

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 DNA polymerase.
3. The composition of claim 2 wherein the DNA polymerase is terminal deoxynucleotidyl transferase (TdT).
4. The composition of claim 1 wherein the nucleic acid polymerase is a RNA polymerase.
5. The composition of claim 4 wherein the RNA polymerase is RNA-specific nucleotidyl transferase.
6. The composition of claim 5 wherein the RNA-specific nucleotidyl transferase is selected from the group consisting of poly(A) polymerase and poly(U) polymerase.
7. 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.
8. The composition of any of the preceding claims wherein the attenuator molecule comprises 10 nucleotides.
9. The composition of any of the preceding claims wherein the attenuator molecule comprises 20 nucleotides.
10. The composition of any of the preceding claims wherein the attenuator molecule comprises 30 nucleotides.
11. The composition of any of the preceding claims wherein the attenuator molecule comprises 50 nucleotides.

12. The composition of any of the preceding claims wherein the attenuator molecule comprises 100 nucleotides.

13. The composition of any of the preceding claims wherein the attenuator molecule comprises a 3' blocking group.

14. The composition of claim 13 wherein the blocking group is 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.

15. The composition of any one of claims 1-14 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.

16. The composition of any of the preceding claims wherein the attenuator molecule is degradable.

17. The composition of any of the preceding claims wherein the attenuator molecule further comprises an adaptor sequence, an identifier tag sequence, or both, located 5' to a homopolymer sequence.

18. The composition of any of the preceding claims wherein a ligase is present.

19. The composition of claim 18 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;

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.

20. The composition of claim 17 wherein the attenuator molecule is immobilized.

21. The composition of any of the preceding claims wherein the attenuator molecule is single stranded.

22. The composition of any of claims 17-21 wherein the attenuator molecule is at least partially double stranded.

23. The composition of any of claims 21-22 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.

24. 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.

25. The composition of claim 16 wherein 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.

26. The composition of claim 16 wherein the attenuator molecule comprises a ribonucleotide and is degradable with a ribonuclease under conditions sufficient for ribonuclease activity.

27. The composition of claim 26 wherein the ribonuclease is selected from the group consisting of RNase H, RNase HII, RNase A, and RNase T1.

28. The composition of claim 16 wherein the attenuator molecule comprises deoxyribonucleotides and is degradable with a DNA-specific nuclease.

29. The composition of claim 28 wherein the DNA-specific nuclease is DNase I.

30. A method of extending a substrate polynucleotide comprising

incubating the substrate polynucleotide with the composition of any of the preceding claims 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.

31. The method of claim 30 further comprising degrading the attenuator molecule following extension of the substrate polynucleotide.

32. The method of claim 30 further comprising destabilizing the attenuator molecule following extension of the substrate polynucleotide.

33. The method of any one of claims 30-33 further comprising isolating the extended substrate polynucleotide.

34. The method of any one of claims 30 through 33 further comprising mixing the composition of any one of claims 1 through 27 with the substrate polynucleotide and a nucleotide that is complementary to the homopolymeric portion of the attenuator molecule.

35. The method of any one of claims 30 through 34 wherein the substrate polynucleotide is a single stranded substrate polynucleotide.

36. The method of any one of claims 30 through 34 wherein the substrate polynucleotide is a double stranded substrate polynucleotide.

37. The method of any one of claims 30 through 34 wherein the substrate polynucleotide is a partially double stranded and partially single stranded substrate polynucleotide.

38. The method of claim 36 or claim 37 wherein the double stranded substrate polynucleotide has a 3' overhanging end.

39. The method of any one of claims 35-37 wherein the substrate polynucleotide comprises a free 3' hydroxyl group.

40. The method of any one of claims 30 through 39 wherein a multiplicity of nucleotides are added to the substrate polynucleotide.

41. The method of claim 40 wherein the attenuator molecule associates with the tail sequence over all or part of the attenuator molecule length.

42. The method of claim 40 or claim 41 wherein the attenuator molecule associates with the tail sequence during the process of adding the tail sequence.

43. The method of any one of claims 40 through 42 wherein association of the attenuator molecule to the tail sequence regulates addition of nucleotides to the substrate polynucleotide.

44. The method of any of claims 30 through 43 wherein the conditions regulate addition of the tail sequence to the substrate polynucleotide.

45. The method of claim 44 wherein the addition of the tail sequence to the substrate polynucleotide is temperature sensitive.

46. The method of claim 45 which is performed in a temperature range of about 4° C to about 50° C.

47. The method of claim 46 wherein the temperature is 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, about 50° C.

48. The method of claim 44 wherein the addition of the tail sequence to the substrate polynucleotide is time sensitive.

49. The method of claim 48 wherein the incubation step is allowed to progress for a length of time in the range of about 0.5 minutes to about 120 minutes.

50. The method of claim 48 or claim 49 wherein the length of time that the incubation step is allowed to progress is about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about

37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, about 51, about 52, about 53, about 54, about 55, about 56, about 57, about 58, about 59, about 60, about 61, about 62, about 63, about 64, about 65, about 66, about 67, about 68, about 69, about 70, about 71, about 72, about 73, about 74, about 75, about 76, about 77, about 78, about 79, about 80, about 81, about 82, about 83, about 84, about 85, about 86, about 87, about 88, about 89, about 90, about 91, about 92, about 93, about 94, about 95, about 96, about 97, about 98, about 99, about 100, about 101, about 102, about 103, about 104, about 105, about 106, about 107, about 108, about 109, about 110, about 111, about 112, about 113, about 114, about 115, about 116, about 117, about 118, about 119 or about 120 minutes.

51. The method of claim 44 wherein the addition of the tail sequence to the substrate polynucleotide is pH sensitive.

52. The method of claim 51 wherein the addition of the tail sequence to the substrate polynucleotide is performed under conditions wherein pH is in the range of about pH 5.0 to about pH 9.0.

53. The method of claim 52 wherein the addition of the tail sequence to the substrate polynucleotide is performed under conditions wherein pH is about pH 5.1, about pH 5.2, about pH 5.3, about pH 5.4, about pH 5.5, about pH 5.6, about pH 5.7, about pH 5.8, about pH 5.9, about pH 6.0, about pH 6.1, about pH 6.2, about pH 6.3, about pH 6.4, about pH 6.5, about pH 6.6, about pH 6.7, about pH 6.8, about pH 6.9, about pH 7.0, about pH 7.1, about pH 7.2, about pH 7.3, about pH 7.4, about pH 7.5, about pH 7.6, about pH 7.7, about pH 7.8, about pH 7.9, about pH 8.0, about pH 8.1, about pH 8.2, about pH 8.3, about pH 8.4, about pH 8.5, about pH 8.6, about pH 8.7, about pH 8.8, about pH 8.9 or about pH 9.0.

54. The method of claim 30 wherein the substrate polynucleotide is DNA.

55. The method of claim 30 wherein the substrate polynucleotide is RNA.

56. The method of any one of claims 30 through 55 wherein at least 3 nucleotides are added to the substrate polynucleotide.

57. The method of any one of claims 30 through 55 wherein at least 10 nucleotides are added to the substrate polynucleotide.

58. The method of any one of claims 30 through 55 wherein at least 20 nucleotides are added to the substrate polynucleotide.

59. The method of any one of claims 30 through 55 wherein at least 30 nucleotides are added to the substrate polynucleotide.

60. The method of any one of claims 30 through 55 wherein at least 50 nucleotides are added to the substrate polynucleotide.

61. The method of any one of claims 30 through 55 wherein at least 100 nucleotides are added to the substrate polynucleotide.

62. The method of any one of claims 30 through 61 wherein the adaptor molecule is ligated by a ligase enzyme to the substrate polynucleotide during addition of nucleotides to the substrate polynucleotide resulting in immobilization of the substrate polynucleotide.

63. The method of claim 62 wherein the ligase is a DNA ligase or a RNA ligase.

64. The method of any one of claims 30 through 63 wherein the adaptor molecule is ligated by a ligase enzyme to the substrate polynucleotide after addition of nucleotides to the substrate polynucleotide.

65. The method of any one of claims 30 through 64 wherein the immobilized attenuator-adaptor molecule is ligated by a DNA or RNA ligase to a substrate polynucleotide during addition of a tail sequence to the substrate polynucleotide.

66. The method of any one of claims 30 through 64 wherein the immobilized attenuator-adaptor molecule is ligated by a DNA or RNA ligase to a substrate polynucleotide after addition of a tail sequence to the substrate polynucleotide.

67. The method of any one of claims 62 through 66 wherein the amount of ligase enzyme added to a reaction is from about 0.1 to about 1000 units (U).

68. A method of extending a DNA substrate polynucleotide comprising
mixing the DNA substrate polynucleotide with TdT enzyme, a degradable
attenuator polynucleotide comprising a 3' phosphate and nucleotides that are complementary to
the homopolymeric portion of the attenuator polynucleotide;

incubating the mixture at about 30° C to about 37° C for about 30 minutes, followed by an additional incubation at 70° C for about 10 minutes;

destabilizing the attenuator molecule by adding a DNA glycosylase and

incubating the mixture at 37° C for about 5 minutes, or degrading the attenuator molecule by incubating the mixture at a temperature above 80° C; and

optionally isolating the extended DNA substrate polynucleotide.

69. A method of extending a DNA substrate polynucleotide comprising

mixing the DNA substrate polynucleotide with TdT enzyme, an attenuator polynucleotide comprising a blocking group, wherein the blocking group optionally comprises at least two ribonucleotides, and deoxynucleotides that are complementary to the homopolymeric portion of the attenuator polynucleotide;

incubating the mixture at about 30° C to about 37° C for 30 minutes, followed by an additional incubation at 70° C for about 10 minutes to inactivate the TdT enzyme; and

optionally isolating the extended DNA substrate polynucleotide.

70. A method of extending a substrate RNA polynucleotide comprising

mixing the substrate RNA polynucleotide with an RNA polymerase, a degradable attenuator polynucleotide and ribonucleotides that are complementary to the homopolymeric portion of the attenuator polynucleotide;

incubating the mixture at about 30° C to about 37° C for about 30 minutes, followed by an additional incubation at 95° C for about 10 minutes;

destabilizing the attenuator molecule by adding a DNA glycosylase and incubating the mixture at 37° C for about 10 minutes, or degrading the attenuator molecule by adding a DNA glycosylase and incubating the mixture at 37° C for about 10 minutes, followed by incubation at a temperature above 80° C; and

optionally isolating the extended substrate polynucleotide.

71. The method of claim 70 wherein the RNA polymerase is poly(A) polymerase.

72. The method of claim 70 wherein the RNA polymerase is poly(U) polymerase.

73. A method of extending a DNA substrate polynucleotide comprising

annealing an attenuator molecule and an adaptor molecule that are at least partially complementary to each other by heating a mixture of the attenuator and the adaptor molecules in a suitable buffer to about 100° C and then cooling to about 25° C, wherein the annealing results in a partially double stranded attenuator-adaptor molecule;

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 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 extended DNA substrate polynucleotide ligated to the attenuator-adaptor molecule.

74. A method of extending a DNA substrate polynucleotide comprising

annealing an attenuator molecule to a biotinylated adaptor molecule that are at least partially complementary to each other by heating a mixture of the attenuator molecule and the biotinylated adaptor molecule in a suitable buffer to about 100° C and then cooling to about 25° C, wherein the annealing results in a partially double stranded biotinylated attenuator-adaptor molecule;

immobilizing the partially double stranded biotinylated attenuator-adaptor molecule by mixing the partially double stranded biotinylated attenuator-adaptor molecule with a solution comprising a streptavidin-coated magnetic bead at about 25° C for about two hours, resulting in immobilization of the partially double stranded biotinylated attenuator-adaptor molecule to the streptavidin-coated magnetic bead;

incubating the immobilized partially double stranded biotinylated attenuator-adaptor molecule attached to the streptavidin-coated magnetic bead with the DNA substrate polynucleotide, TdT enzyme, a ligase enzyme and nucleotides that are complementary to a

single-stranded homopolymeric portion of the partially double stranded attenuator molecule at about 30° C to about 37° C for about 15 minutes to about 30 minutes;

washing the solution with NaOH to remove non-biotinylated single stranded DNA from the beads; and

optionally isolating an extended DNA substrate polynucleotide ligated to the double stranded biotinylated attenuator-adaptor molecule.

75. A method of extending a RNA substrate polynucleotide comprising

annealing an attenuator molecule and an adaptor molecule that are at least partially complementary to each other by heating a mixture of the attenuator and adaptor molecules in a suitable buffer to about 100° C and then cooling to about 25° C, wherein the annealing results in a partially double stranded attenuator-adaptor molecule;

mixing an 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 homopolymeric 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 homopolymeric 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 extended RNA substrate polynucleotide ligated to the attenuator-adaptor molecule.

76. A method of extending and immobilizing an RNA substrate polynucleotide comprising

annealing an attenuator molecule to a biotinylated adaptor molecule that are at least partially complementary to each other by heating a mixture of the two molecules in a suitable buffer to about 100° C and then cooling to about 25° C, wherein the annealing results in a partially double stranded biotinylated attenuator-adaptor molecule;

immobilizing the partially double stranded biotinylated attenuator-adaptor molecule by mixing the partially double stranded biotinylated attenuator-adaptor molecule with a solution comprising a streptavidin-coated magnetic bead at about 25° C for about two hours, resulting in immobilization of the partially double stranded biotinylated attenuator-adaptor molecule to the streptavidin-coated magnetic bead;

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

washing the solution with NaOH to remove non-biotinylated single stranded polynucleotide from the beads; and

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

77. A kit comprising the composition of any of one of claims 1 through 26.

78. A method of extending 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.

79. The method of claim 78 wherein the primer is sufficiently complementary to a sequence in the substrate polynucleotide to hybridize under appropriate conditions to sequence X.

80. The method of claim 78 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.

81. The method of claim 78 wherein the substrate polynucleotide is a single strand DNA polynucleotide.

82. The method of claim 78 wherein the substrate polynucleotide is a ribonucleic acid (RNA).

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).