

WHAT IS CLAIMED IS: CLAIMS

1. A method of modifying a nucleic acid, the method comprising:

(a) providing a single stranded deoxyribonucleic acid (ssDNA) and a nucleoside triphosphate comprising a modified base; and

(b) contacting the ssDNA and the nucleoside triphosphate comprising the modified base with a recombinant terminal deoxynucleotidyl transferase (TdT) to generate a ssDNA scaffold, wherein the ssDNA scaffold comprises the ssDNA incorporated with one or more nucleotides comprising the modified base from the nucleoside triphosphate,

wherein the recombinant TdT comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 1, and wherein the recombinant TdT comprises one or more amino acid substitution mutations at one or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His420 in the *Bos taurus* TdT of SEQ ID NO: 12.

2. The method of claim 1, further comprising:

(c) contacting the ssDNA scaffold with a first adapter oligonucleotide comprising a first adapter sequence and a second adapter oligonucleotide comprising a second adapter sequence to generate a nucleic acid carrier comprising the ssDNA scaffold attached to the first adapter oligonucleotide and the second adapter oligonucleotide.

3. The method of ~~any one of claims 1-2~~ claim 1, wherein contacting the ssDNA scaffold with the first adapter oligonucleotide comprises contacting the ssDNA scaffold with a first adapter comprising the first adapter oligonucleotide and a first polymer.

4. The method of ~~any one of claims 1-3~~ claim 1, wherein contacting the ssDNA scaffold with the second adapter oligonucleotide comprises contacting the ssDNA scaffold with a second adapter comprising the second adapter oligonucleotide and a second polymer.

5. ~~The method of any one of claims 3-4, wherein the first adapter comprises the first adapter oligonucleotide covalently linked, and wherein the second adapter comprises the second adapter oligonucleotide covalently linked.~~

65. The method of ~~any one of claims 2-5~~ claim 2, wherein the ssDNA scaffold comprises a third polymer.

~~76.~~ The method of ~~any one of claims 2-6~~ claim 2, further comprising:

- (d) providing a nucleic acid template comprising the first adapter sequence, or a reverse complement thereof, the second adapter sequence, or a reverse complement thereof, and a nucleic acid hybridization sequence;
- (e) contacting the nucleic acid carrier with the nucleic acid template to generate the nucleic acid carrier having the nucleic acid template hybridized to a template capture site of the nucleic acid carrier via the nucleic acid hybridization sequence of the nucleic acid template;
- (f) performing amplification on the nucleic acid carrier hybridized with the nucleic acid template to generate a plurality of amplified nucleic acids each comprising the first adapter oligonucleotides and the second adapter oligonucleotides extended to comprise a sequence of the nucleic acid template, or a reverse complement thereof; and
- (g) determining the sequence of the nucleic acid template using the plurality of amplified nucleic acids.

~~87.~~ A method of modifying a nucleic acid, the method comprising:

- (a) providing a first nucleic acid and a first nucleoside triphosphate comprising a first modified base; and
- (b) contacting the first nucleic acid and the first nucleoside triphosphate comprising the first modified base with a recombinant terminal deoxynucleotidyl transferase (TdT) for a first reaction time at a first temperature in a first reaction to generate a second nucleic acid, wherein the second nucleic acid comprises the first nucleic acid incorporated with one or more first nucleotides comprising the first modified base from the first nucleoside triphosphate,

wherein the recombinant TdT comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 1, and wherein the recombinant TdT comprises one or more amino acid substitution mutations at one or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His420 in the *Bos taurus* TdT of SEQ ID NO: 12.

~~98.~~ The method of claim ~~87~~, wherein one or more of the first nucleic acid and the second nucleic acid comprise a single stranded nucleic acid, a double stranded nucleic acid with a 3' overhang, a double stranded nucleic acid with a 3' recess, or a combination thereof.

~~10. The method of any one of claims 8-9, wherein one or more of the first nucleic acid and the second nucleic acid comprise deoxyribonucleic acid (DNA).~~

~~11. The method of any one of claims 8-10, wherein at least 50% of nucleotides of one or more of the first nucleic acid and the second nucleic acid comprise deoxyribonucleotides.~~

~~12. The method of any one of claims 8-11, wherein one or more of the first nucleic acid and the second nucleic acid comprise a single stranded deoxyribonucleic acid (ssDNA).~~

~~13. The method of any one of claims 8-11, wherein one or more of the first nucleic acid and the second nucleic acid comprise at least one ribonucleotide.~~

~~14. The method of any one of claims 8-13, wherein the second nucleic acid comprises the first nucleic acid incorporated with two or more, or three or more, of the first nucleotides comprising the first modified base.~~

~~15. The method of claim 14, wherein the two or more, or three or more, of the first nucleotides comprising the first modified base in the second nucleic acid are consecutive.~~

~~169. The method of any one of claims 8-15 claim 7, wherein the first modified base of the first nucleoside triphosphate comprises a modified adenine, a modified guanine, a modified cytosine, a modified thymine, or a modified uracil.~~

~~17. The method of any one of claims 8-16, wherein the first nucleoside triphosphate comprises — 5-(15-Azido-4,7,10,13-tetraoxa-pentadecanoyl-aminoallyl)-2'-deoxyuridine-5'-triphosphate (Azide-PEG4-aminoallyl-dUTP), — N⁶-(6-Azido)hexyl-3'-deoxyadenosine-5'-triphosphate (N⁶-(6-Azido)hexyl-3'-dATP), or a combination thereof.~~

~~1810. The method of any one of claims 8-16 claim 7, wherein the first nucleoside triphosphate comprises the first modified base and a first accessory oligonucleotide covalently linked.~~

~~19. The method of any one of claims 8-17, wherein the first reaction time is at least 1 second.~~

~~20. The method of any one of claims 8-19, wherein the first temperature is at least 16 °C to at least 58 °C.~~

~~21. The method of any one of claims 8-20, wherein a concentration of the first nucleic acid in the first reaction is at least 10 nM.~~

~~22. The method of any one of claims 8-21, wherein a concentration of the first nucleoside triphosphate in the first reaction is at least 0.1 μ M.~~

~~23. The method of any one of claims 8-22, wherein a concentration of the recombinant TdT in the first reaction is at least 10 nM.~~

2411. The method of ~~any one of claims 8-23~~ claim 7,

wherein providing the first nucleic acid and the first nucleoside triphosphate comprising the first modified base comprises: providing the first nucleic acid, the first nucleoside triphosphate comprising the first modified base, and a second nucleoside triphosphate,

wherein contacting the first nucleic acid and the first nucleoside triphosphate comprising the first modified base with the recombinant TdT comprises: contacting the first nucleic acid, the first nucleoside triphosphate comprising the first modified base, and the second nucleoside triphosphate with the recombinant TdT for the first reaction time at the first temperature in the first reaction to generate the second nucleic acid, and

wherein the second nucleic acid comprises the first nucleic acid incorporated with (i) one or more of the first nucleotides comprising the first modified base from the first nucleoside triphosphate and (ii) one or more second nucleotides.

~~25. The method of claim 24, wherein each of the one or more second nucleotides comprises a second modified base from the second nucleoside triphosphate.~~

~~26. The method of claim 25, wherein the second modified base of the second nucleoside triphosphate comprises a modified adenine, a modified guanine, a modified cytosine, a modified thymine, or a modified uracil.~~

~~27. The method of any one of claims 25-26, wherein the first modified base of the first nucleoside triphosphate and the second modified base of the second nucleoside triphosphate comprise modifications of a same unmodified base.~~

~~28. The method of any one of claims 25-26, wherein the first modified base of the first nucleoside triphosphate and the second modified base of the second nucleoside triphosphate~~

comprise modification of different unmodified bases.

~~29. The method of any one of claims 25-28, wherein the second nucleoside triphosphate comprises the second modified base and a second accessory oligonucleotide covalently linked.~~

~~30. The method of claim 24, wherein each of the second nucleotides comprises a second unmodified base from the second nucleoside triphosphate.~~

~~31. The method of claim 30, wherein the second unmodified base of the second nucleoside triphosphate comprises an adenine, a guanine, a cytosine, a thymine, or an uracil.~~

~~32. The method of any one of claims 30-31, wherein the first modified base comprises a modification of the second unmodified base.~~

~~33. The method of any one of claims 24-32, wherein the first nucleoside triphosphate comprising the first modified base and the second nucleoside triphosphate are contacted with the first nucleic acid at a ratio that ranges from about 1:100 to about 100:1.~~

~~34. The method of any one of claims 24-33, wherein the first nucleotide comprising the first modified base and the second nucleotide are incorporated into the second nucleic acid at a ratio that ranges from about 1:100 to about 100:1.~~

~~35~~12. The method of ~~any one of claims 8-20~~ claim 7,

wherein providing the first nucleic acid and the first nucleoside triphosphate comprising the first modified base comprises: providing the first nucleic acid, the first nucleoside triphosphate comprising the first modified base, and a plurality of second nucleoside triphosphates,

wherein contacting the first nucleic acid and the first nucleoside triphosphate comprising the first modified base with the recombinant TdT comprises: contacting the first nucleic acid, the first nucleoside triphosphate comprising the first modified base, and the plurality of second nucleoside triphosphates with the recombinant TdT for the first reaction time at the first temperature in the first reaction to generate the second nucleic acid, and

wherein the second nucleic acid comprises the first nucleic acid incorporated with one or more of the first nucleotides comprising the first modified base and one or more second

nucleotides from the plurality of second nucleoside triphosphates.

~~36. The method of claim 35, wherein the plurality of second nucleoside triphosphates comprises a deoxyribose adenine triphosphate, a deoxyribose guanine triphosphate, a deoxyribose cytosine triphosphate, a deoxyribose thymine triphosphate, a deoxyribose uracil triphosphate, or a combination thereof.~~

~~37. The method of any one of claims 35-36, wherein two of the plurality of second nucleoside triphosphates are contacted with the first nucleic acid at a ratio that ranges from about 1:100 to about 100:1.~~

~~38. The method of any one of claims 35-37, wherein two of the second nucleotides are incorporated into the second nucleic acid at a ratio that ranges from about 1:100 to about 100:1.~~

~~39. The method of any one of claims 35-38, wherein at least one, or each, of the plurality of second nucleoside triphosphates comprises a second unmodified base.~~

~~40. The method of any one of claims 35-39 wherein at least one, or each, of the plurality of second nucleoside triphosphates comprises a second modified base.~~

~~41~~13. The method of ~~any one of claims 8-40~~ claim 7, wherein modified bases and unmodified bases of nucleotides are incorporated into the second nucleic acid at a ratio that ranges from about 1:100 to about 100:1.

~~42. The method of claim 41 or wherein the modified bases comprise the first modified base and a base of at least one, or each, of the plurality of second nucleotides incorporated into the second nucleic acid.~~

~~43. The method of any one of claims 41-42 wherein the unmodified bases comprise the base of at least one, or each, of the plurality of second nucleotides incorporated into the second nucleic acid.~~

~~44. The method of any one of claims 35-43, wherein at least 1% of the nucleotide bases of the second nucleic acid comprise modified bases.~~

~~45. The method of claim 44, wherein the modified bases are distributed throughout the second nucleic acid.~~

~~46~~14. The method of claim ~~45~~7, wherein the modified bases are distributed randomly throughout the second nucleic acid.

~~47. The method of any one of claims 44-46, wherein the second nucleic acid comprises a plurality of two or more consecutive modified bases.~~

~~48. The method of claim 47, wherein the plurality of consecutive modified bases comprises three or more consecutive modified bases.~~

~~49. The method of any one of claims 8-48, wherein at least 1% of the nucleotide bases of the second nucleic acid comprise the first modified base.~~

~~50~~15. The method of ~~any one of claims 8-49~~ claim 7, wherein the first nucleic acid comprises a template capture site capable of binding a nucleic acid template.

~~51. The method of claim 50, wherein the template capture site comprises a template capture sequence, and wherein the nucleic acid template comprises a sequence that has at least 90% sequence identity to a reverse complement of the template capture sequence and is capable of hybridizing to the template capture sequence.~~

~~52. The method of claim 51, wherein the nucleic acid template comprises a single stranded DNA.~~

~~53~~16. The method of ~~any one of claims 8-52~~ claim 7, wherein one or more of the first modified base of the first nucleoside triphosphate and the first nucleotide in the second nucleic acid comprise a functional moiety.

~~54~~17. The method of claim ~~53~~16, wherein the functional moiety of the first modified base is capable of participating in a click chemistry reaction.

~~55. The method of any one of claims 53-54, wherein the first modified base of the~~

~~first nucleoside triphosphate and the first nucleotide in the second nucleic acid comprises a saturated or unsaturated, substituted or unsubstituted, straight or branched aliphatic carbon chain.~~

~~56. The method of claim 55, wherein the functional moiety and the base of the first modified base are on two ends of the first modified base connected by the saturated or unsaturated, substituted or unsubstituted, straight or branched aliphatic carbon chain.~~

~~57~~18. The method of ~~any one of claims 8-56~~ claim 7, further comprising:

providing a first accessory oligonucleotide; and

contacting the second nucleic acid with the first accessory oligonucleotide for a second reaction time at a second temperature in a second reaction to generate a third nucleic acid comprising the second nucleic acid attached to one or more of the first accessory oligonucleotides.

~~58. The method of claim 57,~~

~~wherein providing the first accessory oligonucleotide comprises: providing the first accessory oligonucleotide and a second accessory oligonucleotide, and~~

~~wherein contacting the second nucleic acid with the first accessory oligonucleotide comprises: contacting the second nucleic acid with the first accessory oligonucleotide and the second accessory oligonucleotide for the second reaction time at the second temperature in the second reaction to generate the third nucleic acid comprising the second nucleic acid attached to one or more of the first accessory oligonucleotides and one or more of the second accessory oligonucleotides.~~

~~59~~19. The method of ~~any one of claims 57-58~~ claim 18, wherein the first accessory oligonucleotide comprises a first adapter sequence, or a reverse complement thereof.

~~60. The method of any one of claims 57-59, wherein the second accessory oligonucleotide comprises a second adapter sequence, or a reverse complement thereof.~~

~~61. The method of any one of claims 59-60, wherein the first adapter sequence comprises a P5 sequence.~~

~~62. The method of any one of claims 59-61, wherein the second adapter sequence~~

comprises a P7 sequence.

~~63. The method of any one of claims 57-62, wherein one or more of the first accessory oligonucleotide and the second accessory oligonucleotide is about 10 nucleotides to about 100 nucleotides in length.~~

~~64. The method of any one of claims 57-63, wherein the third nucleic acid comprises about 10 to about 1,000,000 of the first accessory oligonucleotides.~~

~~65. The method of any one of claims 57-64, the third nucleic acid comprises about 10 to about 1,000,000 of the second accessory oligonucleotides.~~

~~66. The method of any one of claims 57-65, wherein the third nucleic acid comprises the second nucleic acid attached to one or more of the first accessory oligonucleotides.~~

~~67. The method of any one of claims 57-66, wherein the third nucleic acid comprises the second nucleic acid attached to one or more of the second accessory oligonucleotides.~~

~~68. The method of any one of claims 57-67, wherein providing the first accessory oligonucleotide comprises providing a first accessory comprising the first accessory oligonucleotide and a first polymer, wherein contacting the second nucleic acid with the first accessory oligonucleotide comprises contacting the second nucleic acid with the first accessory for the second reaction time at the second temperature in the second reaction to generate the third nucleic acid comprising the second nucleic acid attached to one or more of the first accessories.~~

~~69. The method of any one of claims 57-68, wherein providing the second accessory oligonucleotide comprises providing a second accessory comprising the second accessory oligonucleotide and a second polymer, and/or wherein contacting the second nucleic acid with the second accessory comprises contacting the second nucleic acid and the second accessory for the second reaction time at the second temperature in the second reaction to generate the third nucleic acid comprising the second nucleic acid attached to one or more of the second accessories.~~

~~70. The method of any one of claims 68-69, wherein the first accessory comprises the~~

~~first accessory oligonucleotide and the first polymer covalently linked, and wherein the second accessory comprises the second accessory oligonucleotide and the second polymer covalently linked.~~

~~71. The method of any one of claims 57-67, wherein the first accessory oligonucleotide or the first polymer comprises a first functional moiety.~~

~~72. The method of any one of claims 57-67, wherein the second accessory oligonucleotide or the second polymer comprises a second functional moiety.~~

~~73. The method of any one of claims 57-73, wherein the first functional moiety of the first accessory oligonucleotide or the first polymer and the second functional moiety of the second accessory oligonucleotide or the second polymer are identical.~~

~~74. The method of claim 73, wherein the first functional moiety of the first accessory oligonucleotide or the first polymer is capable of reacting with the functional moiety of the first modified base of the first nucleotide to form a covalent linkage.~~

~~75. The method of any one of claims 73-74, wherein the second functional moiety of the second accessory oligonucleotide or the second polymer is capable of reacting with the functional moiety of the first modified base of the first nucleotide to form a covalent linkage.~~

~~76. The method of any one of claims 71-75, wherein the first functional moiety of the first accessory oligonucleotide or the first polymer is capable of participating in a click chemistry reaction.~~

~~77. The method of any one of claims 71-76, wherein the second functional moiety of the second accessory oligonucleotide or the second polymer is capable of participating in a click chemistry reaction.~~

~~78. The method of any one of claims 76-77, wherein the first functional moiety of the first accessory oligonucleotide or the first polymer is capable of participating in a click chemistry reaction with the functional moiety of the first modified base of the first nucleotide.~~

~~79. The method of any one of claims 76-78, wherein the second functional moiety of~~

the second accessory oligonucleotide or the second polymer is capable of participating in a click chemistry reaction with the functional moiety of the first modified base of the first nucleotide.

~~80. The method of any one of claims 76-79, wherein one or more of the first functional moiety of the first accessory oligonucleotide or the first polymer, the second functional moiety of the second accessory oligonucleotide or the second polymer, the functional moiety of the first modified base of the first nucleoside triphosphate, and the functional moiety of the first nucleotide are independently an azide, an alkynyl, an alkenyl, a thiol, or a nitron.~~

~~81. The method of any one of claims 76-80, wherein the functional moiety of the first modified base of the first nucleotide and the first functional moiety of the first accessory oligonucleotide or the first polymer, or the functional moiety of the first modified base of the first nucleotide and the second functional moiety of the second accessory oligonucleotide or the second polymer, or both, are selected from the following pairs:~~

- ~~(i) azido/alkynyl;~~
- ~~(ii) alkynyl/azido;~~
- ~~(iii) thiol/alkynyl;~~
- ~~(iv) alkynyl/thiol;~~
- ~~(v) alkenyl/thiol;~~
- ~~(vi) thiol/alkenyl;~~
- ~~(vii) azido/cyclooctynyl;~~
- ~~(viii) cyclooctynyl/azido;~~
- ~~(ix) nitron/cyclooctynyl; and~~
- ~~(x) cyclooctynyl/nitron.~~

~~82. The method of any one of claims 76-81, wherein the functional moiety of the first modified base of the first nucleotide is an azido, and wherein one or more of the first functional moiety of the first accessory oligonucleotide or the first polymer and the second functional moiety of the second accessory oligonucleotide or the second polymer is independently an alkynyl.~~

~~83. The method of any one of claims 76-82, wherein the click chemistry reaction comprises copper catalyzed azide-alkyne cycloaddition (CuAAC), and wherein the covalent linkage comprises a triazolyl.~~

84. ~~The method of claim 83, wherein the CuAAC comprises a Cu(I) stabilizing ligand.~~

85. ~~The method of claim 84, wherein the Cu(I) stabilizing ligand is selected from the group consisting of: 3-[4-((bis[(1-*tert*-butyl-1H-1,2,3-triazol-4-yl)methyl]amino)methyl)-1H-1,2,3-triazol-1-yl]propanol (BTTP), 3-[4-((bis[(1-*tert*-butyl-1H-1,2,3-triazol-4-yl)methyl]amino)methyl)-1H-1,2,3-triazol-1-yl]propyl hydrogen sulfate (BTTPS), 2-[4-((bis[(1-*tert*-butyl-1H-1,2,3-triazol-4-yl)methyl]amino)methyl)-1H-1,2,3-triazol-1-yl]ethyl hydrogen sulfate (BTTES), 2-[4-((bis[(1-*tert*-butyl-1H-1,2,3-triazol-4-yl)methyl]amino)methyl)-1H-1,2,3-triazol-1-yl]acetic acid (BTTAA), bathophenanthroline disulfonate disodium salt (BPS), N,N,N',N'',N''-Pentamethyldiethylenetriamine (PMDETA), tris-((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA), Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA), N^ε-((1R,2R)-2-azidocyclopentyl)oxy)carbonyl)-L-lysine (ACPK), and 4-*N,N*-dimethyl-amino-1,8-naphthalimide (4-DMN).~~

86. ~~The method of any one of claims 76-82, wherein the click chemistry reaction comprises strain-promoted azide-alkyne cycloaddition (SPAAC), and wherein the covalent linkage comprises a cycloocta-triazolyl.~~

87. ~~The method of any one of claims 76-82, wherein the click chemistry reaction comprises alkyne hydrothiolation, and wherein the covalent linkage comprises an alkenyl sulfide.~~

88. ~~The method of any one of claims 76-82, wherein the click chemistry reaction comprises alkene hydrothiolation, and wherein the covalent linkage comprises an alkyl sulfide.~~

89. ~~The method of any one of claims 76-82, wherein the click chemistry reaction comprises strain-promoted alkyne-nitrone cycloaddition (SPANC), and wherein the covalent linkage comprises an octahydrocycloocta-isoxazolyl.~~

90. ~~The method of claim 89, wherein the cyclooctynyl is dibenzylcyclooctyne (DBCO) or a derivative thereof.~~

91. ~~The method of any one of claims 76-90, wherein the click chemistry reaction is biocompatible.~~

~~92. The method of any one of claims 57-91, wherein the second temperature is about 20°C to about 65°C.~~

~~93. The method of any one of claims 57-91, wherein the second temperature is less than 0°C.~~

~~94. The method of any one of claims 57-91, wherein the second temperature is about -4°C to about -20°C.~~

~~95. The method of any one of claims 57-94, wherein the second reaction time is at least 1 second.~~

~~96~~20. The method of ~~claim 59-95~~ claim 19, further comprising:

(c) providing the nucleic acid template comprising the first adapter sequence, or a reverse complement thereof, the second adapter sequence, or a reverse complement thereof, and a nucleic acid hybridization sequence capable of hybridizing to the template capture site on the third nucleic acid;

(d) contacting the third nucleic acid with the nucleic acid template to generate the third nucleic acid with the nucleic acid template hybridized to the template capture site on the third nucleic acid via the nucleic acid hybridization sequence of the nucleic acid template;

(e) performing amplification on the third nucleic acid hybridized with the nucleic acid template to generate a fourth nucleic acid comprising the third nucleic acid attached to one or more of the first accessory oligonucleotides and one or more of the second accessory oligonucleotides extended to comprise a sequence of the nucleic acid template, or a reverse complement thereof; and

(f) determining the sequence of the nucleic acid template using the fourth nucleic acid.

~~97. The method of claim 96, wherein the nucleic acid hybridization sequence of the nucleic acid template comprises a reverse complement of the template capture site.~~

~~98. The method of any one of claims 8-97, wherein the first nucleic acid, the second nucleic acid, the third nucleic acid, and/or the fourth nucleic acid comprise a third polymer.~~

99. ~~The method of any one of claims 1-98, wherein the amino acid substitution mutation at the position functionally equivalent to Glu191 comprises a nonpolar amino acid, a polar amino acid, a positively charged amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

100. ~~The method of any one of claims 1-98, wherein the amino acid substitution mutation at the position functionally equivalent to Glu191 in the *Bos taurus* TdT of SEQ ID NO: 12 is Glu191Ala, Glu191Arg, Glu191Asn, Glu191Asp, Glu191Cys, Glu191Gln, Glu191Gly, Glu191His, Glu191Ile, Glu191Leu, Glu191Lys, Glu191Met, Glu191Phe, Glu191Pro, Glu191Ser, Glu191Thr, Glu191Trp, Glu191Tyr, or Glu191Val.~~

101. ~~The method of any one of claims 1-98, wherein the amino acid substitution mutation at the position functionally equivalent to Glu191 in the *Bos taurus* TdT of SEQ ID NO: 12 is Glu191Val.~~

102. ~~The method of any one of claims 1-101, wherein the amino acid substitution mutation at the position functionally equivalent to Lys193 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a polar amino acid, a positively charged amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

103. ~~The method of any one of claims 1-101, wherein the amino acid substitution mutation at the position functionally equivalent to Lys193 in the *Bos taurus* TdT of SEQ ID NO: 12 is Lys193Ala, Lys193Arg, Lys193Asn, Lys193Asp, Lys193Cys, Lys193Gln, Lys193Glu, Lys193Gly, Lys193His, Lys193Ile, Lys193Leu, Lys193Met, Lys193Phe, Lys193Pro, Lys193Ser, Lys193Thr, Lys193Trp, Lys193Tyr, or Lys193Val.~~

104. ~~The method of any one of claims 1-101, wherein the amino acid substitution mutation at the position functionally equivalent to Lys193 in the *Bos taurus* TdT of SEQ ID NO: 12 is Lys193Asn.~~

105. ~~The method of any one of claims 1-104, wherein the amino acid substitution mutation at the position functionally equivalent to Glu194 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a polar amino acid, a positively charged~~

~~amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

~~106. The method of any one of claims 1-104, wherein the amino acid substitution mutation at the position functionally equivalent to Glu194 in the *Bos taurus* TdT of SEQ ID NO: 12 is Glu194Ala, Glu194Arg, Glu194Asn, Glu194Asp, Glu194Cys, Glu194Gln, Glu194Gly, Glu194His, Glu194Ile, Glu194Leu, Glu194Lys, Glu194Met, Glu194Phe, Glu194Pro, Glu194Ser, Glu194Thr, Glu194Trp, Glu194Tyr, or Glu194Val.~~

~~107. The method of any one of claims 1-104, wherein the amino acid substitution mutation at the position functionally equivalent to Glu194 in the *Bos taurus* TdT of SEQ ID NO: 12 is Glu194Gly.~~

~~108. The method of any one of claims 1-107, wherein the amino acid substitution mutation at the position functionally equivalent to Asp242 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a polar amino acid, a positively charged amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

~~109. The method of any one of claims 1-107, wherein the amino acid substitution mutation at the position functionally equivalent to Asp242 in the *Bos taurus* TdT of SEQ ID NO: 12 is Asp242Ala, Asp242Arg, Asp242Asn, Asp242Cys, Asp242Gln, Asp242Glu, Asp242Gly, Asp242His, Asp242Ile, Asp242Leu, Asp242Lys, Asp242Met, Asp242Phe, Asp242Pro, Asp242Ser, Asp242Thr, Asp242Trp, Asp242Tyr, or Asp242Val.~~

~~110. The method of any one of claims 1-107, wherein the amino acid substitution mutation at the position functionally equivalent to Asp242 in the *Bos taurus* TdT of SEQ ID NO: 12 is Asp242Tyr.~~

~~111. The method of any one of claims 1-110, wherein the amino acid substitution mutation at the position functionally equivalent to Lys287 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a polar amino acid, a positively charged amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

~~112. The method of any one of claims 1-110, wherein the amino acid substitution mutation at the position functionally equivalent to Lys287 in the *Bos taurus* TdT of SEQ ID NO: 12 is Lys287Ala, Lys287Arg, Lys287Asn, Lys287Asp, Lys287Cys, Lys287Gln, Lys287Glu, Lys287Gly, Lys287His, Lys287Ile, Lys287Leu, Lys287Met, Lys287Phe, Lys287Pro, Lys287Ser, Lys287Thr, Lys287Trp, Lys287Tyr, or Lys287Val.~~

~~113. The method of any one of claims 1-110, wherein the amino acid substitution mutation at the position functionally equivalent to Lys287 in the *Bos taurus* TdT of SEQ ID NO: 12 is Lys287Glu.~~

~~114. The method of any one of claims 1-113, wherein the amino acid substitution mutation at the position functionally equivalent to Phe296 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a hydrophobic amino acid, an aliphatic amino acid, or a branched chain amino acid.~~

~~115. The method of any one of claims 1-113, wherein the amino acid substitution mutation at the position functionally equivalent to Phe296 in the *Bos taurus* TdT of SEQ ID NO: 12 is Phe296Ala, Phe296Arg, Phe296Asn, Phe296Asp, Phe296Cys, Phe296Gln, Phe296Glu, Phe296Gly, Phe296His, Phe296Ile, Phe296Leu, Phe296Lys, Phe296Met, Phe296Pro, Phe296Ser, Phe296Thr, Phe296Trp, Phe296Tyr, or Phe296Val.~~

~~116. The method of any one of claims 1-113, wherein the amino acid substitution mutation at the position functionally equivalent to Phe296 in the *Bos taurus* TdT of SEQ ID NO: 12 is Phe296Leu.~~

~~117. The method of any one of claims 1-116, wherein the amino acid substitution mutation at the position functionally equivalent to Met299 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a hydrophobic amino acid, an aliphatic amino acid, or a branched chain amino acid.~~

~~118. The method of any one of claims 1-116, wherein the amino acid substitution mutation at the position functionally equivalent to Met299 in the *Bos taurus* TdT of SEQ ID NO: 12 is Met299Ala, Met299Arg, Met299Asn, Met299Asp, Met299Cys, Met299Gln, Met299Glu, Met299Gly, Met299His, Met299Ile, Met299Leu, Met299Lys, Met299Phe, Met299Pro, Met299Ser, Met299Thr, Met299Trp, Met299Tyr, or Met299Val.~~

~~119. The method of any one of claims 1-116, wherein the amino acid substitution mutation at the position functionally equivalent to Met299 in the *Bos taurus* TdT of SEQ ID NO: 12 is Met299Lys.~~

~~120. The method of any one of claims 1-119, wherein the amino acid substitution mutation at the position functionally equivalent to Thr342 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a hydrophobic amino acid, an aliphatic amino acid, or a branched chain amino acid.~~

~~121. The method of any one of claims 1-119, wherein the amino acid substitution mutation at the position functionally equivalent to Thr342 in the *Bos taurus* TdT of SEQ ID NO: 12 is Thr342Ala, Thr342Arg, Thr342Asn, Thr342Asp, Thr342Cys, Thr342Gln, Thr342Glu, Thr342Gly, Thr342His, Thr342Ile, Thr342Leu, Thr342Lys, Thr342Met, Thr342Phe, Thr342Pro, Thr342Ser, Thr342Trp, Thr342Tyr, or Thr342Val.~~

~~122. The method of any one of claims 1-119, wherein the amino acid substitution mutation at the position functionally equivalent to Thr342 in the *Bos taurus* TdT of SEQ ID NO: 12 is Thr342Ser.~~

~~123. The method of any one of claims 1-122, wherein the amino acid substitution mutation at the position functionally equivalent to His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a hydrophobic amino acid, an aliphatic amino acid, or a branched chain amino acid.~~

~~124. The method of any one of claims 1-122, wherein the amino acid substitution mutation at the position functionally equivalent to His421 in the *Bos taurus* TdT of SEQ ID NO: 12 is His421Ala, His421Arg, His421Asn, His421Asp, His421Cys, His421Gln, His421Glu, His421Gly, His421Ile, His421Leu, His421Lys, His421Met, His421Phe, His421Pro, His421Ser, His421Thr, His421Trp, His421Tyr, or His421Val.~~

~~125. The method of any one of claims 1-122, wherein the amino acid substitution mutation at the position functionally equivalent to His421 in the *Bos taurus* TdT of SEQ ID NO: 12 is His421Pro.~~

~~126.—The method of any one of claims 1-125, wherein the recombinant TdT comprises two or more amino acid substitution mutations at two or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

~~127.—The method of claim 126, wherein the two or more amino acid substitution mutations at the two or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise two or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

~~128.—The method of any one of claims 1-125, wherein the recombinant TdT comprises three or more amino acid substitution mutations at three or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

~~129.—The method of claim 128, wherein the three or more amino acid substitution mutations at the three or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise three or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

~~130.—The method of any one of claims 1-125, wherein the recombinant TdT comprises four or more amino acid substitution mutations at four or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

~~131.—The method of claim 130, wherein the four or more amino acid substitution mutations at the four or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise four or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

~~132.—The method of any one of claims 1-125, wherein the recombinant TdT comprises five or more amino acid substitution mutations at five or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos*~~

~~*taurus* TdT of SEQ ID NO: 12.~~

~~133. The method of claim 132, wherein the five or more amino acid substitution mutations at the five or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise five or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

~~134. The method of any one of claims 1-125, wherein the recombinant TdT comprises six or more amino acid substitution mutations at six or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

~~135. The method of claim 134, wherein the six or more amino acid substitution mutations at the six or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise six or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

~~136. The method of any one of claims 1-125, wherein the recombinant TdT comprises seven or more amino acid substitution mutations at seven or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

~~137. The method of claim 136, wherein the seven or more amino acid substitution mutations at the seven or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise seven or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

~~138. The method of any one of claims 1-125, wherein the recombinant TdT comprises eight or more amino acid substitution mutations at eight or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

~~139. The method of claim 138, wherein the eight or more amino acid substitution mutations at the eight or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise eight or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

~~140. The method of any one of claims 1-125, wherein the recombinant TdT comprises eight amino acid substitution mutations at eight positions functionally equivalent to Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

~~141. The method of claim 140, wherein the eight amino acid substitution mutations at the eight positions functionally equivalent to Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 are Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

~~142. The method of any one of claims 1-125, wherein the recombinant TdT comprises nine amino acid substitution mutations at positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

~~143. The method of claim 142, wherein the nine amino acid substitution mutations at the positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 are Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

~~144. The method of any one of claims 1-142, wherein the recombinant TdT comprises an amino acid sequence that is at least 85% identical to SEQ ID NO: 1.~~

~~145. The method of any one of claims 1-142, wherein the recombinant TdT comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 1.~~

~~146. The method of any one of claims 1-142, wherein the recombinant TdT comprises~~

an amino acid sequence that is at least 95% identical to SEQ ID NO: 1.

~~147.—The method of any one of claims 1-142, wherein the recombinant TdT comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 11.~~

~~148.—The method of any one of claims 1-142, wherein the recombinant TdT comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 12.~~

~~149.—The method of any one of claims 1-148, wherein the recombinant TdT is stable at a temperature of 47 °C or higher.~~

~~150.—The method of any one of claims 1-148, wherein the recombinant TdT is stable at a temperature of 50 °C or higher.~~

~~151.—The method of any one of claims 1-148, wherein the recombinant TdT is stable at a temperature of 55 °C or higher.~~

~~152.—The method of any one of claims 1-148, wherein the recombinant TdT is stable at a temperature of 58 °C or higher.~~

~~153.—The method of any one of claims 1-152, wherein the terminal deoxynucleotidyl transferase activity of the recombinant TdT is at least 80%, 85%, 90%, 95%, 100%, 105%, 110%, 115%, or 120%, of the terminal deoxynucleotidyl transferase activity of the *Bos taurus* TdT of SEQ ID NO: 12 at a same test temperature.~~

~~154.—The method of claim 153, wherein the test temperature is 37 °C, 47 °C, 50 °C, 55 °C, or 58 °C.~~

~~155.—The method of any one of claims 1-154, wherein the recombinant TdT comprises a small ubiquitin-like modifier (SUMO) fragment.~~

~~156.—The method of claim 155, wherein the SUMO fragment comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 13.~~

~~157.—The method of any one of claims 155-156, wherein the recombinant TdT~~

comprises the SUMO fragment on the N-terminus of the recombinant TdT.

~~158.—The method of claim 156, wherein the recombinant TdT comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 14.~~

~~159.—The method of claim 156, wherein the recombinant TdT comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 15.~~

~~The method of any one of claims 155-156, wherein the recombinant TdT comprises the SUMO fragment on the C-terminus of the recombinant TdT.~~

~~160.—A second nucleic acid obtained by the method of any one of claims 8-159.~~

~~161.—A third nucleic acid obtained by the method of any one of claims 57-159.~~

~~162.—A fourth nucleic acid obtained by the method of any one of claims claim 96-159.~~