METHOD FOR NUCLEIC ACID AMPLIFICATION

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. provisional patent application number 62/623,471 filed on January 29, 2018, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Research methods that utilize nucleic amplification, *e.g.,* Next Generation Sequencing, provide large amounts of information on complex samples, genomes, and other nucleic acid sources. However there is a need for highly accurate, scalable, and efficient nucleic acid amplification and sequencing methods for research, diagnostics, and treatment involving small samples.

BRIEF SUMMARY

[0002a] In one aspect, there is provided a method of amplification comprising:
(a) contacting at least one nucleic acid, at least one primer, a nucleic acid polymerase comprising 3'-5' exonuclease activity, and a mixture of nucleotides, wherein the mixture of nucleotides comprises alpha-thio dideoxy nucleotide triphosphates; and
(b) amplifying the at least one nucleic acid to generate a plurality of amplicons, wherein at least a portion of the plurality of amplicons comprises a 3' terminal alpha-thio dideoxy nucleotide.

[0002b] In another aspect, there is provided a method of amplification comprising:
(a) contacting genomic DNA, at least one primer, a nucleic acid polymerase comprising 3'-5' exonuclease activity, and a mixture of nucleotides, wherein the mixture of nucleotides comprises at least one terminator nucleotide that terminates nucleic acid replication by the polymerase; and
(b) amplifying the genomic DNA to generate a plurality of amplicons, wherein at least a portion of the plurality of amplicons comprises a 3' terminal terminator nucleotide.

[0003] Provided herein are compositions 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. Further provided herein are compositions wherein at least 10% of the polynucleotides are direct copies of the at least one target nucleic acid molecule. Further provided herein are compositions wherein at least 15% of the polynucleotides are direct copies of the at least one target nucleic acid molecule. Further provided herein are compositions wherein at least 25% of the polynucleotides are direct copies of the at least one target nucleic acid molecule. Further provided herein are compositions wherein at least 50% of

the polynucleotides are direct copies of the at least one target nucleic acid molecule. Further provided herein are compositions wherein 5-50% of the polynucleotides are direct copies of the at least one target nucleic acid molecule. Further provided herein are compositions wherein 5-25% of the polynucleotides are direct copies of the at least one target nucleic acid molecule. Further provided herein are compositions 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. Further provided herein are compositions 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. Further provided herein are compositions 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. Further provided herein are compositions wherein the amplicon library has a Gini index of no more

CLAIMS

WHAT IS CLAIMED IS:

1. A method of amplification comprising:

(a) contacting at least one nucleic acid, at least one primer, a nucleic acid polymerase comprising 3'-5' exonuclease activity, and a mixture of nucleotides, wherein the mixture of nucleotides comprises alpha-thio dideoxy nucleotide triphosphates; and

(b) amplifying the at least one nucleic acid to generate a plurality of amplicons, wherein at least a portion of the plurality of amplicons comprises a 3' terminal alpha-thio dideoxy nucleotide.

2. A method of amplification comprising:

(a) contacting genomic DNA, at least one primer, a nucleic acid polymerase comprising 3'-5' exonuclease activity, and a mixture of nucleotides, wherein the mixture of nucleotides comprises at least one terminator nucleotide that terminates nucleic acid replication by the polymerase; and

(b) amplifying the genomic DNA to generate a plurality of amplicons, wherein at least a portion of the plurality of amplicons comprises a 3' terminal terminator nucleotide.

3. The method of claim 1, wherein the at least one nucleic acid is 400 to 600 nucleotides in length.

4. The method of claim 1, wherein the plurality of amplicons are 50 to 5000 nucleotides in length, and wherein the at least one nucleic acid comprises 100 to 5000 nucleic acids.

5. The method of claim 1, wherein the at least one nucleic acid is human genomic DNA.

6. The method of claim 5, wherein the human genomic DNA is isolated from 1 to 10 embryo cells.

7. The method of claim 1, wherein the mixture comprises deoxynucleoside triphosphates and the alpha-thio dideoxy nucleotides in a 5:1 to 20:1 molar ratio.

8. The method of claim 1 or 7, wherein the mixture comprises the alpha-thio dideoxy nucleotides in a concentration of 250 to 1200 micromolar.

9. The method of claim 1 or 2, wherein the nucleic acid polymerase is a bacteriophage phi29 polymerase.

10. The method of claim 1 or 2, wherein the nucleic acid polymerase is selected from the group consisting of a phage M2 DNA polymerase, a phage phiPRD1 DNA polymerase, a T5 DNA polymerase, a T7 DNA polymerase, a T4 DNA polymerase, a Bsu DNA polymerase and DNA polymerase I.

11. The method of claim 1, wherein the 3'-5' exonuclease activity of the polymerase is reduced by at least 85% for an amplicon of the at least a portion of the plurality of amplicons comprising a terminal 3' alpha-thio dideoxy nucleotide relative to an amplicon comprising a terminal 3' deoxynucleotide phosphate.

12. The method of claim 2, wherein the at least one terminator nucleotide comprises a modification to an alpha group.

13. The method of claim 12, wherein the at least one terminator nucleotide with the modification to the alpha group is an alpha-thio dideoxynucleotide.

14. The method of claim 2, wherein the at least one terminator nucleotide is selected from the group consisting of a locked nucleic acid, an inverted nucleic acid, a trans nucleic acid, an acyclonucleotide, a 2′ fluoro nucleotide, a 3′ phosphorylated nucleotide, a 2′-O-Methyl modified nucleotide, a dideoxynucleotide, an inverted dideoxynucleotide, a 3′ biotinylated nucleotide, a 3′ amino nucleotide, a 3′-phosphorylated nucleotide, a 3′-O-methyl nucleotide, a 3′ carbon spacer nucleotide, a 3′ C3 spacer nucleotide, a 3′ C18 nucleotide, and a 3′ Hexanediol spacer nucleotide.

15. The method of claim 13, wherein the genomic DNA is isolated from 1 to 10 human embryonic cells, and wherein the nucleic acid polymerase is a bacteriophage phi29 polymerase.