

CLAIMS**WHAT IS CLAIMED IS:**

1. A composition comprising:
at least one target nucleic acid molecule and
an amplicon library,
wherein the amplicon library comprises a plurality of polynucleotides obtained from
amplification of the at least one target nucleic acid molecule,
wherein at least some of the polynucleotides comprise a terminator nucleotide,
wherein at least 5% of the polynucleotides are direct copies of the at least one target
nucleic acid molecule.
2. The composition of claim 1, wherein at least 10% of the polynucleotides are direct copies of
the at least one target nucleic acid molecule.
3. The composition of claim 1 or 2, wherein at least 15% of the polynucleotides are direct
copies of the at least one target nucleic acid molecule.
4. The composition of any one of claims 1-3, wherein at least 25% of the polynucleotides are
direct copies of the at least one target nucleic acid molecule.
5. The composition of any one of claims 1-4, wherein at least 50% of the polynucleotides are
direct copies of the at least one target nucleic acid molecule.
6. The composition of claim 1, wherein 5-50% of the polynucleotides are direct copies of the
at least one target nucleic acid molecule.
7. The composition of claim 1, wherein 5-20% of the polynucleotides are direct copies of the
at least one target nucleic acid molecule.
8. The composition of any one of claims 1-7, wherein no more than 50% of a cumulative
fraction of polynucleotides comprises sequences of at least 80% of a cumulative fraction of
sequences of the at least one target nucleic acid molecule.
9. The composition of any one of claims 1-8, wherein no more than 50% of the cumulative
fraction of the plurality of polynucleotides comprises sequences of the at least 85% of the
cumulative fraction of target nucleic acid sequences.
10. The composition of any one of claims 1-9, wherein no more than 50% of the cumulative
fraction the plurality of polynucleotides comprises sequences of the at least 90% of the
cumulative fraction of target nucleic acid sequences.
11. The composition of any one of claims 1-9, wherein the amplicon library has a Gini index of
no more than 0.5.

12. The composition of any one of claims 1-9, wherein the amplicon library has a Gini index of no more than 0.4.
13. The composition of any one of claims 1-12, wherein the plurality of polynucleotides are between about 50 and about 2000 nucleotides in length.
14. The composition of any one of claims 1-13, wherein polynucleotides are between about 400 and about 600 nucleotides in length.
15. The composition of any one of claims 1-14, wherein the number of polynucleotides is 100-5000.
16. The composition of any one of claims 1-14, wherein the number of polynucleotides is 250-1250.
17. The composition of any one of claims 1-14, wherein the number of polynucleotides is at least 100.
18. The composition of any one of claims 1-14, wherein the number of polynucleotides is at least 500.
19. The composition of any one of claims 1-14, wherein the number of polynucleotides is at least 1000.
20. The composition of any one of claims 1-19, wherein at least some of the polynucleotides comprise a barcode.
21. The composition of claim 16, wherein the barcode comprises a cell barcode.
22. The composition of claim 16 or 21, wherein the barcode comprises a sample barcode.
23. The composition of any one of claims 1-22, wherein at least some of the polynucleotides comprise a unique molecular identifier.
24. The composition of any one of claims 1-23, wherein the plurality of polynucleotides comprises sequences at least partially representative of a genome.
25. The composition of any one of claims 1-23, wherein the plurality of polynucleotides comprises sequences at least partially representative of at least two genomes.
26. The composition of any one of claims 1-23, wherein the plurality of polynucleotides comprises sequences from cDNA.
27. The composition of any one of claims 1-26, wherein at least 90% of the polynucleotides comprise a terminator nucleotide.
28. The composition of any one of claims 1-27, wherein at least 98% of the polynucleotides comprise a terminator nucleotide.
29. The composition of any one of claims 1-28, wherein the terminator nucleotide is attached to the 3' terminus of the at least some polynucleotides.

30. The composition of any one of claims 1-29, wherein the terminator nucleotide is selected from the group consisting of nucleotides with modification to the alpha group, C3 spacer nucleotides, locked nucleic acids (LNA), inverted nucleic acids, 2' fluoro nucleotides, 3' phosphorylated nucleotides, 2'-O-Methyl modified nucleotides, and trans nucleic acids.
31. The composition of any one of claims 1-30, wherein the nucleotides with modification to the alpha group are alpha-thio dideoxynucleotides.
32. The composition of any one of claims 1-31, wherein the terminator nucleotide comprises modifications of the r group of the 3' carbon of the deoxyribose.
33. The composition of any one of claims 1-32, wherein the terminator nucleotide is selected from the group consisting of 3' blocked reversible terminator containing nucleotides, 3' unblocked reversible terminator containing nucleotides, terminators containing 2' modifications of deoxynucleotides, terminators containing modifications to the nitrogenous base of deoxynucleotides, and combinations thereof.
34. The composition of any one of claims 1-32, wherein the terminator nucleotides is selected from the group consisting of dideoxynucleotides, inverted dideoxynucleotides, 3' biotinylated nucleotides, 3' amino nucleotides, 3'-phosphorylated nucleotides, 3'-O-methyl nucleotides, 3' carbon spacer nucleotides including 3' C3 spacer nucleotides, 3' C18 nucleotides, 3' Hexanediol spacer nucleotides, acyclonucleotides, and combinations thereof.
35. The composition of any one of claims 1-34, wherein the composition is contained in a droplet.
36. A composition comprising:
at least one target nucleic acid molecule and
an amplicon library,
wherein the amplicon library comprises a plurality of polynucleotides obtained from amplification of the at least one target nucleic acid molecule,
wherein at least some of the polynucleotides comprise a terminator nucleotide,
wherein no more than 50% of a cumulative fraction of polynucleotides comprises sequences of at least 80% of a cumulative fraction of sequences of the at least one target nucleic acid molecule.
37. The composition of claim 36, wherein no more than 50% of the cumulative fraction of the plurality of polynucleotides comprises sequences of the at least 85% of the cumulative fraction of target nucleic acid sequences.

38. The composition of claim 36 or 37, wherein no more than 50% of the cumulative fraction the plurality of polynucleotides comprises sequences of the at least 90% of the cumulative fraction of target nucleic acid sequences.
39. The composition of any one of claims 36-38, wherein the plurality of polynucleotides are between about 50 and about 2000 nucleotides in length.
40. The composition of any one of claims 36-39, wherein polynucleotides are between about 400 and about 600 nucleotides in length.
41. The composition of any one of claims 36-40, wherein at least some of the polynucleotides comprise a barcode.
42. The composition of claim 41, wherein the barcode comprises a cell barcode.
43. The composition of claim 41 or 42, wherein the barcode comprises a sample barcode.
44. The composition of any one of claims 36-43, wherein at least some of the polynucleotides comprise a unique molecular identifier.
45. The composition of any one of claims 36-44, wherein the plurality of polynucleotides comprises sequences at least partially representative of a genome.
46. The composition of any one of claims 36-44, wherein the plurality of polynucleotides comprises sequences at least partially representative of at least two genomes.
47. The composition of any one of claims 36-44, wherein the plurality of polynucleotides comprises sequences from cDNA.
48. The composition of any one of claims 36-47, wherein at least 90% of the polynucleotides comprise a terminator nucleotide.
49. The composition of any one of claims 36-48, wherein at least 98% of the polynucleotides comprise a terminator nucleotide.
50. The composition of any one of claims 36-49, wherein the terminator nucleotide is attached to the 3' terminus of the at least some polynucleotides.
51. The composition of any one of claims 36-50, wherein the terminator nucleotide is selected from the group consisting of nucleotides with modification to the alpha group, C3 spacer nucleotides, locked nucleic acids (LNA), inverted nucleic acids, 2' fluoro nucleotides, 3' phosphorylated nucleotides, 2'-O-Methyl modified nucleotides, and trans nucleic acids.
52. The composition of any one of claims 36-51, wherein the nucleotides with modification to the alpha group are alpha-thio dideoxynucleotides.
53. The composition of any one of claims 36-52, wherein the terminator nucleotide comprises modifications of the r group of the 3' carbon of the deoxyribose.

54. The composition of any one of claims 36-53, wherein the terminator nucleotide is selected from the group consisting of 3' blocked reversible terminator containing nucleotides, 3' unblocked reversible terminator containing nucleotides, terminators containing 2' modifications of deoxynucleotides, terminators containing modifications to the nitrogenous base of deoxynucleotides, and combinations thereof.
55. The composition of any one of claims 36-53, wherein the terminator nucleotides is selected from the group consisting of dideoxynucleotides, inverted dideoxynucleotides, 3' biotinylated nucleotides, 3' amino nucleotides, 3'-phosphorylated nucleotides, 3'-O-methyl nucleotides, 3' carbon spacer nucleotides including 3' C3 spacer nucleotides, 3' C18 nucleotides, 3' Hexanediol spacer nucleotides, acyclonucleotides, and combinations thereof.
56. The composition of any one of claims 36-54, wherein the composition is contained in a droplet.
57. A method of amplifying a target nucleic acid molecule, the method comprising:
- contacting a sample comprising the target nucleic acid molecule, at least one amplification primer, at least one nucleic acid polymerase, and a mixture of nucleotides, wherein the mixture of nucleotides comprises at least one terminator nucleotide which terminates nucleic acid replication by the polymerase, and
 - amplifying the target nucleic acid molecule to generate a plurality of terminated amplification products, wherein the replication proceeds by strand displacement replication.
58. The method of claim 37, wherein the amplification is performed under substantially isothermal conditions.
59. The method of claim 37 or 58, wherein the amplification is performed under conditions wherein the temperature varies by no more than 10 degrees C.
60. The method of any one of claims 37-59, wherein the amplification is performed under conditions wherein the temperature varies by no more than 5 degrees C.
61. The method of any one of claims 37-60, wherein the nucleic acid polymerase is a DNA polymerase.
62. The method of claim 61, wherein the DNA polymerase is a strand displacing DNA polymerase.
63. The method of any one of claims 37-62, wherein the nucleic acid polymerase is bacteriophage phi29 (Φ 29) polymerase, genetically modified phi29 (Φ 29) DNA polymerase, Klenow Fragment of DNA polymerase I, phage M2 DNA polymerase, phage phiPRD1 DNA polymerase, Bst DNA polymerase, Bst large fragment DNA polymerase,

- exo(-) Bst polymerase, exo(-)Bca DNA polymerase, Bsu DNA polymerase, Vent_R DNA polymerase, Vent_R (exo-) DNA polymerase, Deep Vent DNA polymerase, Deep Vent (exo-) DNA polymerase, IsoPol DNA polymerase, DNA polymerase I, Terminator DNA polymerase, T5 DNA polymerase, Sequenase, T7 DNA polymerase, T7-Sequenase, or T4 DNA polymerase.
64. The method of any one of claims 37-63, wherein the nucleic acid polymerase comprises 3'->5' exonuclease activity and the at least one terminator nucleotide inhibits the 3'->5' exonuclease activity.
65. The method of any one of claims 37-63, wherein the nucleic acid polymerase does not comprise 3'->5' exonuclease activity.
66. The method of any one of claims 37-63, wherein the polymerase is Bst DNA polymerase, exo(-) Bst polymerase, exo(-) Bca DNA polymerase, Bsu DNA polymerase, Vent_R (exo-) DNA polymerase, Deep Vent (exo-) DNA polymerase, Klenow Fragment (exo-) DNA polymerase, or Terminator DNA polymerase.
67. The method of any one of claims 37-66, wherein the least one terminator nucleotide comprises modifications of the r group of the 3' carbon of the deoxyribose.
68. The method of any one of claims 37-67, wherein the at least one terminator nucleotide is selected from the group consisting of 3' blocked reversible terminator containing nucleotides, 3' unblocked reversible terminator containing nucleotides, terminators containing 2' modifications of deoxynucleotides, terminators containing modifications to the nitrogenous base of deoxynucleotides, and combinations thereof.
69. The method of any one of claims 37-68, wherein the at least one terminator nucleotide is selected from the group consisting of dideoxynucleotides, inverted dideoxynucleotides, 3' biotinylated nucleotides, 3' amino nucleotides, 3'-phosphorylated nucleotides, 3'-O-methyl nucleotides, 3' carbon spacer nucleotides including 3' C3 spacer nucleotides, 3' C18 nucleotides, 3' Hexanediol spacer nucleotides, acyclonucleotides, and combinations thereof.
70. The method of any one of claims 37-69, wherein the at least one terminator nucleotide are selected from the group consisting of nucleotides with modification to the alpha group, C3 spacer nucleotides, locked nucleic acids (LNA), inverted nucleic acids, 2' fluoro nucleotides, 3' phosphorylated nucleotides, 2'-O-Methyl modified nucleotides, and trans nucleic acids.
71. The method of any one of claims 37-70, wherein the nucleotides with modification to the alpha group are alpha-thio dideoxynucleotides.

72. The method of any one of claims 37-71, wherein the amplification primers are 4 to 70 nucleotides in length.
73. The method of any one of claims 37-72, wherein the at least one amplification primer is 4 to 20 nucleotides in length.
74. The method of any one of claims 37-73, wherein the at least one amplification primer comprises a randomized region.
75. The method of claim 74, wherein the randomized region is 4 to 20 nucleotides in length.
76. The method of claim 74 or 75, wherein the randomized region is 8 to 15 nucleotides in length.
77. The method of any one of claims 37-76, wherein the amplification products are between about 50 and about 2000 nucleotides in length.
78. The method of any one of claims 37-77, wherein the amplification products are between about 200 and about 1000 nucleotides in length.
79. The method of any one of claims 37-78, wherein the method further comprising an additional amplification step using PCR.
80. A method of sequencing a target nucleic acid molecule, the method comprising:
- a. contacting a sample comprising the target nucleic acid molecule, at least one amplification primer, at least one nucleic acid polymerase, and a mixture of nucleotides, wherein the mixture of nucleotides comprises at least one terminator nucleotide which terminates nucleic acid replication by the polymerase, and
 - b. amplifying the target nucleic acid molecule to generate a plurality of terminated amplification products, wherein the replication proceeds by strand displacement replication;
 - c. removing at least one terminator nucleotide from the terminated amplification products;
 - d. ligating the molecules obtained in step (c) to adaptors, thereby generating a library of amplification products; and
 - e. sequencing the library of amplification products.
81. The method of claim 80, further comprising repairing ends and A-tailing.
82. The method of any one of claims 80 or 81, wherein the target nucleic acid is DNA.
83. The method of claim 82, wherein the DNA is a cDNA.
84. The method of claim 82, wherein the DNA is a genomic DNA.
85. The method of any one of claims 80-84, wherein the at least one amplification primer comprises two or more primers.

86. The method of any one of claims 80-85, wherein the at least one amplification primer is a random primer.
87. The method of any one of claims 80-86, wherein the at least one amplification primer comprise a barcode.
88. The method of claim 87, wherein the barcode comprises a cell barcode.
89. The method of claim 87 or 88, wherein the barcode comprises a sample barcode.
90. The method of any one of claims 80-89, wherein the method further comprising an additional amplification step using PCR.
91. The method of any one of claims 80-90, wherein the amplification primers comprise a unique molecular identifier (UMI).
92. The method of any one of claims 80-91, wherein the method further comprises denaturing the target nucleic acid or genomic DNA before the initial primer annealing.
93. The method of claim 92, wherein denaturation is conducted under alkaline conditions followed by neutralization.
94. The method of any one of claims 80-93, wherein the sample, the amplification primers, the nucleic acid polymerase, and the mixture of nucleotides are contained in a microfluidic device.
95. The method of any one of claims 80-94, wherein the sample, the amplification primers, the nucleic acid polymerase, and the mixture of nucleotides are contained in a droplet.
96. The method of any one of claims 80-95, wherein the sample is selected from tissue(s) samples, cells, biological fluid samples, bone marrow samples, semen samples, biopsy samples, cancer samples, tumor samples, cell lysate samples, forensic samples, archaeological samples, paleontological samples, infection samples, production samples, whole plants, plant parts, microbiota samples, viral preparations, soil samples, marine samples, freshwater samples, household or industrial samples, and combinations and isolates thereof.
97. The method of claim 96, wherein the biological fluids are selected from blood, urine, saliva, lymphatic fluid, cerebrospinal fluid (CSF), amniotic fluid, pleural fluid, pericardial fluid, ascites, and aqueous humor.
98. A method of sequencing a single cell, the method comprising:
 - a. providing a cell lysate from the single cell;
 - b. contacting the cell lysate with at least one amplification primer, at least one nucleic acid polymerase, and a mixture of nucleotides, wherein the mixture of nucleotides

comprises at least one terminator nucleotide which terminates nucleic acid replication by the polymerase, and

- c. amplifying the target nucleic acid molecule to generate a plurality of terminated amplification products, wherein the replication proceeds by strand displacement replication;
- d. removing at least one terminator nucleotide from the terminated amplification products;
- e. ligating the molecules obtained in step (d) to adaptors, thereby generating a library of amplification products; and
- f. sequencing the library of amplification products.

99. The method of claim 98, wherein cell lysis is accompanied by proteolysis.

100. The method of claim 98 or 99, wherein the sample, at least one amplification primer, the nucleic acid polymerase, and the mixture of nucleotides are contained in a microfluidic device.

101. The method of any one of claims 98-100, wherein the sample, the at least one amplification primer, the nucleic acid polymerase, and the mixture of nucleotides are contained in a droplet.

102. The method of any one of claims 98-101, wherein the at least one amplification primer is attached to a solid support.

103. The method of claim 102, wherein the solid support is a bead.

104. The method of claims 102 or 103, wherein the at least one amplification primer is attached to a solid support via a cleavable linker.

105. The method of any one of claims 98-104, wherein the at least one amplification primer comprises a barcode.

106. The method of claim 104, wherein the method further comprises cleaving the cleavable linker prior to amplification.

107. The method of any one of claims 98-106, wherein the method further comprising an additional amplification step using PCR.

108. The method of any one of claims 98-107, wherein the cell is selected from an animal cell, a plant cell, a fungal cell, a bacterial cell, and a protozoal cell.

109. The method of claim 108, wherein the animal cell is a human cell.

110. The method of any one of claims 98-109, wherein the cell is selected from a cell from a preimplantation embryo, a stem cell, a fetal cell, a tumor cell, a suspected cancer cell, a cancer cell, a cell subjected to a gene editing procedure, a cell from a pathogenic organism,

a cell obtained from a forensic sample, a cell obtained from an archeological sample, and a cell obtained from a paleontological sample.

111. The method of claim 110, wherein the preimplantation embryo cell is a blastomere.
112. The method of claim 111, wherein the blastomere is obtained from an eight-cell stage embryo produced by in vitro fertilization.
113. The method of any one of claims 110-112, further comprising determining the presence of disease predisposing germline or somatic variants in the embryo cell.
114. The method of claim 110, wherein the pathogenic organism is a bacterium, a fungus or a protozoan.
115. The method claim 114, wherein the cell obtained from a pathogenic organism is obtained from fluid taken from a patient, microbiota sample or an indwelling medical device.
116. The method of claim 114 or 115, further comprising the step of determining the identity of the pathogenic organism.
117. The method of any one of claims 110-116, further comprising determining the presence of genetic variants responsible for resistance of the pathogenic organism to a treatment.
118. The method of any one of claims 98-110, wherein the cell is a tumor cell, a suspected cancer cell, or a cancer cell.
119. The method of any one of claims 98-110 or 118, further comprising determining the presence of one or more diagnostic or prognostic mutations.
120. The method of any one of claims 98-110, 118, or 119, further comprising determining the presence of germline or somatic variants responsible for resistance to a treatment.
121. The method any one of claims 98-110, wherein the cell is a cell subjected to a gene editing procedure.
122. The method of claim 121, further comprising determining the presence of unplanned mutations caused by the gene editing process.
123. The method any one of claims 98-122, further comprising determining the history of a cell lineage.
124. A use of the method of any one of claims 37-123 for identifying low frequency sequence variants.
125. The use of claim 124, wherein the low frequency sequence variants constitute $\geq 0.01\%$ of the total sequences.
126. The use of claim 124, wherein the low frequency sequence variants constitute $\geq 0.05\%$ of the total sequences.

127. The use of claim 124, wherein the low frequency sequence variants constitute $\geq 0.10\%$ of the total sequences.
128. A method of determining the mutagenicity of an environmental condition, the method comprising:
- exposing cells to the environmental condition;
 - isolating single cells from the population;
 - providing a cell lysate from a single cell;
 - contacting the cell lysate with at least one amplification primer, at least one nucleic acid polymerase, and a mixture of nucleotides, wherein the mixture of nucleotides comprises at least one terminator nucleotide which terminates nucleic acid replication by the polymerase, and
 - amplifying the target nucleic acid molecule to generate a plurality of terminated amplification products, wherein the replication proceeds by strand displacement replication;
 - removing at least one terminator nucleotide from the terminated amplification products;
 - ligating the molecules obtained in step (f) to adaptors, thereby generating a library of amplification products; and
 - sequencing the library of amplification products, and
 - comparing the sequences of amplification products to at least one reference sequence to identify mutations.
129. The method of claim 128, wherein the single cell is a human cell.
130. The method of claim 128 or 129, wherein environmental condition comprises a chemical substance.
131. The method of claim 128 or 129, wherein the environmental condition comprises radiation.
132. The method of claim 128 or 129, wherein the environmental condition comprises ultraviolet light.
133. The method of any one of claims 128-132, wherein the single cells originate from liver, skin, kidney, blood, or lung.
134. The method of any one of claims 128-133, wherein at least some of the amplification products comprise a barcode.
135. The method of claim 134, wherein the barcode comprises a cell barcode.
136. The method of claim 134 or 135, wherein the barcode comprises a sample barcode.

137. The method of any one of claims 128-136, wherein at least some of the amplification primers comprise a unique molecular identifier (UMI).
138. The method of any one of claims 128-137, wherein the method further comprising an additional amplification step using PCR.