/106/108/141, 14/34/150/153, 14/106, 14/140, 14/141/161, 14/142, 14/142/161/162, 14/153, 14/161, 20/21/24/33/58/104/106/124/155/156, 20/21/33/58/101/104/106/124/155, 20/21/58/104/106/155/156, 20/33/104/106/124/156, 20/58/101/104/106/156, 20/58/101/106, 20/101/106/156, 21/33/58/101/106, 21/33/58/106/155/156, 21/33/101/104/106, 21/33/106, 21/58/101/104/106/155, 21/58/106/155/156,

21/101/104/106/156, 21/104/106, 21/104/106/124, 21/104/106/156, 30/33/58/104/106/155/156, 30/33/101/106/156, 30/33/104/106/155/156, 30/101/104/106/155/156, 30/104/106/155, 30/104/106/155/156, 30/106/155, 33/58/104/106, 33/101/104/106/155, 34/37, 34/37/92, 34/37/140/141/142/145, 34/37/141/142, 34/37/150/153, 34/92/94/141/142, 34/141/142/145, 34/150/152/153, 37/92/142, 37/141/142, 37/153, 58/101/104/106/156, 58/101/106/155, 58/104/106/155/156, 92/94/106/142/145, 101/104/106, 101/104/106/155/156, 101/104/106/156, 101/106, 101/106/124/155, 101/106/155/156, 104/106, 104/106/124, 104/106/155, 104/106/155/156, 104/106/156, 106/107/108/142/220, 106/108/140/141/152/153, 106/108/140/142/150/153, 106/156, 140/141/142, 140/145, 140/145/150/152, 141, 141/152, and 161, and/or any combinations thereof.

9. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 660 at amino acid positions selected from: 16/29/30, 16/29/30/33/153, 16/29/30/101/104, 16/30/104, 16/33, 29/30, 58, 92/94/108/141/155/392, 92/101/137/155/476, 94/101/156/476, 101, 101/104, 101/137/155, 101/141/155/156, 108, 195, 197, 204/342, 205, 236/297, 258, 261, 262, 264, 268, 269, 276, 278, 280, 281, 282, 290, 291, 297, 300, 303, 306, 308, 309, 310, 312, 315, 316, 342, 344, 353, 360, 385, 391, 410, 413, 419, 421, 448, 454, 456, 473, 476, 515, 525, and/or any combinations thereof.

10. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 882 at amino acid positions selected from 175, 179, 196, 199, 201, 203, 208, 272, 273, 275, 313, 314, 317, 319, 321, 322, 324, 325, 329/462, 350, 376, 379, 394, 397, 403/462, 404, 406, 408, 457, 461, 462, 469, 477, 481, 484, 491, 492, and 495, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 882.

11. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 1100 at amino acid positions selected from 101/137/264/476/525 and 264, and/or any combinations thereof.

12. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 1336 at amino acid positions selected from 61, 152/503, 160, 162, 165, 177, 200, 200/425, 213, 217, 219, 223, 236, 246, 248, 292, 292/411, 295, 326, 329, 330, 333, 334, 338, 340, 340/438, 363, 369, 370, 372, 373, 383, 400/401/402, 425, 427, 435, 435/503, 437, 440, 441, 442, 443, 444, 446, 459, 460, 488, 490, 501, 502, 503, 504, and 506,

15/199/203/394, 15/199/394, 28/344/353/395, 195/199/203/278/297/394, 195/199/203/278/314/353/394, 195/203/278/394/395, 195/203/297/314/394, 195/203/297/394/419, 195/203/394, 195/278/297/394, 195/278/297/394/395, 195/314/344, 195/394/395, 199/203/297/394/395, 203/278/297/394, 203/297/314/394/395, 203/310/314/394/395/419, 203/344/394, 203/353, 203/394, 203/394/395, 297/394, 314/394/395, 344/394/395, 353, and 394, and/or any combinations thereof.

13. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 1348 at amino acid positions selected from 3/175/213/313/325/340/457/481/485, 3/307/321/340/353/406/408/445, 148/175/201/457/485, 175/201/333/412/425/457/485, 175/325/397, 175/333, 175/333/369/481, 175/333/485, 175/485, 199/307/321/340/406/408/445/484, 201/213/333/344/397/425/481/485, 201/333/344/457/481, 201/333/481, 201/406/408/462/484/502, 213, 213/333/397, 307/333/340/408/445/462/502, 307/373/406/408/484, 321/333/340, 325/333/369/425, 325/425/457/481, 333, 333/344/369/397, 333/344/369/485, 340/484, 344/485, 353/406, 373, and 406/408, and/or any combinations thereof.

14. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 1596 at amino acid positions selected from 161, 162, 163, 165, 171, 177, 179, 188, 200, 205, 208, 231, 233, 251, 252, 253, 260, 261, 277, 306, 307, 321, 325, 327, 329, 330, 353, 368, 370, 371, 376, 380, 382, 393, 400, 402, 405, 406, 407, 408, 410, 413, 414, 419, 426, 441, 442, 446, 460, 464, 484, 488, 490, 495, 506, 508, 518, 520, 160/219/460/503/506, 160/165/203/205/219/353/406/408/442/446/460/488, 160/165/205/219/406/408/441/442/446, 160/203/205/406/408/441/442/446, 160/219/330/406/408/442/446/484, 205/307/406/408/441/442/446/460/488, 252/333, 406/408/441/442/446, 406/408/442/446/219/307/326, 406/408, 406/408/442/446, and 406/408/490, and/or any combinations thereof.

15. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 1654 at amino acid positions selected from 14/53/300, 14/53/419, 106/300/415/419/456, 140, 160, 160/163/165/203/205/219/353/414/441/460/488, 160/165/203/205/219/353/460/488, 160/165/179/203/205/208/219/353/376/413/414/441/488, 160/165/179/203/307/376/413/495, 160/165/203/205/219/353/460/488, 160/165/203/205/307/371/376/413/414, 160/165/203/208/371/376/413/414, 160/165/205, 160/165/205/208, 160/165/205/219/441, 160/165/205/414/441/495, 160/165/307/353/413/414/441/488/495, 160/179/203/205/208/219/371/414/484/506, 160/179/208/219/307/413/414/503/506, 160/179/208/307/371/376/414, 160/179/307/376/441/488/503,

160/203/205/208/219/414/460/506, 160/203/205/208/413/460/484/488, 160/203/205/441, 160/203/208/307/353/495, 160/203/208/371/413/414, 160/203/208/413/414/441/484, 160/203/326/353/413/414/484/495, 160/205/208/219/326/441/484/488/503, 160/208/326/376/414/441/484/488, 160/208/371/441/484/506, 160/208/414/441/452/480/488/495, 160/219/307/371/506, 160/219/330/484, 165/179/203/205/219/414/418/441/488/503, 165/179/203/205/484/503, 165/179/205/413/441, 165/179/208/353/413/414/503, 165/203/205, 165/203/205/307/414/441/484/495/503/506, 165/203/205/484/488, 165/203/208/326/376/503, 165/205, 165/208/326/413/414/484/495/506, 179, 179/203/205, 179/203/208/326/353/376/484, 179/205/208/353/414/441/460/484/488, 179/205/353, 179/208/353/460, 179/353, 203/205/208/307/330/353/441/460/503/506, 203/205/208/307/441, 203/205/208/353, 203/208/219/376/441, 203/208/219/441, 203/208/326/353, 203/413/503/506, 205/208/307/353/376/413, 205/208/414, 205/219/307/353, 205/307, 205/307/376/414/441/495, 205/307/441/460/488, 205/326/488/503/506, 208/488/506, 300/395/4193/160/205/208/219/307/353/371/376/413/414/441/488, 326/353/371/376/414, and 441, and/or any combinations thereof.

16. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 1830 at amino acid positions selected from 163/179/277/338/340, 163/414/441, 171/200/334/406/490, 177, 194, 196, 266, 267, 268, 269, 273, 273/501, 274, 277, 278, 292/406, 297, 298, 299, 301, 302, 309, 312, 347, 359, 390, 392, 394, 407, 408, 413, 416, 454, 468, 473, 477, 479, and 493, and/or any combinations thereof.

17. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 1950 at amino acid positions selected from 163/169, 185, 186, 192, 193, 194, 197, 198, 245, 251, 258, 259, 261, 263, 271, 274, 278, 280, 284, 286, 290, 291, 297, 304, 306, 308, 316, 347, 352, 359, 362, 378, 393, 396, 398, 399, 405, 407, 409/414, 410/414, 411/414, 413/414, 414, 414/415, 414/417, 415, 455, 465, 466, 468, 494, and 509, and/or any combinations thereof.

18. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 2008 at amino acid positions selected from 3, 8, 9, 11, 12, 15, 16, 26, 26/27, 36, 38, 39, 50, 58, 62, 70, 76, 79, 81, 90, 116, 147, 151, 157, 158, 158/274/411/413/414, 185/274/413/414, 185/411/413/414, 189, 196, 205, 206, 246, 248, 249, 253, 255, 262, 263/411/413/414/468, 263/413/414, 274/286/411/413/417/468, 274/411/417/468, 274/411/468, 274/413/414/417/468, 274/468, 278/411/413/468, 307, 314, 318, 324, 353, 397, 408, 410, 411/413/417,

411/413/417/468, 411/413/468, 411/414, 413, 413/414, 413/414/468, 413/417/468, 413/468, 414, and 468, 469, 473, 480, 481, 491, and 493, and/or any combinations thereof.

19. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 2254 at amino acid positions selected from 197/407/455, 197/455, 284/398/466, 362/407/455, 396/398/410/466, 398/466, 399/411/416, and 466, and/or any combinations thereof.

20. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 2514 at amino acid positions selected from 26/90/94/248/261/266/362/455, 26/90/246, 26/90/246/248, 26/90/246/248/261, 26/90/246/248/362/455, 26/90/246/248/455, 26/90/246/266/362, 26/90/246/362, 26/90/246/455, 26/90/248, 26/90/248/266/455, 26/90/248/455, 26/90/248/455/459, 26/90/266, 26/90/362/455, 26/173/248, 26/246, 26/246/248/362, 26/246/248/362/455, 26/246/248/455, 26/248, 26/248/261/266, 26/248/261/266/362/455, 26/248/266/362, 26/248/362/455, 26/248/455, 26/362, 26/362/455, 58/197/249/407/410, 62/249, 90/246/248, 90/246/248/261/266/362/455, 90/246/248/266/362/455, 246/248, 246/248/362, 246/266/455, 248, 248/266/362, 248/362/455, 248/455, and 362, and/or any combinations thereof.

21. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 2524 at amino acid positions selected from 9/16/62/157/246/249/362, 11/58/227/246, 12/16/158/246/248/249, 30/189/261/266/353/465/468, 47/326, 30/189/266, 30/261/266/353/468, 30/266, 30/266/303, 30/266/353, 38, 38/81/318, 38/197, 39, 39/79, 58/157/158/362, 70/353, 79/81, 81, 189, 189/261, 189/353, 246/249, 261/353, 266/307/353/468, 266/353/468, 266/468, 169, 175, 179, 191/413, 200, 203, 292, 293, 304/329, 325, 327/406, 329, 340, 353/459, 373, 379, 382, 402, 403, 404, 406, 427, 429, 459, 461, 484, 490, 495, 504, 506, and 508, and/or any combinations thereof.

22. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 2638 at amino acid positions selected from 11/30/79/189/480, 30/58/79/189/307/480, 79/189/307/410, and 79/307, and/or any combinations thereof.

23. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as

compared to the reference sequence of SEQ ID NO: 2804 at amino acid positions selected from 169/304/340/402/427, 169/304/340/427/429/504, 169/304/340/429/506, 169/304/340/504, 169/304/402/403/427/506, 169/304/403/427/504, 169/304/427/504/506, 169/304/504, 169/340/402/403/427/429/504, 169/340/402/403/427/504/506, 169/340/402/504, 169/340/427, 169/340/506, 169/402/403/504/506, 169/402/427/429/504, 169/402/504, 169/402/504/506, 169/403/427/506, 266/327/329/404/410, 292/327/329/468, 304, 304/340/402, 304/340/402/403/427/429/504/506, 304/340/402/403/504/506, 304/340/402/403/506, 304/340/402/427/504/506, 304/340/402/506, 304/340/403/427/429/504/506, 304/340/427, 304/402/403, 304/402/403/427/504, 304/402/403/429, 304/402/403/504/506, 304/402/403/506, 304/403/504, 304/504, 304/504/506, 327, 327/329, 327/329/379/404/406/410, 327/329/404/410, 327/329/465/484, 327/382/406/410/484, 327/410/484, 340, 340/402/403, 340/402/427/429/506, 340/402/429/504/506, 340/402/504, 340/403/504, 340/504, 340/506, 379/382/468, 379/404/410, 379/410, 379/465/468/484, 379/468, 402/403/427/504/506, 402/403/429/504, 402/427/504, 402/504, 403/427, 427, 484, 504, 504/506, and 506, and/or any combinations thereof.

24. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 2812 at amino acid positions selected from 30, 186, 188, 198, 231, 233, 235, 243, 248, 253, 264, 266, 278, 287, 297/440, 326, 366, 367, 368, 370, 396, 414, 433, 435, 437, 439, 442, 444, 446, 485, 499, 503, 515, 520, 525, 30/179/200/373/403, 30/179/373/379, 30/184/246/325/379/429/495, 30/184/246/327/329/459, 30/184/246/379/404, 30/184/246/459/461/495/500/504, 30/200/373, 30/200/373/379, 30/246/325/327/329/404/461, 30/246/325/379/404/427/429/461, 30/246/427/459/461, 30/325/327, 30/325/327/379/404/429/495, 30/327/404/504, 30/329/379, 30/373, 30/373/403, 30/379/429/459/461, 30/379/459/461/504, 30/403/441/460, 200/373/379, 246/325/329/379/461, 246/327/404/461/495, 327/329/379/504, 327/459/461/495, 353/403, 373/379, 373/379/403, 379/403/406/468, and 403/441, and/or any combinations thereof.

25. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 2956 at amino acid positions selected from 140/142/153/177/427/434/441/444/461/502, 140/142/153/427/484, 140/142/177, 140/142/177/441, 140/142/365/373/404/427/484, 140/142/373/427/484, 140/148/161/177/404, 140/150/153/365/373/427/484, 140/150/177/404/436/441/484/502, 140/161/177/404/427/484, 140/177/404, 140/177/404/484, 142/150/158/177/427/445, 142/150/177/404, 142/153/177/441/444, 142/177/373, 142/177/373/441, 142/461/484/502, 150/177, 153, 153/161/325/404/427/441/484, 153/484, 177, 177/365/427/434, 325/427, 427, and 484, and/or any combinations thereof.

26. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 3174 at amino acid positions selected from 122/473, 189, 190, 193, 194, 196, 263, 264, 266, 267, 268, 269, 273, 273/501, 274, 278, 279, 281, 297, 298, 299, 300, 301, 302, 304, 309, 312, 315, 347, 350, 352, 353, 359, 390, 392, 394, 407, 408, 410, 411, 413, 414, 416, 436, 454, 468, 472, 473, 477, 479, 480, 493, 186/188/248/253/365/366/444/445, 186/231/248/253/484, 186/231/248/365/366/368/484, 186/231/248/366/368/444, 186/231/248/484, 186/231/368/416/441/442/444/484/485, 186/231/441/444/445, 186/484/485, 188/231/248/253/365/441/442/444/445/484, 198/243/264/431/441, 198/243/396/414/431/433/441/499, 198/243/396/414/433/437/441/515, 198/264/266/414, 198/264/396/414/433/441/515, 198/266/396/414/433/441/499/501, 198/266/414/433/441/515, 198/266/437/441/499, 198/414/431/441/499/520, 198/414/433/441/515/520, 231/248/441/484, 243/264/515/520, 243/396/414/433/441/515/520, 243/414/433/437/441, 243/414/437/441/515, 248/442/444/445/484, 264/266/396/414/433/441/515, 264/266/414/441/499/501/515/520, 264/414, 264/414/441, 264/433/441, 266/437/441/499/515/520, 365/366/441/484/485, 396/414/441, 396/414/441/515, and 414/441/520, and/or any combinations thereof.

27. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 3222 at amino acid positions selected from 186/194/248/396, 186/198/243/248/366/368/394/501, 186/231/243/368/394/485/499, 186/231/248/485, 186/231/366/368/394/485, 186/243, 186/243/248/366/368/394, 186/243/248/394/484/485, 186/243/484/520, 186/248, 186/365, 186/365/366/368/394/499, 186/365/366/394/485, 186/366/368/394/396/484, 186/366/368/394/396/484/485, 186/394/396/485, 194/198/243/366/368/499, 194/515/520, 198/231/243/248/485, 198/243/248/365/394/501, 198/248/394/396/484/485/499, 198/394/396/484/485/499, 198/394/396/499/515/520, 198/394/499/501, 231/365/368/394/499/520, 231/368/394, 231/484/485/499/501, 243/248/394/396/484/485, 243/484, 243/484/485/499, 248/365/366/368/394/484/520, 248/394/484, 365/366/368/394, 365/368/394/396/520, 394/396, and 394/499, and/or any combinations thereof.

28. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 3670 at amino acid positions selected from 168, 198, 267, 273, 273/493, 273/493/499, 273/309/493/499, 273/309/413/499, 273/499, 274/299/408/416, 274/408/416, 274/416, 288/299/416, 298/299/416, 301, 303/396, 307, 308, 308/361, 309, 309/413,

309/499, 313, 372, 392, 397, 413, 413/493/499, 413/499, 415, 416, 419, 451, 452, 456, 472, 473, 475, 493/499, and 528, and/or any combinations thereof.

29. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 3674 at amino acid positions selected from 194/196/390, 194/196/390/394/460/480, 194/196/390/394/480, 194/196/390/454/480, 194/196/390/480, 194/196/394/454/480, 194/196/454, 194/390, 194/394, 194/394/454, 194/394/454/480, 196, 196/390, 196/390/394, 196/390/394/454, 196/390/394/454/480, 196/390/394/480, 196/390/454, 196/394, 196/394/454, 196/394/454/480, 196/394/480, 196/454, 297/470/473, 297/473/493, 390, 390/394, 390/394/454, 390/394/454/480, 390/394/480, 390/454, 390/480, 394, 394/480, and 454, and/or any combinations thereof.

30. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 3796 at amino acid positions selected from 198, 198/267/313/451/475/494/499, 198/267/314/451, 198/267/314/475, 198/267/409/451, 198/267/475, 198/451/493, 208/308, 208/308/461, 267, 267/314/328/451/494/499, 267/451, 267/451/494/499, 308, 314/328/451/499, 314/451, 328/409/451, 328/451, 409, 409/475/494, 451, 451/493/494, 451/493/499, 451/494, and 475, and/or any combinations thereof.

31. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 2 or 660 at amino acid positions selected from 26, 30, 38, 79, 81, 90, 92, 94, 101, 108, 137, 140 141, 142, 153, 155, 160, 163, 165, 177, 184, 189, 194, 196, 201, 203, 205, 213, 219, 231, 246, 248, 258, 263, 264, 266, 267, 301, 304, 307, 314, 318, 325, 333, 340, 344, 353, 362, 379, 390, 392, 394, 395, 396, 397, 398, 402, 403, 406, 408, 410, 411, 413, 414, 416, 425, 427, 429, 433, 434, 441, 442, 444, 446, 451, 455, 460, 461, 466, 468, 476, 481, 484, 485, 488, 495, 499, 501, 502, 506, 515, 525, 26/30/38/79/81/90/92/94/101/108/137/140/141/142/153/155/160/163/165/177/184/189/194/196/201/203/205/213/219/231/248/258/263/264/266/267/301/304/307/314/318/325/333/340/344/353/362/379/390/392/394/395/397/398/402/403/406/408/410/411/413/414/416/425/427/429/433/434/441/442/444/446/451/455/460/461/466/468/476/481/484/485/488/495/499/501/502/506/515/525, 26/30/38/79/81/90/92/94/101/108/137/140/141/142/153/155/160/163/165/177/184/189/201/203/205/213/219/231/248/258/263/264/266/304/307/314/318/325/333/340/344/353/362/379/392/394/395/397/398/402/403/406/408/410/411/413/414/416/425/427/429/433/434/441/442/444/446/455/460/461/466/468/476/481/484/485/488/495/499/501/502/506/515/525,

26/38/79/81/90/92/94/101/108/137/141/155/160/163/165/189/201/203/205/213/219/246/248/258/263/264/304/307/314/318/333/340/344/353/362/392/394/395/396/397/398/402/403/406/408/410/411/413/414/425/441/442/446/455/460/461/466/468/476/481/485/488/506/525,
92/94/101/108/137/141/155/160/163/165/201/203/205/213/219/258/263/264/314/333/344/353/392/394/395/397/406/408/411/413/414/425/441/442/446/460/461/468/476/481/485/488/525, and
92/94/101/108/137/141/155/201/213/264/314/333/344/392/394/395/397/406/408/425/442/446/461/476/481/485/525, and/or any combinations thereof.

32. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 3870 at amino acid positions selected from 165, 169, 171, 173, 175, 176, 179, 183, 187, 191, 192, 195, 197, 199, 200, 203, 204, 210, 257, 259, 267, 291, 293, 295, 301, 319, 325, 340, 341, 342, 374, 387, 398, 399, 403, 404, 406, 429, 480, 481, 483, 484, 490, 491, 493, 494, 495, 521, 507, 508, 509, and 522, and/or any combinations thereof.

33. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 3918 at amino acid positions selected from 3, 7/400/459/504, 164, 173/367/459/500, 184, 203, 203/367/459, 203/367/459/500/501, 203/400/459/501, 203/459, 203/459/499/504, 203/459/500, 215, 218, 294, 335, 335/402/481/484, 335/402/512, 335/481/484, 335/481/493, 335/481/512, 336, 338, 339, 367, 367/459/500, 370, 373, 376, 380, 384, 390, 395, 395/402, 395/402/481/484, 395/481, 395/481/512, 395/484, 400, 400/459, 400/459/499/500/504, 400/459/500/501, 400/459/501/504, 400/501, 400/504, 402, 402/481, 402/481/484, 402/481/484/512, 402/481/493, 402/484/493, 402/512, 458, 459, 459/501, 460, 481, 481/512, 484, 485, 493, 499, 500, 501, 504, 512, 515, and 516, and/or any combinations thereof.

33. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 4266 at amino acid positions selected from 95/428/480, 163/190, 163/203/366, 163/328/363/480, 163/363/480/485, 172/174/178/340, 178, 188, 190/202/203/363/366/480/483/485, 190/202/203/480, 190/480/485, 192, 192/498/499/503, 202, 202/203/328/362/363/366/428/480/485/498/499/503, 202/203/485, 203/328/363/428/483, 203/328/428, 203/328/480/485, 203/362/366, 203/498/499/503, 272, 280, 280/498/499/503, 296, 297, 299, 299/498/499/503, 301, 301/503, 308, 308/503, 311, 328/428, 328/480/483, 328/485, 343, 346, 349, 358, 359/498/499/503, 362/363, 362/363/366/428/480/483/498/499/503, 392/498/499/503, 406, 407, 410, 411, 418/498/499/503, 418/503, 419/503, 465, 471, 472/498/499/503, 473, 480/483, 491, and 498/499/503, and/or any combinations thereof.

34. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 4558 at amino acid positions selected from 94/365/367, 172/174/178/401/403, 172/174/178/401/403/507, 172/174/178/402/508, 172/174/401/403/507, 172/178, 172/178/401, 174/178, 178/401/403, 178/402/403, 318/375/380, 324/379/405/483, 340, 340/394, 365, 365/367/428, 365/389/394, 367, 375/376, 375/379/483, 375/380, 375/380/400/483, 375/380/483, 376/483, 379/483, 389/394, 394, 401, 401/402/403/507, 402/403, 405/483, and 483, and/or any combinations thereof.

35. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 4442 at amino acid positions selected from 174/296/299, 182, 185/190, 189/190, 190, 190/193, 192/280, 192/402/507, 257, 259, 260, 281, 289, 296/299, 305, 306, 307, 308, 312, 313, 316, 318, 327, 374, 381, 394, 395, 402, 402/507, 404, 405, 414, 432, 451, 455, 460, 461, 476/480, 480, 480/481, 493, 494, and 522, and/or any combinations thereof.

36. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 4654 at amino acid positions selected from 190, 190/197/308, 190/308/380/405, 190/375, 190/375/380, 190/380/405, 190/405/406, 272/301/393/394/480, 272/318/480/483, 301/394/480, 318, 375, 375/380, 375/405, 375/405/406, 380, 394, 394/480, and 480/483, and/or any combinations thereof.

37. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 4850 at amino acid positions selected from 189, 189/193/207/307/353, 190/322, 193, 193/307, 261/322/421, 297/298/300/392, 297/300, 297/300/328, 298/300/328, 298/300/328/395, 298/300/360, 298/300/392/395, 298/300/392/395/492, 298/300/395, 298/300/481, 300, 300/392/395, 319, 322, 392, 421, and 492, and/or any combinations thereof.

38. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 4856 at amino acid positions selected from 10/413, 260, 268, 302/307, 317, 353, 354, 362, 364, 392, 393, 394, 395, 397, 402, 404, 412, 413, 419, 436/512, 460, 477, 486, 490, 495, and 518, and/or any combinations thereof.

39. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 4904 at amino acid positions selected from 190, 190/287/300/302, 190/300/477/490, 194/300/302/413, 194/300/302/481, 297/298/308/392/395, 298/392/525, 300, 300/317, 300/490, and 395, and/or any combinations thereof.

40. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 5002 at amino acid positions selected from 11/523, 190/194, 194, 194/198, 202, 208, 264, 273, 273/347/354, 274, 281, 290, 298/300/302, 298/302/392/393/394/433, 298/302/392/394, 298/392/393/394, 298/392/393/394/477, 298/392/394/490, 298/393/394/395/433/477, 298/393/394/477/495, 298/394/433, 300/302/303, 308/402/460, 309, 313, 314, 324, 352, 359, 360, 361, 392/393/394/433, 392/393/394/477, 392/393/394/477/495, 392/393/394/490, 392/394, 392/394/395, 392/394/433/477, 392/394/433/495, 392/394/477/495, 392/394/495, 393/394, 393/394/433/477/490, 394/477, 394/490, 405, 408/413, 411/413, 413, 460/525, 463, 466, 467, 472, 473, 477, 492, 523, and 526, and/or any combinations thereof.

41. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 5028 at amino acid positions selected from 82/194/198/313, 194, 194/198, 194/198/208/313, 194/198/309, 194/198/313, 194/198/411, 194/208/411, 194/309, 194/313, 194/411, 198, 198/208, 198/208/309/411, 198/208/313/411, 273/274, 274, 274/281/526, 274/359/526, 274/523, 309, 309/313/411, 324/526, 411, 466, 466/526, 523, and 526, and/or any combinations thereof.

42. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 5192 at amino acid positions selected from 96/295, 169, 176, 177, 179, 184, 187, 193, 195, 197, 197/307, 198, 199, 200, 203, 292, 295, 300/394, 304, 325, 326, 326/380, 329, 373, 376, 377, 383, 394, 403, 409, 430, 485, 508, and 520/526, and/or any combinations thereof.

43. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution or substitution set comprising substitutions as compared to the reference sequence of SEQ ID NO: 5246 at amino acid positions selected from 177/198/200, 177/200/203, 177/200/203/295, 177/200/295/326, 180, 184/198/200/203/295, 184/200/295/326, 190/198/200/203, 190/200/203/295/380, 190/200/295, 197, 198, 198/200, 198/200/203, 198/200/203/295,

200/326, 200/380, 203, 203/380, 233, 252, 295, 336, 364, 365, 367, 381, 384, 441, 459, and 485, and/or any combinations thereof.

44. The engineered terminal deoxynucleotidyl transferase of any of Claims 1-43, wherein the polypeptide sequence further comprises an N-terminal truncation of 1-156 amino acids.

45. The engineered terminal deoxynucleotidyl transferase of any of Claims 1-44, wherein the engineered terminal deoxynucleotidyl transferase polypeptide is fused with a second polypeptide; optionally, wherein the second polypeptide has inorganic pyrophosphatase (IPP) activity

46. The engineered terminal deoxynucleotidyl transferase Claim 45, wherein the second polypeptide with IPP activity comprises an amino acid sequence selected from SEQ ID NO: 3942 and 3944.

47. The engineered terminal deoxynucleotidyl transferase Claim 45, wherein the engineered terminal deoxynucleotidyl transferase polypeptide fused with the second polypeptide comprises a sequence selected from SEQ ID NO: 5468, 5470, 5472, and 5474.

48. The engineered terminal deoxynucleotidyl transferase of any of Claims 1-47, wherein said engineered terminal deoxynucleotidyl transferase is capable of template-independent synthesis.

49. The engineered terminal deoxynucleotidyl transferase of any of Claims 1-47, having at least one improved property, as compared to a wild-type or reference terminal deoxynucleotidyl transferase or template-independent polymerase.

50. The engineered terminal deoxynucleotidyl transferase of Claim 49, wherein said improved property is selected from increased thermostability, increased activity at elevated temperatures, increased soluble expression or isolated protein yield, decreased by-product formation, increased specific activity on one or more NTP-3'-O-RBG or natural or modified NTP substrates, increased incorporation efficiency in extension of oligo acceptor substrates, and increased activity on one or more oligo acceptor substrates.

51. The engineered terminal deoxynucleotidyl transferase of any of Claims 1-50, wherein said engineered terminal deoxynucleotidyl transferase comprises increased soluble expression or isolated protein yield as compared to a wild-type or reference terminal deoxynucleotidyl transferase or template-independent polymerase.

52. The engineered terminal deoxynucleotidyl transferase of any of Claims 1-51, wherein said engineered terminal deoxynucleotidyl transferase comprises increased thermal stability as compared to a wild-type or reference terminal deoxynucleotidyl transferase or template-independent polymerase.

53. The engineered terminal deoxynucleotidyl transferase of any of Claims 1-52, wherein said engineered terminal deoxynucleotidyl transferase comprises increased activity on one or more NTP-3'-O-RBG or natural or modified NTP substrates as compared to a wild-type or reference terminal deoxynucleotidyl transferase or template-independent polymerase.

54. The engineered terminal deoxynucleotidyl transferase of any of Claims 1-53, wherein said terminal deoxynucleotidyl transferase is purified.

55. A polynucleotide sequence encoding at least one engineered terminal deoxynucleotidyl transferase of any of Claims 1-54.

56. A polynucleotide sequence comprising at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the reference sequence of SEQ ID NOs: 1, 7, 11, 15, 23, 35, 53, 267, 647, 659, 881, 1099, 1335, 1347, 1595, 1653, 1829, 1949, 2007, 2253, 2513, 2523, 2637, 2803, 2811, 2955, 3173, 3221, 3669, 3673, 3795, 3869, 3917, 4265, 4441, 4653, 4849, 4855, 4903, 5001, 5027, 5191, and 5245, and/or a functional fragment thereof, wherein said polynucleotide sequence encodes an engineered polypeptide comprising at least one substitution at one or more amino acid positions.

57. The polynucleotide sequence of Claim 56, wherein said polynucleotide sequence encodes at least one engineered terminal deoxynucleotidyl transferase comprising a sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the reference sequence of SEQ ID NOs: 2, 8, 12, 16, 24, 36, 54, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192, and 5246.

58. The polynucleotide sequence of Claim 56, wherein said sequence comprises SEQ ID NOs: 7, 11, 15, 23, 35, 53, 267, 647, 659, 881, 1099, 1335, 1347, 1595, 1653, 1829, 1949, 2007, 2253, 2513, 2523, 2637, 2803, 2811, 2955, 3173, 3221, 3669, 3673, 3795, 3869, 3917, 4265, 4441, 4653, 4849, 4855, 4903, 5001, 5027, 5191, and 5245.

59. The polynucleotide sequence of Claim 56, wherein said polynucleotide sequence is operably linked to a control sequence.

60. The polynucleotide sequence of Claim 56, wherein said polynucleotide sequence is codon-optimized.
61. An expression vector comprising at least one polynucleotide sequence of any of Claims 55-60.
62. A host cell comprising at least one expression vector of Claim 61.
63. A method of producing an engineered terminal deoxynucleotidyl transferase polypeptide in a host cell comprising culturing a host cell of Claim 62, under suitable culture conditions, such that at least one engineered terminal deoxynucleotidyl transferase is produced.
64. The method of Claim 63, further comprising recovering at least one engineered terminal deoxynucleotidyl transferase from the culture and/or host cells.
65. The method of Claim 63 or 64, further comprising the step of purifying said at least one engineered terminal deoxynucleotidyl transferase.
66. A composition comprising at least one engineered terminal deoxynucleotidyl transferase of any of Claims 1-54.
67. A method for template-independent synthesis of an oligonucleotide, the method comprising:
- a) providing at least one terminal deoxynucleotidyl transferase or template-independent polymerase;
 - b) providing at least one oligo acceptor substrate, wherein the oligo acceptor substrate comprises a 3'-OH, or an equivalent thereof;
 - c) contacting the oligo acceptor substrate, the terminal deoxynucleotidyl transferase or template-independent polymerase, and one or more nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBG under conditions sufficient for the addition of the nucleotide, modified nucleotide, or nucleotide-3'-O-RBG to the 3' end of the oligo acceptor substrate.
68. The method of Claim 67, wherein the method further comprises (d) deblocking the oligonucleotide formed in step (c) at the protected 3'-O-position of the oligonucleotide product.
69. The method of Claims 67 or 68, wherein the method further comprises e) deactivating

unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs.

70. The method of any of Claims 67-69, wherein the method further comprises an optional step (f) of removing excess nucleoside and/or excess inorganic phosphate or pyrophosphate from the reaction.

71. The method of any of Claims 67-70, wherein the method further comprises repeating steps (a)-(c) or (a)-(d) or (a)-(e) or (a)-(f) until a desired oligonucleotide sequence is obtained.

72. The method of any of Claims 67-71, wherein the method further comprises (g) cleaving or releasing the growing or completed oligonucleotide chain.

73. The method of any of Claims 68-72, wherein step (d) deblocking the oligonucleotide formed in step (c) at the protected 3'-O-position and step (e) deactivating the unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs occur simultaneously.

74. The method of any of Claims 67-73, wherein a phosphatase is used to deblock the NTP-3'-O-RBG and to deactivate unreacted NTP-3'-O-RBGs by removing at least one of the 5' phosphates.

75. The method of Claim 74, wherein the phosphatase comprises an alkaline phosphatase set forth in SEQ ID NO: 3922, 3924, 3926, 3928, 3930, 3932, or 3934.

76. The method of any of Claims 67-75, wherein step (c) optionally includes contacting the oligo acceptor substrate, the terminal deoxynucleotidyl transferase or template-independent polymerase, and either a nucleotide triphosphate, or a modified nucleotide triphosphate, or an NTP-3'-O-RBG with a phosphatase, such as an inorganic pyrophosphatase, to convert pyrophosphate to inorganic phosphate.

77. The method of Claim 76, wherein the phosphatase comprises an inorganic pyrophosphatase set forth in SEQ ID NO: 3936, 3938, 3940, 3942, 3944, 3946, or 3948.

78. The method of any of Claims 67-77, further comprising an NTP-3'-O-RBG comprising a carbonitrile, phosphate, carbonate, carbamate, ester, ether, borate, nitrate, sugar, phosphoramidate, phenylsulfenate, or sulfate.

79. The method of any of Claims 67-78, wherein the nucleotide triphosphate, the modified nucleotide triphosphate, or the NTP-3'-O-RBG comprise a 2' modification.

80. The method of Claim 79, wherein the 2' modification comprises a 2'-F, 2'-O-alkyl, 2'-O-methoxyethyl, or a locked or constrained ethyl.

81. The method of Claim 80, wherein the modification is 2'-F and the 2' modified nucleotide triphosphate, the modified nucleotide triphosphate, or the NTP-3'-O-RBG is selected from: 2'-fluoro-2'-deoxyadenosine-5'-triphosphate, 2'-fluoro-2'-deoxycytidine-5'-triphosphate, 2'-fluoro-2'-deoxyguanosine-5'-triphosphate, and 2'-fluoro-2'-deoxyuridine-5'-triphosphate.

82. The method of claim 80, wherein the modification is 2'-O-alkyl and the 2' modified nucleotide triphosphate, the modified nucleotide triphosphate, or the NTP-3'-O-RBG is selected from: 2'-O-methyladenosine-5'-triphosphate, 2'-O-methylcytidine-5'-triphosphate, 2'-O-methylguanosine-5'-triphosphate, 2'-O-methyluridine-5'-triphosphate, and 2'-O-methylinosine-5'-triphosphate.

83. The method of any of Claims 67-82, wherein the oligonucleotide chain comprises one or more phosphorothioate linkages.

84. The method of any of Claims 67-83, wherein the oligonucleotide, oligonucleotide sequence, or oligonucleotide chain comprises RNA.

85. The method of any of Claims 67-84, wherein the oligonucleotide, oligonucleotide sequence, or oligonucleotide chain comprises DNA.

86. The method of any of Claims 67-85, wherein the terminal deoxynucleotidyl transferase or template-independent polymerase comprises one or more engineered terminal deoxynucleotidyl transferases.

87. The method of Claim 86, wherein the engineered terminal deoxynucleotidyl transferase of any one of claims 1-54.

88. The method of Claim 86, wherein the engineered terminal deoxynucleotidyl transferase comprises a polypeptide sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a reference sequence of SEQ ID NO: 2, 8, 12, 16, 24, 36, 54, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192, and/or 5246, or a functional fragment thereof, and comprises an amino acid residue difference or set of amino acid residue differences in its polypeptide sequence relative to the reference sequence, wherein the amino acid residue difference or set of differences is selected from the amino acid differences listed in:

Tables 5.1, 6.2, 7.2, 8.2, 9.2, 10.2, 11.2, 12.2, 13.2, 14.2, 15.2, 16.2, 17.2, 18.2, 19.2, 20.2, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 26.3, 26.4, 27.2, 27.3, 27.4, 27.5, 28.1, 28.2, 28.3, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35.2, 36.2, 37.2, 38.2, 39.2, 40.2, 41.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 49.2, 50.2, 51.2, 52.2, 53.2, 54.2, 55.2, 56.2, 56.3, 56.4, 61.2, 63.2, 64.2, 65.2, 66.2, 67.2, 68.2, 69.2, 70.2, 71.2, 72.2, 73.2, 74.2, 75.2, 76.2, 77.2, 78.2, 79.2, and 80.1.

89. The method of Claim 86, wherein the engineered terminal deoxynucleotidyl transferase comprises a polypeptide sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity to an amino acid sequence selected from the even-numbered sequences SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476.

90. The method of any of Claims 67-89, wherein the terminal deoxynucleotidyl transferase or template-independent polymerase is immobilized.

91. The method of Claim 90, wherein the method further comprises a column solid support for immobilization of the terminal deoxynucleotidyl transferase or template-independent polymerase.

92. The method of Claim 90, wherein the method further comprises a batch method with a solid support for immobilization of the terminal deoxynucleotidyl transferase or template-independent polymerase.

93. The method of any of Claims 67-89, wherein the oligoacceptor substrate and subsequent oligonucleotide products are immobilized.

94. The method of any of Claims 67-89, wherein neither the oligoacceptor substrate nor the terminal deoxynucleotidyl transferase or template-independent polymerase are immobilized.

95. The method of any Claims 67-94, further comprising an aqueous liquid phase.

96. The engineered terminal deoxynucleotidyl transferase of any of Claims 1-54, wherein the engineered terminal deoxynucleotidyl transferase is immobilized.