

WHAT IS CLAIMED IS:

1. A composition comprising a nucleic acid polymerase and an 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 base analog, 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 one of claims 1-6 wherein the attenuator molecule is selected from the group consisting of a polynucleotide, a polypeptide, a polysaccharide, a circular molecule, a peptide nucleic acid, a Schizophyllan polysaccharide, a locked nucleic acid and combinations thereof.
8. The composition of any of the preceding claims wherein the attenuator molecule is degradable.
9. The composition of any of the preceding claims wherein the attenuator molecule is an attenuator-adaptor molecule which comprises an attenuator sequence and further

comprises a sequence W positioned adjacent the attenuator sequence and is complementary to a sequence X on a separate polynucleotide, optionally wherein the adaptor comprises a 5' phosphate and a 3' biotin.

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 wherein the attenuator molecule is an attenuator-adaptor molecule which comprises an attenuator sequence and further comprises a sequence W positioned 5' to the attenuator sequence and is complementary to a sequence X on a separate polynucleotide;

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.

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

13. The composition of any of the preceding claims wherein the attenuator molecule is single stranded or the attenuator molecule is at least partially double stranded.

14. The composition of claim 13 wherein the at least partially double stranded attenuator molecule is produced by annealing the attenuator molecule to an adaptor molecule which comprises the adaptor sequence.

15. The composition of any of the preceding claims wherein the attenuator molecule comprises 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 or dC; (ii) dA or dT; (iii) dG or dT; (iv) dG or dA; (v) dA or dC; or (vi) dC or dT.

16. 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/apyrimidinic 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; and/or

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

17. A method of attenuated tailing of a substrate polynucleotide comprising mixing the composition of any one of claims 1 through 16 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.

18. The method of claim 17 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.

19. The method of claim 18 wherein the substrate polynucleotide comprises a free 3' hydroxyl group.

20. The method of claim 18 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.

21. The method of claim 20 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.

22. The method of claim 17 wherein the substrate polynucleotide is DNA or the substrate polynucleotide is RNA.

23. The method of any one of claims 17 through 22 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.

24. 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;

destabilizing the attenuator molecule by adding (i) a DNA glycosylase, (ii) an RNase, (iii) a DNase to degrade the attenuator, or (iv) a primer polynucleotide capable of displacing the attenuator from the attenuated and tailed DNA substrate polynucleotide, and

optionally isolating the attenuated and tailed DNA substrate polynucleotide.

25. A method of attenuated tailing of a substrate RNA polynucleotide comprising mixing the substrate RNA polynucleotide with an RNA polymerase, a degradable or displaceable attenuator polynucleotide and ribonucleotides that are complementary to the attenuator polynucleotide;

incubating the mixture at about 30° C to about 37° C for about 30 minutes;;

destabilizing the attenuator molecule by adding (i) a DNA glycosylase, (ii) a DNase to degrade the attenuator, or (iii) a primer polynucleotide capable of displacing the attenuator from the attenuated and tailed RNA substrate polynucleotide; and

optionally isolating the extended substrate polynucleotide.

26. The method of claim 25 wherein the RNA polymerase is poly(A) polymerase or poly(U) polymerase.

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

mixing the DNA substrate polynucleotide with TdT enzyme, a ligase enzyme, the partially double stranded attenuator-adaptor molecule and nucleotides that are complementary to the homopolymeric or dinucleotide portion of the attenuator-adaptor molecule;

incubating at about 30° C to about 37° C for about 15 minutes to about 30 minutes; and

optionally isolating the attenuated and tailed DNA substrate polynucleotide ligated to the attenuator-adaptor molecule.

28. The method of claim 27 wherein the attenuator-adaptor molecule is ligated by a ligase enzyme to the substrate polynucleotide during addition of nucleotides to the substrate polynucleotide.

29. The method of claim 27 wherein the attenuator-adaptor molecule is ligated by a ligase enzyme to the substrate polynucleotide after addition of nucleotides to the substrate polynucleotide.

30. The method of claim 27 wherein the DNA substrate polynucleotide is immobilized after the attenuated tailing and simultaneous ligation reaction.

31. A method of attenuated tailing, simultaneous adaptor ligation, and immobilization of a DNA substrate polynucleotide comprising

incubating an immobilized partially double stranded-attenuator-adaptor molecule with the DNA substrate polynucleotide, TdT enzyme, a ligase enzyme and nucleotides that are complementary to a single-stranded attenuator portion of the partially double stranded attenuator-adaptor molecule; and

optionally isolating the attenuated and immobilized DNA substrate polynucleotide ligated to the double stranded attenuator-adaptor molecule.

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

mixing the RNA substrate polynucleotide with poly (A) or poly(U) enzyme, a ligase enzyme, the partially double stranded attenuator-adaptor molecule and ribonucleotides that are complementary to the single-stranded portion of the partially double stranded attenuator-adaptor molecule;

incubating the RNA substrate polynucleotide with poly (A) or poly(U) enzyme, a ligase enzyme, the partially double stranded attenuator-adaptor molecule and ribonucleotides that are complementary to the single-stranded portion of the partially double stranded attenuator-adaptor molecule at about 30° C to about 37° C for about 15 to about 30 minutes; and

optionally isolating an attenuated and tailed RNA substrate polynucleotide ligated to the attenuator-adaptor molecule.

33. A method of attenuated tailing, simultaneous adaptor ligation, and immobilization of an RNA substrate polynucleotide comprising

incubating an immobilized partially double strandedattenuator-adaptor molecule with the RNA substrate polynucleotide, poly(A) or poly(U) polymerase, a ligase

enzyme and ribonucleotides that are complementary to the single stranded attenuator portion of the partially double stranded attenuator-adaptor molecule; and

optionally isolating the attenuated and immobilized RNA substrate polynucleotide ligated to the double stranded attenuator-adaptor molecule.

34. A kit comprising the composition of any of one of claims 1 through 16.

35. 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 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 on a separate polynucleotide; 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;

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

(6) optionally degrading the second strand polynucleotide.

36. The method of claim 35 wherein the primer is sufficiently complementary to a sequence in the substrate polynucleotide to hybridize under appropriate conditions to sequence X.

37. The method of claim 35 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.

38. The method of claim 35 wherein the substrate polynucleotide is a single strand DNA polynucleotide or a ribonucleic acid (RNA).

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/apyrimidinic 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.