

ribonucleotide, a deoxyribonucleotide, or a combination thereof, is used in combination with a substrate polynucleotide that is either DNA or RNA.

[0149] In certain instances, the art uses the term "nucleobase" which embraces naturally-occurring nucleotides as well as modifications of nucleotides that can be polymerized. Thus, nucleotide or nucleobase means the naturally occurring nucleobases adenine (A), guanine (G), cytosine (C), thymine (T) and uracil (U) as well as non-naturally occurring nucleobases such as xanthine, diaminopurine, 8-oxo-N6-methyladenine, 7-deazaxanthine, 7-deazaguanine, N4,N4-ethanocytosin, N',N'-ethano-2,6-diaminopurine, 5-methylcytosine (mC), 5-(C₃—C₆)-alkynyl-cytosine, 5-fluorouracil, 5-bromouracil, pseudoisocytosine, 2-hydroxy-5-methyl-4-triazolopyridin, isocytosine, isoguanine, inosine and the "non-naturally occurring" nucleobases described in Benner *et al.*, U.S. Pat. No. 5,432,272 and Susan M. Freier and Karl-Heinz Altmann, 1997, *Nucleic Acids Research*, vol. 25: pp 4429-4443. The term "nucleobase" also includes not only the known purine and pyrimidine heterocycles, but also heterocyclic analogues and tautomers thereof. Further naturally and non-naturally occurring nucleobases include those disclosed in U.S. Pat. No. 3,687,808 (Merigan, *et al.*), in Chapter 15 by Sanghvi, in *Antisense Research and Application*, Ed. S. T. Crooke and B. Lebleu, CRC Press, 1993, in Englisch *et al.*, 1991, *Angewandte Chemie, International Edition*, 30: 613-722 (see especially pages 622 and 623, and in the *Concise Encyclopedia of Polymer Science and Engineering*, J. I. Kroschwitz Ed., John Wiley & Sons, 1990, pages 858-859, Cook, *Anti-Cancer Drug Design* 1991, 6, 585-607; each of which are hereby incorporated by reference in their entirety). In various aspects, polynucleotides also include one or more "nucleosidic bases" or "base units" which include compounds such as heterocyclic compounds that can serve like nucleobases, including certain "universal bases" that are not nucleosidic bases in the most classical sense but serve as nucleosidic bases. Universal bases include 3-nitropyrrole, optionally substituted indoles (*e.g.*, 5-nitroindole), and optionally substituted hypoxanthine. Other desirable universal bases include, pyrrole, diazole or triazole derivatives, including those universal bases known in the art.

[0150] Polynucleotides may also include modified nucleobases. A "modified base" is understood in the art to be one that can pair with a natural base (*e.g.*, adenine, guanine, cytosine, uracil, and/or thymine) and/or can pair with a non-naturally occurring base. Exemplary modified bases are described in EP 1 072 679 and WO 97/12896, the disclosures of which are incorporated herein by reference. Modified nucleobases include without limitation, 5-

methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further modified bases include tricyclic pyrimidines such as phenoxazine cytidine(1H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido[5,4-b][1,4]benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (*e.g.* 9-(2-aminoethoxy)-H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5-b]indol-2-one), pyridoindole cytidine (H-pyrido[3',2':4,5]pyrrolo[2,3-d]pyrimidin-2-one). Modified bases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Additional nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. I., ed. John Wiley & Sons, 1990, those disclosed by Englisch *et al.*, 1991, Angewandte Chemie, International Edition, 30: 613, and those disclosed by Sanghvi, Y. S., Chapter 15, Antisense Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., ed., CRC Press, 1993. Certain of these bases are useful for increasing the binding affinity and include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2° C and are, in certain aspects combined with 2'-O-methoxyethyl sugar modifications. See, U.S. Pat. Nos. 3,687,808, U.S. Pat. Nos. 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,645,985; 5,830,653; 5,763,588; 6,005,096; 5,750,692 and 5,681,941, the disclosures of which are incorporated herein by reference.

[0151] Methods of making polynucleotides of a predetermined sequence are well-known. See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd ed. 1989) and F. Eckstein (ed.) *Oligonucleotides and Analogues*, 1st Ed. (Oxford University Press, New York, 1991). Solid-phase synthesis methods are preferred for both polyribonucleotides and polydeoxyribonucleotides (the well-known methods of synthesizing DNA are also useful for synthesizing RNA). Polyribonucleotides can also be prepared enzymatically. Non-naturally occurring nucleobases can be incorporated into the polynucleotide, as well. See, *e.g.*, U.S. Patent No. 7,223,833; Katz, *J. Am. Chem. Soc.*, 74:2238 (1951); Yamane, *et al.*, *J. Am. Chem. Soc.*, 83:2599 (1961); Kosturko, *et al.*, *Biochemistry*, 13:3949 (1974); Thomas, *J. Am. Chem. Soc.*, 76:6032 (1954); Zhang, *et al.*, *J. Am. Chem. Soc.*, 127:74-75 (2005); and Zimmermann, *et al.*, *J. Am. Chem. Soc.*, 124:13684-13685 (2002).

MODIFIED POLYNUCLEOTIDES

[0152] Modified polynucleotides are contemplated for use in an attenuator molecule or in a substrate polynucleotide wherein both one or more sugar and/or one or more internucleotide linkage of the nucleotide units in the polynucleotide is replaced with "non-naturally occurring" groups. In one aspect, this embodiment contemplates a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of a polynucleotide is replaced with an amide containing backbone. See, for example US Patent Nos. 5,539,082; 5,714,331; and 5,719,262, and Nielsen *et al.*, *Science*, 1991, 254, 1497-1500, ~~the disclosures of which are herein incorporated by reference.~~

[0153] Other linkages between nucleotides and unnatural nucleotides contemplated for the disclosed polynucleotides include those described in U.S. Patent Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,792,747; and 5,700,920; U.S. Patent Publication No. 20040219565; International Patent Publication Nos. WO 98/39352 and WO 99/14226; Mesmaeker *et. al.*, *Current Opinion in Structural Biology* 5:343-355 (1995) and Susan M. Freier and Karl-Heinz Altmann, *Nucleic Acids Research*, 25:4429-4443 (1997), ~~the disclosures of which are incorporated herein by reference.~~

[0154] Specific examples of polynucleotides include those containing modified backbones or non-natural internucleoside linkages. Polynucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. Modified polynucleotides that do not have a phosphorus atom in their internucleoside backbone are considered to be within the meaning of "polynucleotide."

[0155] Modified polynucleotide backbones containing a phosphorus atom include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage. Also contemplated are polynucleotides having inverted polarity comprising a single 3' to 3' linkage at the 3'-most internucleotide linkage, *i.e.* a single inverted nucleoside residue which may be abasic (the nucleotide is missing or has a hydroxyl group in place thereof). Salts, mixed salts and free acid forms are also contemplated.

[0156] Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,194,599; 5,565,555; 5,527,899; 5,721,218; 5,672,697 and 5,625,050, the disclosures of which are incorporated by reference herein.

[0157] Modified polynucleotide backbones that do not include a phosphorus atom have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages; siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; riboacetyl

backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts. In still other embodiments, polynucleotides are provided with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and including —CH₂—NH—O—CH₂—, —CH₂—N(CH₃)—O—CH₂—, —CH₂—O—N(CH₃)—CH₂—, —CH₂—N(CH₃)—N(CH₃)—CH₂— and —O—N(CH₃)—CH₂—CH₂— described in US Patent Nos. 5,489,677, and 5,602,240. See, for example, U.S. Patent Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; 5,792,608; 5,646,269 and 5,677,439, the disclosures of which are incorporated herein by reference in their entireties.

[0158] In various forms, the linkage between two successive monomers in the polynucleotide consists of 2 to 4, desirably 3, groups/atoms selected from —CH₂—, —O—, —S—, —NRH—, >C=O, >C=NRH, >C=S, —Si(R'')₂—, —SO—, —S(O)₂—, —P(O)₂—, —PO(BH₃)—, —P(O,S)—, —P(S)₂—, —PO(R'')—, —PO(OCH₃)—, and —PO(NHRH)—, where RH is selected from hydrogen and C1-4-alkyl, and R'' is selected from C1-6-alkyl and phenyl.

Illustrative examples of such linkages are —CH₂—CH₂—CH₂—, —CH₂—CO—CH₂—, —CH₂—CHOH—CH₂—, —O—CH₂—O—, —O—CH₂—CH₂—, —O—CH₂—CH= (including R₅ when used as a linkage to a succeeding monomer), —CH₂—CH₂—O—, —NRH—CH₂—CH₂—, —CH₂—CH₂—NRH—, —CH₂—NRH—CH₂—, —O—CH₂—CH₂—NRH—, —NRH—CO—O—, —NRH—CO—NRH—, —NRH—CS—NRH—, —NRH—C(=NRH)—NRH—, —NRH—CO—CH₂—NRH—O—CO—O—, —O—CO—CH₂—O—, —O—CH₂—CO—O—, —CH₂—CO—NRH—, —O—CO—NRH—, —NRH—CO—CH₂—, —O—CH₂—CO—NRH—, —O—CH₂—CH₂—NRH—, —CH=N—O—, —CH₂—NRH—O—, —CH₂—O—N= (including R₅ when used as a linkage to a succeeding monomer), —CH₂—O—NRH—, —CO—NRH—CH₂—, —CH₂—NRH—O—, —CH₂—NRH—CO—, —O—NRH—CH₂—, —O—NRH—, —O—CH₂—S—, —S—CH₂—O—, —CH₂—CH₂—S—, —O—CH₂—CH₂—S—, —S—CH₂—CH= (including R₅ when used as a linkage to a succeeding monomer), —S—CH₂—CH₂—, —S—CH₂—CH₂—O—, —S—CH₂—CH₂—S—, —CH₂—S—CH₂—, —CH₂—SO—CH₂—, —CH₂—SO₂—CH₂—, —O—SO—O—, —O—S(O)₂—O—, —O—

$\text{S(O)}_2\text{—CH}_2\text{—}$, $\text{—O—S(O)}_2\text{—NRH—}$, $\text{—NRH—S(O)}_2\text{—CH}_2\text{—}$; $\text{—O—S(O)}_2\text{—CH}_2\text{—}$, $\text{—O—P(O)}_2\text{—O—}$, —O—P(O,S)—O— , $\text{—O—P(S)}_2\text{—O—}$, $\text{—S—P(O)}_2\text{—O—}$, —S—P(O,S)—O— , $\text{—S—P(S)}_2\text{—O—}$, $\text{—O—P(O)}_2\text{—S—}$, —O—P(O,S)—S— , $\text{—O—P(S)}_2\text{—S—}$, $\text{—S—P(O)}_2\text{—S—}$, —S—P(O,S)—S— , $\text{—S—P(S)}_2\text{—S—}$, —O—PO(R'')—O— , $\text{—O—PO(OCH}_3\text{)—O—}$, $\text{—O—PO(O—CH}_2\text{CH}_3\text{)—O—}$, $\text{—O—PO(O—CH}_2\text{CH}_2\text{S—R)—O—}$, $\text{—O—PO(BH}_3\text{)—O—}$, —O—PO(NHRN)—O— , $\text{—O—P(O)}_2\text{—NRH—}$, $\text{—NRH—P(O)}_2\text{—O—}$, —O—P(O,NRH)—O— , $\text{—CH}_2\text{—P(O)}_2\text{—O—}$, $\text{—O—P(O)}_2\text{—CH}_2\text{—}$, and $\text{—O—Si(R'')}_2\text{—O—}$; among which $\text{—CH}_2\text{—CO—NRH—}$, $\text{—CH}_2\text{—NRH—O—}$, $\text{—S—CH}_2\text{—O—}$, $\text{—O—P(O)}_2\text{—O—O—P(O,S)—O—}$, $\text{—O—P(S)}_2\text{—O—}$, $\text{—NRH—P(O)}_2\text{—O—}$, —O—P(O,NRH)—O— , —O—PO(R'')—O— , $\text{—O—PO(CH}_3\text{)—O—}$, and —O—PO(NHRN)—O— , where RH is selected from hydrogen and C1-4-alkyl, and R'' is selected from C1-6-alkyl and phenyl, are contemplated. Further illustrative examples are given in Mesmaeker et. al., 1995, Current Opinion in Structural Biology, 5: 343-355 and Susan M. Freier and Karl-Heinz Altmann, 1997, Nucleic Acids Research, vol 25: pp 4429-4443.

[0159] Still other modified forms of polynucleotides are described in detail in U.S. Patent Application No. 20040219565, the disclosure of which is incorporated by reference herein in its entirety.

[0160] Modified polynucleotides may also contain one or more substituted sugar moieties. In certain aspects, polynucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Other embodiments include $\text{O}[(\text{CH}_2)_n\text{O}]_m\text{CH}_3$, $\text{O}(\text{CH}_2)_n\text{OCH}_3$, $\text{O}(\text{CH}_2)_n\text{NH}_2$, $\text{O}(\text{CH}_2)_n\text{CH}_3$, $\text{O}(\text{CH}_2)_n\text{ONH}_2$, and $\text{O}(\text{CH}_2)_n\text{ON}[(\text{CH}_2)_n\text{CH}_3]_2$, where n and m are from 1 to about 10. Other polynucleotides comprise one of the following at the 2' position: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of a polynucleotide, or a group for improving the pharmacodynamic properties of a polynucleotide, and other substituents having similar properties. In one aspect, a modification includes 2'-methoxyethoxy (2'-O-CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin

et al., 1995, *Helv. Chim. Acta*, 78: 486-504) *i.e.*, an alkoxyalkoxy group. Other modifications include 2'-dimethylaminooxyethoxy, *i.e.*, a $\text{O}(\text{CH}_2)_2\text{ON}(\text{CH}_3)_2$ group, also known as 2'-DMAOE, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethyl-amino-ethoxy-ethyl or 2'-DMAEOE), *i.e.*, $2'\text{-O}-\text{CH}_2-\text{O}-\text{CH}_2-\text{N}(\text{CH}_3)_2$.

[0161] Still other modifications include 2'-methoxy ($2'\text{-O}-\text{CH}_3$), 2'-aminopropoxy ($2'\text{-OCH}_2\text{CH}_2\text{CH}_2\text{NH}_2$), 2'-allyl ($2'\text{-CH}_2-\text{CH}=\text{CH}_2$), 2'-O-allyl ($2'\text{-O}-\text{CH}_2-\text{CH}=\text{CH}_2$) and 2'-fluoro (2'-F). The 2'-modification may be in the arabino (up) position or ribo (down) position. In one aspect, a 2'-arabino modification is 2'-F. Similar modifications may also be made at other positions on the polynucleotide, for example, at the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked polynucleotides and the 5' position of 5' terminal nucleotide. Polynucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. See, for example, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,792,747; and 5,700,920, the disclosures of which are incorporated by reference in their entireties herein.

[0162] Further modifications include those that extend the genetic code such as, without limitation, Iso-dC and Iso-dG. Iso-dC and Iso-dG are chemical variants of cytosine and guanine, respectively. Iso-dC will hydrogen bond with Iso-dG but not with dG. Similarly, Iso-dG will base pair with Iso-dC but not with dC [Switzer *et al.*, *Biochemistry* 32:10489-96 (1993)]. In these aspects, controlled tailing by addition of Iso-dC bases is achieved by using a poly (iso-dG) attenuator molecule and vice versa.

[0163] In one aspect, a modification of the sugar includes Locked Nucleic Acids (LNAs) in which the 2'-hydroxyl group is linked to the 3' or 4' carbon atom of the sugar ring, thereby forming a bicyclic sugar moiety. The linkage is in certain aspects a methylene ($-\text{CH}_2-$)_n group bridging the 2' oxygen atom and the 4' carbon atom wherein n is 1 or 2. LNAs and preparation thereof are described in WO 98/39352 and WO 99/14226, the disclosures of which are incorporated herein by reference.

LABELS

WHAT IS CLAIMED IS:

1. A composition comprising a template-independent nucleic acid polymerase, an attenuator molecule that is a polynucleotide, and nucleotides complementary to the attenuator molecule.
2. The composition of claim 1 wherein the nucleic acid polymerase is a terminal deoxynucleotidyl transferase (TdT).
3. The composition of claim 1 wherein the nucleic acid polymerase is an RNA-specific nucleotidyl transferase selected from the group consisting of poly(A) polymerase and poly(U) polymerase.
4. The composition of any of the preceding claims wherein the attenuator molecule comprises a nucleotide selected from the group consisting of 2'-deoxythymidine 5'-monophosphate (dTMP), 2'-deoxyguanosine 5'-monophosphate (dGMP), 2'-deoxyadenosine 5'-monophosphate (dAMP), 2'-deoxycytidine 5'-monophosphate (dCMP), 2'-deoxyuridine 5'-monophosphate (dUMP), thymidine monophosphate (TMP), guanosine monophosphate (GMP), adenosine monophosphate (AMP), cytidine monophosphate (CMP), uridine monophosphate (UMP), a modified base, and combinations thereof.
5. The composition of any of the preceding claims wherein the attenuator molecule comprises 10 nucleotides, 20 nucleotides, 30 nucleotides, 50 nucleotides, or 100 nucleotides.
6. The composition of any of the preceding claims wherein the attenuator molecule comprises a 3' blocking group selected from the group consisting of at least one ribonucleotide, at least one deoxynucleotide, a C3 spacer, a phosphate, a dideoxynucleotide, an amino group, and an inverted deoxythymidine.
7. The composition of any of the preceding claims wherein the attenuator molecule comprises a degradable base.
8. The composition of any of the preceding claims wherein the attenuator molecule is an attenuator-adaptor molecule that comprises a single-stranded attenuator sequence and further comprises a partially double-stranded adaptor sequence W positioned 5' to the attenuator sequence and is complementary to a sequence X, wherein the adaptor comprises a 5' phosphate.

9. The composition of claim 8 wherein sequence X is on a separate polynucleotide.
10. The composition of any of the preceding claims wherein a DNA ligase or an RNA ligase is present.
11. The composition of claim 10 further comprising a primer, a polymerase and an adaptor molecule comprising a sequence Y complementary to a sequence V, wherein sequence V is the same length as Y or is less than the same length as sequence Y, the adaptor molecule being a separate molecule from the attenuator-adaptor molecule.
12. The composition of claim 9 wherein the attenuator-adaptor molecule is immobilized.
13. The composition of any one of claims 8-12 wherein the attenuator portion of the attenuator-adaptor molecule comprises (1) a homopolymeric sequence selected from the group consisting of poly (dA), poly (dT), poly (dC), poly (dG), poly (dU), poly (rA), poly (U), poly (rC), poly (rG) and a heteropolymeric sequence comprising combinations of:
- (i) dA and rA bases,
 - (ii) dT, dU and U bases,
 - (iii) dC and rC bases, or
 - (iv) dG and rG bases,
- or the attenuator molecule comprises a dinucleotide sequence comprising a plurality of random sequences comprised of the following dinucleotide combinations (i) dG and dC; (ii) dA and dT; (iii) dG and dT; (iv) dG and dA; (v) dA and dC; or (vi) dC and dT,
- or the attenuator molecule comprises a dinucleotide sequence comprising a mixture of a ribonucleotide and a deoxyribonucleotide.
14. The composition of claim 8 wherein
- (i) the attenuator molecule comprises dU bases and incubation with a dU-glycosylase destabilizes the attenuator molecule, or incubation with a dU-glycosylase and subsequent incubation at a temperature that is above 80° C degrades the attenuator molecule, or the attenuator molecule is incubated with a mixture of dU-glycosylase and an apurinic/aprimidinic endonuclease;

(ii) the attenuator molecule comprises a ribonucleotide and is degradable with a ribonuclease under conditions sufficient for ribonuclease activity, wherein the ribonuclease is selected from the group consisting of RNase H, RNase HII, RNase A, and RNase T1; or

(iii) the attenuator molecule comprises deoxyribonucleotides and is degradable with a DNA-specific nuclease, optionally wherein the DNA-specific nuclease is DNase I.

15. A method of attenuated tailing and simultaneous adaptor ligation of a substrate polynucleotide comprising

mixing the composition of any one of claims 1 through 14 with the substrate polynucleotide, a nucleotide that is complementary to the attenuator molecule, and a ligase, and

incubating the mixture under conditions sufficient to allow addition of a tail sequence to the 3' end of the substrate polynucleotide; wherein the addition of the tail sequence allows association between the tail sequence and the attenuator molecule to form a complex, and ligating the adaptor sequence to the substrate polynucleotide.

16. The method of claim 15 wherein the substrate polynucleotide is

(i) a single stranded substrate polynucleotide;

(ii) a double stranded substrate polynucleotide; or

(iii) a partially double stranded and partially single stranded substrate polynucleotide,

wherein the double stranded substrate polynucleotide has a 3' overhanging end

(iv) bisulfite-treated or

(v) a product of a primer extension reaction.

17. The method of claim 16 wherein the substrate polynucleotide comprises a free 3' hydroxyl group.

18. The method of claim 16 wherein the attenuator molecule associates with the tail sequence during the process of adding the tail sequence, wherein association of the attenuator molecule to the tail sequence regulates addition of nucleotides to the substrate polynucleotide.

19. The method of claim 18 wherein the addition of the tail sequence to the substrate polynucleotide is temperature sensitive, and wherein the temperature is about 4° C, about 5° C, about 6° C, about 7° C, about 8° C, about 9° C, about 10° C, about 11° C, about 12° C, about 13° C, about 14° C, about 15° C, about 16° C, about 17° C, about 18° C, about 19° C, about 20° C, about 21° C, about 22° C, about 23° C, about 24° C, about 25° C, about 26° C, about 27° C, about 28° C, about 29° C, about 30° C, about 31° C, about 32° C, about 33° C, about 34° C, about 35° C, about 36° C, about 37° C, about 38° C, about 39° C, about 40° C, about 41° C, about 42° C, about 43° C, about 44° C, about 45° C, about 46° C, about 47° C, about 48° C, about 49° C, or about 50° C.

20. The method of claim 15 wherein the substrate polynucleotide is DNA or the substrate polynucleotide is RNA.

21. The method of any one of claims 15 through 20 wherein the attenuator molecule comprises at least 5 nucleotides and up to about 1000 nucleotides.

22. A method of attenuated tailing of a DNA substrate polynucleotide comprising mixing the DNA substrate polynucleotide with (i) TdT enzyme, (ii) an attenuator polynucleotide comprising a degradable base and a 3' blocking group and (iii) nucleotides that are complementary to the attenuator polynucleotide;

incubating the mixture at about 30° C to about 37° C for about 30 minutes; and destabilizing the attenuator molecule by adding (i) a DNA glycosylase, or (ii) an RNase.

23. The method of claim 22 further comprising the step of isolating the attenuated and tailed DNA substrate polynucleotide.

24. The method of claim 15 wherein the DNA substrate polynucleotide is immobilized after the attenuated tailing and simultaneous ligation reaction.

25. A kit comprising the composition of any of one of claims 1 through 14.

26. A method of attenuated tailing and simultaneous adaptor ligation of a substrate polynucleotide comprising:

(1) incubating a mixture comprising the substrate polynucleotide with (i) a template-independent polymerase enzyme; (ii) a composition comprising an attenuator molecule comprising an attenuator sequence and further comprises a sequence W positioned adjacent the attenuator sequence and is complementary to an adaptor sequence X; the composition further comprising an adaptor molecule comprising a sequence Y complementary to a sequence V, wherein sequence V is the same length as Y or is less than the same length as sequence Y, the adaptor molecule being a separate molecule from the attenuator-adaptor molecule; and (iii) deoxynucleotides that are complementary to the attenuator sequence of the attenuator molecule, under conditions that allow extension of the substrate polynucleotide to tail the substrate;

(2) ligating the adaptor sequence X to the substrate polynucleotide and dissociating the attenuator molecule from the separate polynucleotide;

(3) adding a primer complementary to a sequence in the substrate polynucleotide under conditions wherein the primer hybridizes to the substrate polynucleotide;

(4) adding a polymerase and deoxynucleotides to perform polymerase extension from the primer to produce a second strand polynucleotide complementary to the substrate polynucleotide and create a double stranded substrate molecule; and

(5) ligating the adaptor molecule to the double stranded substrate molecule.

27. The method of claim 26 wherein the primer is sufficiently complementary to a sequence in the substrate polynucleotide to hybridize under appropriate conditions to sequence X.

28. The method of claim 26 wherein the primer is a target-specific primer sufficiently complementary to hybridize under appropriate conditions to a sequence in the substrate molecule other than sequence X.

29. The method of claim 26 wherein the substrate polynucleotide is a single strand DNA polynucleotide or a ribonucleic acid (RNA).

30. The method of claim 26 wherein sequence X is on a separate polynucleotide.

WHAT IS CLAIMED IS:

1. A composition comprising a template-independent nucleic acid polymerase, an attenuator molecule that is a polynucleotide, and nucleotides complementary to the attenuator molecule.
2. The composition of claim 1 wherein the nucleic acid polymerase is a terminal deoxynucleotidyl transferase (TdT).
3. The composition of claim 1 wherein the nucleic acid polymerase is an RNA-specific nucleotidyl transferase selected from the group consisting of poly(A) polymerase and poly(U) polymerase.
4. The composition of any of the preceding claims wherein the attenuator molecule comprises a nucleotide selected from the group consisting of 2'-deoxythymidine 5'-monophosphate (dTMP), 2'-deoxyguanosine 5'-monophosphate (dGMP), 2'-deoxyadenosine 5'-monophosphate (dAMP), 2'-deoxycytidine 5'-monophosphate (dCMP), 2'-deoxyuridine 5'-monophosphate (dUMP), thymidine monophosphate (TMP), guanosine monophosphate (GMP), adenosine monophosphate (AMP), cytidine monophosphate (CMP), uridine monophosphate (UMP), a modified base, and combinations thereof.
5. The composition of any of the preceding claims wherein the attenuator molecule comprises 10 nucleotides, 20 nucleotides, 30 nucleotides, 50 nucleotides, or 100 nucleotides.
6. The composition of any of the preceding claims wherein the attenuator molecule comprises a 3' blocking group selected from the group consisting of at least one ribonucleotide, at least one deoxynucleotide, a C3 spacer, a phosphate, a dideoxynucleotide, an amino group, and an inverted deoxythymidine.
7. The composition of any of the preceding claims wherein the attenuator molecule is degradable or displaceable.
8. The composition of any of the preceding claims wherein the attenuator molecule is an attenuator-adaptor molecule that comprises a single-stranded attenuator sequence and further comprises a partially double-stranded adaptor sequence W positioned 5' to the attenuator sequence and is complementary to a sequence X, wherein the adaptor comprises a 5' phosphate.

9. The composition of claim 8 wherein sequence X is on a separate polynucleotide.

10. The composition of any of the preceding claims wherein a DNA ligase or an RNA ligase is present.

11. The composition of claim 10 further comprising a primer, a polymerase and an adaptor molecule comprising a sequence Y complementary to a sequence V, wherein sequence V is the same length as Y or is less than the same length as sequence Y, the adaptor molecule being a separate molecule from the attenuator-adaptor molecule.

12. The composition of claim 9 wherein the attenuator-adaptor molecule is immobilized.

13. The composition of any one of claims 8-12 wherein the attenuator portion of the attenuator-adaptor molecule comprises (1) a homopolymeric sequence selected from the group consisting of poly (dA), poly (dT), poly (dC), poly (dG), poly (dU), poly (rA), poly (U), poly (rC), poly (rG) and a heteropolymeric sequence comprising combinations of:

- (i) dA and rA bases,
- (ii) dT, dU and U bases,
- (iii) dC and rC bases, or
- (iv) dG and rG bases,

or the attenuator molecule comprises a dinucleotide sequence comprising a plurality of random sequences comprised of the following dinucleotide combinations (i) dG and dC; (ii) dA and dT; (iii) dG and dT; (iv) dG and dA; (v) dA and dC; or (vi) dC and dT,

or the attenuator molecule comprises a dinucleotide sequence comprising a mixture of a ribonucleotide and a deoxyribonucleotide.

14. The composition of claim 8 wherein

- (i) the attenuator molecule comprises dU bases and incubation with a dU-glycosylase destabilizes the attenuator molecule, or incubation with a dU-glycosylase and subsequent incubation at a temperature that is above 80° C degrades the attenuator molecule, or the attenuator molecule is incubated with a mixture of dU-glycosylase and an apurinic/aprimidinic endonuclease;

(ii) the attenuator molecule comprises a ribonucleotide and is degradable with a ribonuclease under conditions sufficient for ribonuclease activity, wherein the ribonuclease is selected from the group consisting of RNase H, RNase HII, RNase A, and RNase T1; or

(iii) the attenuator molecule comprises deoxyribonucleotides and is degradable with a DNA-specific nuclease, optionally wherein the DNA-specific nuclease is DNase I.

15. A method of attenuated tailing and simultaneous adaptor ligation of a substrate polynucleotide comprising

mixing the composition of any one of claims 1 through 14 with the substrate polynucleotide and a nucleotide that is complementary to the attenuator molecule, and

incubating the mixture under conditions sufficient to allow addition of a tail sequence to the 3' end of the substrate polynucleotide; wherein the addition of the tail sequence allows association between the tail sequence and the attenuator molecule to form a complex, and ligating the adaptor sequence to the substrate polynucleotide.

16. The method of claim 15 wherein the substrate polynucleotide is

(i) a single stranded substrate polynucleotide;

(ii) a double stranded substrate polynucleotide; or

(iii) a partially double stranded and partially single stranded substrate polynucleotide,

wherein the double stranded substrate polynucleotide has a 3' overhanging end

(iv) bisulfite-treated or

(v) a product of a primer extension reaction.

17. The method of claim 16 wherein the substrate polynucleotide comprises a free 3' hydroxyl group.

18. The method of claim 16 wherein the attenuator molecule associates with the tail sequence during the process of adding the tail sequence, wherein association of the attenuator molecule to the tail sequence regulates addition of nucleotides to the substrate polynucleotide.

19. The method of claim 18 wherein the addition of the tail sequence to the substrate polynucleotide is temperature sensitive, and wherein the temperature is about 4° C, about 5° C, about 6° C, about 7° C, about 8° C, about 9° C, about 10° C, about 11° C, about 12° C, about 13° C, about 14° C, about 15° C, about 16° C, about 17° C, about 18° C, about 19° C, about 20° C, about 21° C, about 22° C, about 23° C, about 24° C, about 25° C, about 26° C, about 27° C, about 28° C, about 29° C, about 30° C, about 31° C, about 32° C, about 33° C, about 34° C, about 35° C, about 36° C, about 37° C, about 38° C, about 39° C, about 40° C, about 41° C, about 42° C, about 43° C, about 44° C, about 45° C, about 46° C, about 47° C, about 48° C, about 49° C, or about 50° C.

20. The method of claim 15 wherein the substrate polynucleotide is DNA or the substrate polynucleotide is RNA.

21. The method of any one of claims 15 through 20 wherein at least 3, at least 10, at least 20, at least 30, at least 50, or at least 100 nucleotides are added to the substrate polynucleotide.

22. A method of attenuated tailing of a DNA substrate polynucleotide comprising mixing the DNA substrate polynucleotide with TdT enzyme, a degradable or displaceable attenuator polynucleotide comprising a 3' blocking group and nucleotides that are complementary to the attenuator polynucleotide;

incubating the mixture at about 30° C to about 37° C for about 30 minutes; and destabilizing the attenuator molecule by adding (i) a DNA glycosylase, or (ii) an RNase.

23. The method of claim 22 further comprising the step of isolating the attenuated and tailed DNA substrate polynucleotide.

24. The method of claim 15 wherein the DNA substrate polynucleotide is immobilized after the attenuated tailing and simultaneous ligation reaction.

25. A kit comprising the composition of any of one of claims 1 through 14.

26. A method of attenuated tailing and simultaneous adaptor ligation of a substrate polynucleotide comprising:

(1) incubating a mixture comprising the substrate polynucleotide with (i) a template-independent polymerase enzyme; (ii) a composition comprising an attenuator molecule comprising an attenuator sequence and further comprises a sequence W positioned adjacent the attenuator sequence and is complementary to an adaptor sequence X; the composition further comprising an adaptor molecule comprising a sequence Y complementary to a sequence V, wherein sequence V is the same length as Y or is less than the same length as sequence Y, the adaptor molecule being a separate molecule from the attenuator-adaptor molecule; and (iii) deoxynucleotides that are complementary to the attenuator sequence of the attenuator molecule, under conditions that allow extension of the substrate polynucleotide to tail the substrate;

(2) ligating the adaptor sequence X to the substrate polynucleotide and dissociating the attenuator molecule from the separate polynucleotide;

(3) adding a primer complementary to a sequence in the substrate polynucleotide under conditions wherein the primer hybridizes to the substrate polynucleotide;

(4) adding a polymerase and deoxynucleotides to perform polymerase extension from the primer to produce a second strand polynucleotide complementary to the substrate polynucleotide and create a double stranded substrate molecule; and

(5) *ligating the adaptor molecule to the double stranded substrate molecule.*

27. The method of claim 26 further comprising the step of degrading the second strand polynucleotide.

28. The method of claim 26 wherein the primer is sufficiently complementary to a sequence in the substrate polynucleotide to hybridize under appropriate conditions to sequence X.

29. The method of claim 26 wherein the primer is a target-specific primer sufficiently complementary to hybridize under appropriate conditions to a sequence in the substrate molecule other than sequence X.

30. The method of claim 26 wherein the substrate polynucleotide is a single strand DNA polynucleotide or a ribonucleic acid (RNA).

31. The method of claim 25 wherein sequence X is on a separate polynucleotide.