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.

SUMMARY

[0002a] In one aspect, the present disclosure refers to a composition comprising: a polymerase comprising 3'-5' exonuclease activity; at least one target nucleic acid molecule which is a genomic DNA, and an amplicon library, wherein the amplicon library comprises at least 100 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 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, wherein the terminator nucleotide is an irreversible terminator, and wherein at least 3% of the polynucleotides are direct copies of the at least one target nucleic acid molecule.

[0002b] In another aspect, the present disclosure refers to a method of amplifying a target nucleic acid molecule, the method comprising: a. contacting a sample comprising the target nucleic acid molecule which is a genomic DNA, at least one amplification primer, at least one nucleic acid polymerase comprising 3' to 5' exonuclease activity, and a mixture of nucleotides, wherein the mixture of nucleotides comprises at least one terminator nucleotide which terminates nucleic acid replication by the polymerase, 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, and b. amplifying the target nucleic acid molecule to generate a plurality of terminated amplification products, wherein the replication proceeds by strand displacement replication and wherein the amplification is performed under conditions wherein the temperature varies by no more than 10 degrees C.

[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 composition comprising:

a polymerase comprising 3'-5' exonuclease activity;

at least one target nucleic acid molecule which is a genomic DNA, and

an amplicon library,

wherein the amplicon library comprises at least 100 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 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, wherein the terminator nucleotide is an irreversible terminator, and wherein at least 3% of the polynucleotides are direct copies of the at least one target nucleic acid molecule.

2. The composition of claim 1, wherein at least 5% of the polynucleotides are direct copies of the at least one target nucleic acid molecule.

~~3. The composition of claim 1, wherein the at least 100 polynucleotides are direct copies obtained from at least 5 cycles of amplification of the at least one target nucleic acid molecule.~~

~~4.~~3. The composition of claim 1, wherein 5-50% of the polynucleotides are direct copies of the at least one target nucleic acid molecule.

~~5.~~4. The composition of claim 1, 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.

~~6.~~5. The composition of claim 1, wherein the plurality of polynucleotides are between ~~about~~ 50 and ~~about~~ 2000 nucleotides in length.

~~7.~~6. The composition of claim 1, wherein the number of polynucleotides is 100-5000.

~~8.~~7. The composition of claim 1, wherein the number of polynucleotides is at least 1000.

~~9.~~8. The composition of claim 1, wherein the plurality of polynucleotides comprises sequences at least partially representative of a genome.

~~10.~~9. The composition of claim 1, wherein at least 90% of the polynucleotides comprise a terminator nucleotide.

~~11.~~10. The composition of claim 1, wherein the terminator nucleotide is attached to the 3’ terminus of the at least some polynucleotides.

~~12. The composition of claim 1, 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.~~

~~13.~~11. The composition of claim 1, wherein the nucleotides with modification to the alpha group are alpha-thio dideoxynucleotides.

~~14.~~12. The composition of claim 1, wherein the terminator nucleotide comprises modifications of the r group of the 3’ carbon of the deoxyribose.

~~15.~~13. The composition of claim 1, 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.

~~16.~~14. A method of amplifying a target nucleic acid molecule, the method comprising:

a. contacting a sample comprising the target nucleic acid molecule which is a genomic DNA, at least one amplification primer, at least one nucleic acid polymerase comprising 3’ to 5’ exonuclease activity, and a mixture of nucleotides, wherein the mixture of nucleotides comprises at least one terminator nucleotide which terminates nucleic acid replication by the polymerase, 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, and

b. amplifying the target nucleic acid molecule to generate a plurality of terminated amplification products, wherein the replication proceeds by strand displacement replication and wherein the amplification is performed under conditions wherein the temperature varies by no more than 10 degrees C.

~~17.~~15. The method of claim ~~16~~14, 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.

~~18.~~16. The method of claim ~~16~~14, 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.

~~19. The method of claim 16, 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.~~

~~20.~~17. The method of claim ~~16~~14, wherein the nucleotides with modification to the alpha group are alpha-thio dideoxynucleotides.