transcripts, are not polyadenylated and are therefore lost from the library with oligo dT priming. While NSR priming strategies can be effective when designed to specific organisms, NSR priming can cause distortions in the sample populations when a less optimized set of primers is employed across a broader range of sample types.

[0004] There is a need for improved methods for removal of specific non-desired DNA fragments from NGS libraries. Such methods would ideally enable starting with an unbiased template population and eliminating non-desired DNA fragments in a sequence-specific manner after the NGS library has been generated. The invention described herein fulfills this need.

SUMMARY OF THE INVENTION

[0005] The present invention provides novel methods, compositions and kits for construction of NGS libraries in which non-desired nucleic acid sequences have been depleted or substantially reduced. Specifically, an important aspect of this invention is the methods and compositions that allow for elimination or reduction of non-desired DNA sequences in a sequence-specific manner after the generation of a NGS library in which all sequences of the starting nucleic acid sequence population (for example, a transcriptome) are represented in an undistorted, unbiased manner. The methods of the invention can be employed for the elimination of non-desired nucleic acid sequences, such as ribosomal RNA, from a nucleic acid library, and consequently, for the enrichment of nucleic acid sequences of interest in the library.

[0005a] In one aspect, there is provided a method of reducing an abundance ratio of non-desired and desired nucleic acid sequence from a single-stranded nucleic acid library, the method comprising:

a) annealing a sequence-specific oligonucleotide probe to a non-desired nucleic acid sequence in the single-stranded nucleic acid library, wherein the single-stranded nucleic acid library comprises single stranded nucleic acid fragments comprising an adaptor sequence;

b) extending the oligonucleotide probe to produce a partial double-stranded nucleic acid;

c) selectively cleaving the partial double-stranded nucleic acid at a cleavage site in the adaptor; and

d) amplifying the desired nucleic acid sequences, thereby reducing the abundance ratio of non-desired and desired nucleic acid sequence from the single-stranded nucleic acid library.

[0005b] In one aspect, there is provided a method of depleting a non-desired nucleic acid sequences from a directional single-stranded nucleic acid library, the method comprising:

a) ligating a double stranded nucleic acid template to a first double-stranded adapter oligonucleotide and a second double-stranded adaptor oligonucleotide, wherein the double stranded nucleic acid template comprises:

(i) a first product strand that does not comprise a non-canonical nucleotide, and

(ii) a second product strand comprising one or more non-canonical nucleotides,

wherein the first double-stranded adaptor oligonucleotide comprises:

(i) a first adapter strand that does not comprise one or more non-canonical nucleotides, and

(ii) a second adapter strand comprising one or more non-canonical nucleotides,

and wherein the second double-stranded adapter oligonucleotide comprises a recognition sequence for a restriction endonuclease specific for double-stranded DNA, a third adapter strand that does not comprise one or more non-canonical nucleotides, and a fourth adapter strand that does not comprises one or more non-canonical nucleotides,

wherein the ligating creates a double stranded product comprising the first product strand ligated to the first adapter strand and the fourth adapter strand; and

b) selectively cleaving the second product strand and second adapter strand, thereby generating a directional single-stranded nucleic acid library;

c) hybridizing a probe to a non-desired sequence in the directional single-stranded nucleic acid library,

d) extending the probe with a DNA polymerase, thereby producing a partial duplex nucleic acid and

e) treating the partial duplex nucleic acid with a restriction endonuclease specific for double-stranded DNA, thereby cleaving double-stranded DNA fragments at the recognition sequence.

[0005c] In one aspect, there is provided a method of creating a strand retained library of nucleic acids, the method comprising:

a) annealing a partial duplex primer to a single-stranded nucleic acid template, wherein the single-stranded nucleic acid template comprises a non-canonical nucleotide, wherein the partial duplex primer comprises a 3' overhang,

b) extending the partial duplex primer to form a primer extension product strand, thereby forming a double-stranded nucleic acid comprising (i) the primer extension product strand and (ii) the template strand;

c) ligating an adapter to a 3' end of the primer extension product strand; and

d) cleaving the template strand from the double-stranded nucleic acid, wherein the cleaving comprises use of a cleavage agent specific for the one or more non-canonical nucleotides, thereby creating a strand retained library of nucleic acids.

[0006] In one aspect, the invention provides a method for the selective removal of non-desired nucleic acid sequences in a sequence-specific manner from a nucleic acid library with single-stranded DNA templates. In some embodiments, the method comprises: a) annealing a sequence-specific oligonucleotide primer or a set of sequence-specific oligonucleotide primers to the single-stranded DNA templates with adaptors of fixed orientation attached at each end, wherein the sequence-specific oligonucleotides are designed to be complementary to the non-desired nucleic acid sequences or regions adjacent to the non-desired nucleic acid sequences, and wherein one of the two adaptor sequences contains a recognition sequence for a restriction endonuclease specific for double-stranded DNA; b) extending the sequence-specific primers with a DNA polymerase beyond the adaptor-DNA template junction, thereby creating double-stranded DNA fragments where the oligonucleotide primers are complementary to the single-stranded DNA templates ; c) treating the population of DNA fragments (both single-stranded and double-stranded) with a restriction endonuclease specific for double-stranded DNA, thereby cleaving only double-stranded DNA fragments at the

CLAIMS

WHAT IS CLAIMED IS:

1. A method for depleting or reducing specific non-desired nucleic acid sequences from a nucleic acid library, the method comprising:

a. generating a nucleic acid library comprising single-stranded DNA fragments with adaptors of fixed orientation attached to each end of each DNA fragment;

b. annealing sequence-specific oligonucleotide probes to the single-stranded DNA fragments with adaptors of fixed orientation attached at each end, wherein the sequence-specific oligonucleotide probes are designed to be complementary to the non-desired nucleic acid sequences, and wherein at least one of the two adaptors comprises a recognition sequence for a restriction endonuclease specific for double-stranded DNA;

c. extending the sequence-specific oligonucleotide probes with a DNA polymerase, thereby creating double-stranded DNA fragments comprising at least a portion of the non-desired nucleic acid sequences;

d. treating the population of DNA fragments comprising double-stranded and single-stranded DNA with a restriction endonuclease specific for double-stranded DNA, thereby cleaving double-stranded DNA fragments at the restriction endonuclease site; and

e. performing PCR with a set of primers specific to the adaptor sequences, thereby amplifying the DNA fragments comprising the desired nucleic acid sequences.

2. The method of claim 1, further comprising an additional step of sequencing the amplified products.

3. The method of claim 1, wherein the nucleic acid library originates from a population of sorted cells.

4. The method of claim 3, wherein the nucleic acid library originates from a single cell.

5. The method of claim 3, further comprising sorting cells into a multiwell plate, microarray, microfluidic device, or slide and thereby generating the population of sorted cells.

6. The method of claim 5, wherein the sorting is performed according to a cell surface marker.

7. The method of claim 5, wherein the sorting is performed according to optical properties of the cells.

8. The method of claim 5, wherein the sorting is performed according to cell size.

9. The method of claim 1, wherein the non-desired nucleic acid sequences comprise bacterial ribosomal RNA, mitochondrial DNA, human globin mRNA, human cytoplasmic rRNA, human mitochondrial rRNA, grape cytoplasmic rRNA, grape mitochondrial rRNA, or grape chloroplast rRNA.

10. The method of claim 1, wherein the restriction endonuclease of step d. is BspQI.

11. The method of claim 1, wherein the DNA polymerase comprises a hot start polymerase.

12. The method of claim 11, wherein the DNA polymerase is MyTaq polymerase.

13. The method of claim 1, wherein step a. comprises the following steps:

i. reverse transcribing a RNA sample;

ii. generating double-stranded cDNA from the reverse transcribed RNA sample, wherein at least one of the four dNTPs dATP, dCTP, dGTP or dTTP is replaced by a non-canonical dNTP during second strand synthesis and incorporated into the second strand;

iii. performing end repair on the double-stranded cDNA;

iv. ligating adaptors to a 5' end of the double-stranded cDNA, wherein one of the adaptor strands has the non-canonical nucleotide incorporated into a ligation strand of the adaptor;

v. performing gap repair; and

vi. selectively removing the second strand by a cleavage agent.

14. The method of claim 5, wherein the non-canonical nucleotide comprises uridine or inosine.

15. The method of claim 13, wherein step vi comprises cleaving a base portion of the one or more non-canonical nucleotides, thereby forming an abasic site.

16. The method of claim 13, wherein the cleavage agent comprises a glycosylase.

17. The method of claim 16, wherein the glycosylase is UNG or UDG.

18. The method of claim 13, wherein the cleavage agent comprises a primary amine.

19. The method of claim 13, wherein the cleavage agent comprises a polyamine.

20. The method of claim 19, wherein the polyamine is DMED.

21. The method of claim 13, wherein the cleavage agent comprises a glycosylase and a polyamine.

22. The method of claim 13, wherein the cleavage agent comprises endonuclease V.

23. A method of adapter ligation to a pool of nucleic acids, comprising:

a. ligating a nucleic acid comprising a first nucleic acid strand comprising a 5' phosphate, a second nucleic acid strand comprising a 5' phosphate and one or more non-

canonical nucleotides, with at least a first adapter comprising a first adapter strand lacking a 5' phosphate and a second adapter strand lacking a 5' phosphate and one or more non-canonical nucleotides;

b. performing a 3' extension reaction; and

c. performing a cleavage reaction with an agent comprising one or more cleavage reagents, thereby cleaving at least one nucleic acid strand comprising one or more non-canonical nucleotides; wherein one of the one or more cleavage agents is specific for nucleic acid strands comprising the one or more non-canonical nucleotides.

24. The method of claim 23, further comprising ligating the nucleic acid with a second adapter comprising a third adapter strand lacking a 5' phosphate and a fourth adapter strand lacking a 5' phosphate and one or more non-canonical nucleotides, wherein the first and the second adapters are different.

25. The method of claims 23 or 24, wherein the nucleic acid is ligated with a first or second adapter at each end.

26. The method of claim 23, wherein the non-canonical nucleotide is selected from uracil and inosine.

27. The method of claim 23, wherein step c comprises cleaving a base portion of the one or more non-canonical nucleotides, thereby forming an abasic site.

28. The method of claim 23, wherein the one or more cleavage reagents comprises a glycosylase.

29. The method of claim 28, wherein the glycosylase is UNG or UDG.

30. The method of claim 23, wherein the one or more cleavage reagents comprises a primary amine.

31. The method of claim 23, wherein the one or more cleavage reagents comprises a polyamine.

32. The method of claim 31, wherein the polyamine is DMED.

33. The method of claim 23, wherein the one or more cleavage reagents comprises a glycosylase and a polyamine.

34. The method of claim 23, wherein the one or more cleavage reagents comprises endonuclease V.

35. The method of claim 24, further comprising performing an amplification reaction comprising a first primer and second primer, wherein the first primer is hybridizable to the first adapter strand and the second primer is hybridizable to the fourth adapter strand, thereby generating amplified products.

36. The method of any one of claims 23-35, wherein the first adapter comprises a recognition sequence for a restriction endonuclease specific for double-stranded DNA.

37. The method of claim 36, further comprising,

d. hybridizing a probe to a sequence on the first nucleic acid strand,

e. extending the probe with a DNA polymerase, thereby producing a partial duplex nucleic acid, and

f. treating the partial duplex nucleic acid with a restriction endonuclease specific for double-stranded DNA, thereby cleaving double-stranded DNA fragments at the recognition sequence.

38. The method of claim 37, further comprising performing PCR with a set of primers specific to the adaptor sequences, thereby amplifying at least a second nucleic acid in the pool of nucleic acids.

39. The method of claim 38, wherein the second nucleic acid lacks the sequence in step d.

40. The method of claim 38, further comprising sequencing a portion of the second nucleic acid.

41. The method of claim 23, wherein the nucleic acid is generated by

i. performing a first strand synthesis on an RNA, thereby forming a first strand synthesis product; and

ii. performing a second strand synthesis on the first strand in the presence of a non-canonical nucleotide, thereby forming a second strand synthesis product.

42. The method of claim 41, further comprising selectively cleaving the RNA.

43. The method of claim 42, wherein selectively cleaving the RNA comprises treatment with RNAse H.

44. The method of claim 41, further comprising

iii. fragmenting the first and second strand synthesis products, thereby generating fragmented first and second strand synthesis products;

iv. performing end repair; and

v. performing 5' phosphorylation.

45. The method of claim 23, wherein the pool of nucleic acids originates from a population of sorted cells.

46. The method of claim 45, wherein the pool of nucleic acids originates from a single cell.

47. The method of claim 45, further comprising sorting cells into a multiwell plate, microarray, microfluidic device, or slide and thereby generating the population of sorted cells.

48. The method of claim 47, wherein the sorting is performed according to a cell surface marker.

49. The method of claim 47, wherein the sorting is performed according to optical properties of the cells.

50. The method of claim 47, wherein the sorting is performed according to cell size.

51. The method of claim 23, wherein the pool of nucleic acids comprises bacterial ribosomal RNA, mitochondrial DNA, human globin mRNA, human cytoplasmic rRNA, human mitochondrial rRNA, grape cytoplasmic rRNA, grape mitochondrial rRNA, or grape chloroplast rRNA.

52. The method of claim 36, wherein the restriction endonuclease is BspQI.

53. The method of claim 23, wherein the 3' extension reaction is performed using a hot start polymerase.

54. The method of claim 53, wherein the 3' extension reaction is performed using MyTaq polymerase.

55. A method of adapter ligation to create a strand retained library of nucleic acids comprising desired and undesired nucleic acids, comprising:

a. mixing pool of templates comprising an undesired nucleic acid comprising one or more non-canonical nucleotides and a desired nucleic acid comprising one or more non-canonical nucleotides with a plurality of partial duplex primers each comprising a 3' overhang,

b. annealing to the templates the plurality of partial duplex primers;

c. performing primer extension reaction along the templates, thereby forming double-stranded nucleic acids each comprising a primer extension product;

d. ligating an adapter to at least one 5' end of the primer extension products; and

e. cleaving the templates from the double-stranded nucleic acids with a cleavage agent that is specific for nucleic acids comprising the one or more nucleotides.

56. The method of claim 55, wherein the plurality of partial duplex primers comprise at least two partial duplex primers with dissimilar 3' overhang sequences.

57. The method of claim 55 or 56, wherein the plurality of partial duplex primers comprise a shared sequence within a double-stranded portion.

58. The method of claim 55, further comprising a step comprising performing a primer extension reaction along the adapter.

59. The method of claim 55, wherein step e comprises cleaving a base portion of the one or more non-canonical nucleotides, thereby forming an abasic site.

60. The method of claim 55, wherein the cleavage agent comprises a glycosylase.

61. The method of claim 60, wherein the glycosylase is UNG or UDG.

62. The method of claim 55, wherein the cleavage agent comprises a primary amine.

63. The method of claim 55, wherein the cleavage agent comprises a polyamine.

64. The method of claim 63, wherein the polyamine is DMED.

65. The method of claim 55, wherein the cleavage agents comprises a glycosylase and a polyamine.

66. The method of claim 55, wherein the cleavage agent comprises endonuclease V.

67. The method of claim 55, wherein the one or more non-canonical nucleotides comprise uracil or inosine.

68. The method of claim 55, wherein the one or more non-canonical nucleotides comprise uracil and inosine.

69. The method of claim 55, wherein the pool of templates is generated by

i. performing a first strand synthesis on an RNA in the presence of the one or more non-canonical nucleotides, thereby forming a first strand synthesis product;

ii. and performing a fragmentation reaction.

70. The method of claim 69, further comprising selectively cleaving the RNA.

71. The method of claim 70, wherein selectively cleaving the RNA comprises treatment with RNAse H.

72. The method of claim 69, wherein the fragmentation reaction comprises utilizing a cleavage agent targeting the one or more non-canonical nucleotides.

73. The method of claim 72, wherein fragmentation reaction comprises cleaving a base portion of the one or more non-canonical nucleotides, thereby forming an abasic site.

74. The method of claim 72, wherein the cleavage agent comprises a glycosylase.

75. The method of claim 74, wherein the glycosylase is UNG or UDG.

76. The method of claim 72, wherein the cleavage agent comprises a primary amine.

77. The method of claim 72, wherein the cleavage agent comprises a polyamine.

78. The method of claim 77, wherein the polyamine is DMED.

79. The method of claim 72, wherein the cleavage agents comprises a glycosylase and a polyamine.

80. The method of claim 72, wherein the cleavage agent comprises endonuclease V.

81. The method of any one of claims 55-80, wherein the adapter comprises a recognition sequence for a restriction endonuclease specific for double-stranded DNA.

82. The method of claim 81, further comprising,

f. hybridizing a probe to a sequence of the primer extension product;

g. extending the probe with a DNA polymerase, thereby producing a partial duplex nucleic acid,

h. treating the partial duplex nucleic acid with a restriction endonuclease specific for double-stranded DNA, thereby cleaving double-stranded DNA fragments at the recognition sequence.

83. The method of claim 82, further comprising performing PCR with a primer that is hybridizable a sequence reverse complimentary to the adapter, thereby amplifying a desired nucleic acid in the pool of templates.

84. The method of claim 83, further comprising sequencing a portion of the desired nucleic acid.

85. The method of claim 55, wherein the pool of templates originates from a population of sorted cells.

86. The method of claim 85, wherein the pool of templates originates from a single cell.

87. The method of claim 85, further comprising sorting cells into a multiwell plate, microarray, microfluidic device, or slide and thereby generating the population of sorted cells.

88. The method of claim 87, wherein the sorting is performed according to a cell surface marker.

89. The method of claim 87, wherein the sorting is performed according to optical properties of the cells.

90. The method of claim 87, wherein the sorting is performed according to cell size.

91. The method of claim 55, wherein the pool of templates comprises bacterial ribosomal RNA, mitochondrial DNA, human globin mRNA, human cytoplasmic rRNA, human mitochondrial rRNA, grape cytoplasmic rRNA, grape mitochondrial rRNA, or grape chloroplast rRNA.

92. The method of claim 81, wherein the restriction endonuclease is BspQI.

93. The method of claim 55, wherein the primer extension reaction is performed using a hot start polymerase.

94. The method of claim 93, wherein the primer extension reaction is performed using MyTaq polymerase.

95. A method of adapter ligation to create a strand retained library of nucleic acids with desired and undesired nucleic acids, comprising:

a. mixing a pool of templates comprising an undesired nucleic acid and a desired nucleic acid with a plurality of partial duplex primers each comprising a 3' overhang;

b. annealing to the templates the plurality of partial duplex primers;

c. performing primer extension reaction along the templates, thereby forming double-stranded nucleic acids each comprising a primer extension product;

d. ligating an adapter to at least one 5' end of the primer extension product; and

e. cleaving the primer extension products from the double-stranded nucleic acids with a cleavage agent that is specific for nucleic acids comprising the one or more nucleotides.

96. The method of claim 95, wherein the plurality of partial duplex primers comprise at least two partial duplex primers with dissimilar 3' overhang sequences.

97. The method of claim 95 or 96, wherein the plurality of partial duplex primers comprise a shared sequence within a double-stranded portion.

98. The method of claim 97, wherein the strand of the plurality of partial duplex primers with the 3' overhang lacks adenines in the shared sequence within the double-stranded portion.

99. The method of claim 95, further comprising a step comprising performing a primer extension reaction along the adapter.

100. The method of claim 99, wherein the primer extension reaction is performed in the presence of one or more non-canonical nucleotides.

101. The method of claim 95, wherein step e comprises cleaving a base portion of the one or more non-canonical nucleotides, thereby forming an abasic site.

102. The method of claim 95, wherein the cleavage agent comprises a glycosylase.

103. The method of claim 102, wherein the glycosylase is UNG or UDG.

104. The method of claim 95, wherein the cleavage agent comprises a primary amine.

105. The method of claim 95, wherein the cleavage agent comprises a polyamine.

106. The method of claim 105, wherein the polyamine is DMED.

107. The method of claim 95, wherein the cleavage agents comprises a glycosylase and a polyamine.

108. The method of claim 95, wherein the cleavage agent comprises endonuclease V.

109. The method of claim 95 or 100, wherein the one or more non-canonical nucleotides comprise uracil or inosine.

110. The method of claim 95, wherein the pool of templates is generated by

i. performing a first strand synthesis on an RNA in the presence of the one or more non-canonical nucleotides, thereby forming a first strand synthesis product;

ii. and performing a fragmentation reaction.

111. The method of claim 110, further comprising selectively cleaving the RNA.

112. The method of claim 111, wherein selectively cleaving the RNA comprises treatment with RNAse H.

113. The method of claim 110, wherein the fragmentation reaction comprises utilizing a cleavage agent targeting the one or more non-canonical nucleotides.

114. The method of claim 113, wherein the fragmentation reaction comprises cleaving a base portion of the one or more non-canonical nucleotides, thereby forming an abasic site.

115. The method of claim 113, wherein the cleavage agent comprises a glycosylase.

116. The method of claim 115, wherein the glycosylase is UNG or UDG.

117. The method of claim 113, wherein the cleavage agent comprises a primary amine.

118. The method of claim 113, wherein the cleavage agent comprises a polyamine.

119. The method of claim 118, wherein the polyamine is DMED.

120. The method of claim 113, wherein the cleavage agents comprises a glycosylase and a polyamine.

121. The method of claim 113, wherein the cleavage agent comprises endonuclease V.

122. The method of any one of claims 95-121, wherein the adapter comprises a recognition sequence for a restriction endonuclease specific for double-stranded DNA.

123. The method of claim 122, further comprising,

f. hybridizing a probe to a sequence of the undesired nucleic acid;

g. extending the probe with a DNA polymerase, thereby producing a partial duplex nucleic acid,

h. treating the partial duplex nucleic acid with a restriction endonuclease specific for double-stranded DNA, thereby cleaving double-stranded DNA fragments at the recognition sequence.

124. The method of claim 123, further comprising performing PCR with a set of primers that are hybridizable to a sequence reverse complimentary to the adapter and the shared sequence of the partial duplex primers opposite the 3' overhang, thereby amplifying a desired nucleic acid in the pool of templates.

125. The method of claim 124, further comprising sequencing a portion of the desired nucleic acid.

126. The method of claim 95, wherein the pool of templates originates from a population of sorted cells.

127. The method of claim 126, wherein the pool of templates originates from a single cell.

128. The method of claim 126, further comprising sorting cells into a multiwell plate, microarray, microfluidic device, or slide and thereby generating the population of sorted cells.

129. The method of claim 128, wherein the sorting is performed according to a cell surface marker.

130. The method of claim 128, wherein the sorting is performed according to optical properties of the cells.

131. The method of claim 128, wherein the sorting is performed according to cell size.

132. The method of claim 95, wherein the pool of templates comprises bacterial ribosomal RNA, mitochondrial DNA, human globin mRNA, human cytoplasmic rRNA, human mitochondrial rRNA, grape cytoplasmic rRNA, grape mitochondrial rRNA, or grape chloroplast rRNA.

133. The method of claim 122, wherein the restriction endonuclease is BspQI.

134. The method of claim 95, wherein the primer extension reaction is performed using a hot start polymerase.

135. The method of claim 134, wherein the primer extension reaction is performed using MyTaq polymerase

136. A kit comprising;

a. a restriction endonuclease;

b. a first adapter comprising one or more non-canonical nucleotides on one strand and lacking 5' phosphates;

c. a second adapter lacking said one or more non-canonical nucleotides and lacking 5' phosphates;

d. a ligase;

e. a polymerase;

f. a cleavage agent;

g. a library of probes; and

h. a set of primers specific to the adaptor sequences;

wherein the second adapter comprises a recognition sequence for the restriction endonuclease.

137. A kit comprising;

a. a restriction endonuclease;

b. a first adapter lacking 5' phosphates;

c. a plurality of partial duplex primers each comprising a 3' overhang and comprising a shared sequence within a double-stranded portion;

d. a ligase;

e. a polymerase;

f. a cleavage agent;

g. a library of probes capable of acting as a primer for a primer extension reaction; and

h. a primer that is hybridizable a sequence reverse complimentary to the adapter;

wherein the first adapter comprises a recognition sequence for the restriction endonuclease; and

wherein the plurality of partial duplex primers comprise at least two partial duplex primers with dissimilar 3' overhang sequences.

138. A kit comprising;

a. a restriction endonuclease;

b. a first adapter lacking 5' phosphates;

c. a plurality of partial duplex primers each comprising a 3' overhang, comprising a shared sequence within a double-stranded portion, and the strand of the plurality of partial duplex primers with the 3' overhang lacking adenines in the shared sequence within the double-stranded portion

d. a ligase;

e. a polymerase;

f. a cleavage agent;

g. a library of probes capable of acting as a primer for a primer extension reaction; and

h. a set of primers that are hybridizable to a sequence reverse complimentary to the adapter and the shared sequence of the partial duplex primers opposite the 3' overhang;

wherein the first adapter comprises a recognition sequence for the restriction endonuclease; and

wherein the plurality of partial duplex primers comprise at least two partial duplex primers with dissimilar 3' overhang sequences.

139. The kit as in one of claims 136-138, wherein the restriction endonuclease is BspQI.

140. The kit as in one of claims 136-138, wherein the polymerase is a hot start polymerase

141. The kit of claim 140, wherein the polymerase is MyTaq.

142. The kit as in one of claims 136-138, further comprising one or more non-canonical nucleotides.

143. The kit as in one of claims 136-138, wherein the one or more non-canonical nucleotides comprise uracil or inosine.

144. The kit as in one of claims 136-138, wherein the cleavage agent comprises a glycosylase.

145. The kit as in claim 144, wherein the glycosylase is UNG or UDG.

146. The kit as in one of claims 136-138, wherein the cleavage agent comprises a primary amine.

147. The kit as in one of claims 136-138, wherein the cleavage agent comprises a polyamine.

148. The kit of claim 147, wherein the polyamine is DMED.

149. The kit as in one of claims 136-138, wherein the cleavage agent comprises a glycosylase and a polyamine.

150. The kit as in one of claims 136-138, wherein the cleavage agent comprises endonuclease V.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of reducing an abundance ratio of non-desired and desired nucleic acid sequence from a single-stranded nucleic acid library, the method comprising:

a) annealing a sequence-specific oligonucleotide probe to a non-desired nucleic acid sequence in the single-stranded nucleic acid library, wherein the single-stranded nucleic acid library comprises single stranded nucleic acid fragments comprising an adaptor sequence;

b) extending the oligonucleotide probe to produce a partial double-stranded nucleic acid;

c) selectively cleaving the partial double-stranded nucleic acid at a cleavage site in the adaptor; and

d) amplifying the desired nucleic acid sequences, thereby reducing the abundance ratio of non-desired and desired nucleic acid sequence from the single-stranded nucleic acid library.

2. The method of claim 1, wherein the single stranded nucleic acid fragments comprise an adaptor sequence at both ends.

3. The method of claim 2, wherein the amplifying comprises amplifying with a primer that is specific for the adaptor sequence.

4. The method of claim 1, wherein the cleavage site is capable of recognition by a cleavage agent.

5. The method of claim 4, wherein the cleavage site comprises a recognition sequence for the restriction endonuclease.

6. The method of claim 1, wherein the selectively cleaving comprises cleavage with a cleavage agent specific for a double-stranded nucleic acid sequence in the partial double-stranded nucleic acid.

7. The method of claim 6, wherein the cleavage agent is a restriction endonuclease.

8. The method of claim 1, wherein the non-desired nucleic acid sequence comprises a sequence of ribosomal RNA (rRNA), mitochondrial DNA, or human globin mRNA.

9. The method of claim 1, further comprising sequencing the amplified products.

10. A method of depleting a non-desired nucleic acid sequences from a directional single-stranded nucleic acid library, the method comprising:

a) ligating a double stranded nucleic acid template to a first double-stranded adapter oligonucleotide and a second double-stranded adaptor oligonucleotide, wherein the double stranded nucleic acid template comprises:

(i) a first product strand that does not comprise a non-canonical nucleotide, and

(ii) a second product strand comprising one or more non-canonical nucleotides,

wherein the first double-stranded adaptor oligonucleotide comprises:

(i) a first adapter strand that does not comprise one or more non-canonical nucleotides, and

(ii) a second adapter strand comprising one or more non-canonical nucleotides,

and wherein the second double-stranded adapter oligonucleotide comprises a recognition sequence for a restriction endonuclease specific for double-stranded DNA, a third adapter strand that does not comprise one or more non-canonical nucleotides, and a fourth adapter strand that does not comprises one or more non-canonical nucleotides,

wherein the ligating creates a double stranded product comprising the first product strand ligated to the first adapter strand and the fourth adapter strand; and

b) selectively cleaving the second product strand and second adapter strand, thereby generating a directional single-stranded nucleic acid library;

c) hybridizing a probe to a non-desired sequence in the directional single-stranded nucleic acid library,

d) extending the probe with a DNA polymerase, thereby producing a partial duplex nucleic acid and

e) treating the partial duplex nucleic acid with a restriction endonuclease specific for double-stranded DNA, thereby cleaving double-stranded DNA fragments at the recognition sequence.

11. The method of claim 10, wherein the non-canonical nucleotide is a non-canonical dNTP.

12. The method of claim 11, wherein the non-canonical dNTP comprises uracil or inosine.

13. The method of claim 10, wherein the selectively cleaving comprises cleaving with a glycosylase and a polyamine.

14. The method of claim 10, further comprising performing an amplification reaction comprising a first primer and second primer, wherein the first primer is hybridizable to the first adapter strand and the second primer is hybridizable to sequence complementary to the fourth adapter strand, thereby generating amplified products.

15. The method of claim 14, further comprising sequencing a portion of the amplified products.

16. The method of claim 10, wherein the directional single-stranded nucleic acid library comprises bacterial ribosomal RNA, mitochondrial DNA, human globin mRNA, human cytoplasmic rRNA, human mitochondrial rRNA, grape cytoplasmic rRNA, grape mitochondrial rRNA, or grape chloroplast rRNA.

17. The method of claim 10, wherein the restriction endonuclease is BspQI.

18. A method of creating a strand retained library of nucleic acids, the method comprising:

a) annealing a partial duplex primer to a single-stranded nucleic acid template, wherein the single-stranded nucleic acid template comprises a non-canonical nucleotide, wherein the partial duplex primer comprises a 3' overhang,

b) extending the partial duplex primer to form a primer extension product strand, thereby forming a double-stranded nucleic acid comprising (i) the primer extension product strand and (ii) the template strand;

c) ligating an adapter to a 3' end of the primer extension product strand; and

d) cleaving the template strand from the double-stranded nucleic acid, wherein the cleaving comprises use of a cleavage agent specific for the one or more non-canonical nucleotides, thereby creating a strand retained library of nucleic acids.

19. The method of claim 18, wherein the cleavage agent comprises a glycosylase and a polyamine.

20. The method of claim 18, wherein the one or more non-canonical nucleotides comprise uracil or inosine.

21. The method of claim 18, wherein the single-stranded nucleic acid template is generated by:

i. performing a first strand synthesis on an RNA in a presence of the one or more non-canonical nucleotides, thereby forming a first strand synthesis product; and

ii. performing a fragmentation reaction.

22. The method of any one of claims 18-21, wherein the adapter comprises a recognition sequence for a restriction endonuclease specific for double-stranded DNA.

23. The method of claim 22, further comprising,

e) hybridizing a probe to a sequence of the primer extension product;

f) extending the probe with a DNA polymerase, thereby producing a partial duplex nucleic acid; and

g) treating the partial duplex nucleic acid with a restriction endonuclease specific for double-stranded DNA, thereby cleaving double-stranded DNA fragments at the recognition sequence.

24. The method of claim 23, further comprising performing PCR with a primer that is hybridizable to a sequence of the adapter, thereby amplifying a desired nucleic acid.

25. The method of claim 24, further comprising sequencing a portion of the desired nucleic acid.

26. The method of claim 18, wherein the single-stranded nucleic acid template comprises bacterial ribosomal RNA, mitochondrial DNA, human globin mRNA, human cytoplasmic rRNA, human mitochondrial rRNA, grape cytoplasmic rRNA, grape mitochondrial rRNA, or grape chloroplast rRNA.