CLAIMS

1. A method for reducing non-specific nucleic acid binding to a substrate, comprising:

contacting the substrate with an oligonucleotide, wherein the oligonucleotide comprises a backbone comprising a phosphorothioate bond, and wherein non-specific nucleic acid binding to the substrate is reduced compared to a substrate not contacted with the oligonucleotide, optionally, wherein the substrate comprises a bead; optionally wherein the oligonucleotide comprises one or more of:

(i) at least 50% of the backbone comprises phosphorothioate bonds;

(ii)a length greater than 20 consecutive nucleotides;

(iii) a sequence lacking the capability of forming a hairpin structure at a temperature less than 25°C;

(iv) a nucleotide sequence motif of [AAA(CT)X]Y, wherein X is 2 to 5, and Y is 2 to 6; and

(v) the oligonucleotide is single-stranded.

2. The method of claim 1, comprising one or more of:

(i) wherein the oligonucleotide comprises at least 90% sequence identity to the nucleotide sequence set forth in any one of SEQ ID NOs:02-04;

(ii) further comprising an agent bound to a surface of the bead, wherein the agent is selected from streptavidin, biotin, or a derivative thereof;

(iii) wherein the nucleic acid comprises DNA; optionally, wherein the nucleic acid comprises genomic DNA; and

(iv) further comprising a transposome bound to the bead.

3. The method of claim 1 or 2, wherein the contacting comprises one or more of: (i) contacting the substrate with the oligonucleotide for a period greater than 30 minutes; optionally, wherein the contacting is for a period greater than 1 hour, 6 hours, or 12 hours; (ii) contacting the substrate with the oligonucleotide at room temperature or at about 4°C; (iii) contacting the substrate with a plurality of transposomes; and (iv) contacting the substrate with genomic DNA.

4. A blocked bead composition comprising a magnetic bead in contact with an oligonucleotide, wherein the oligonucleotide comprises a backbone comprising a phosphorothioate bond, and wherein non-specific nucleic acid binding to the blocked bead is reduced compared to non-specific nucleic acid binding to a bead not in contact with the oligonucleotide, optionally wherein the oligonucleotide comprises one or more of:

(i) at least 50% of the backbone comprises phosphorothioate bonds;

(ii)a length greater than 20 consecutive nucleotides;

(iii) a sequence lacking the capability of forming a hairpin structure at a temperature less than 25°C;

(iv) a nucleotide sequence motif of [AAA(CT)X]Y, wherein X is 2 to 5, and Y is 2 to 6; and

(v) the oligonucleotide is single-stranded.

5. The blocked bead composition of claim 4, comprising one or more of:

(i) wherein the oligonucleotide comprises at least 90% sequence identity to the nucleotide sequence set forth in any one of SEQ ID NOs:02-04;

(ii) further comprising an agent bound to a surface of the bead, wherein the agent is selected from streptavidin, biotin, or a derivative thereof;

(iii) wherein the nucleic acid comprises DNA; optionally, wherein the nucleic acid comprises genomic DNA; and

(iv) further comprising a transposome bound to the bead.

6. A method for preparing a nucleic acid library, comprising:

(a) obtaining a plurality of transposomes comprising transposon adaptors, wherein the plurality of transposomes are immobilized on a plurality beads comprising the blocked bead composition of claim 4 or 5;

(b) contacting a plurality of nucleic acid fragments with the plurality of transposomes to obtain a plurality of polynucleotides;

(c) amplifying the plurality of polynucleotides to obtain amplified polynucleotides; and

(d) adding library adapters to each end of the amplified polynucleotides, thereby obtaining the nucleic acid library.

7. The method of claim 6, wherein step (c) or (d) comprises contacting the plurality of polynucleotides with the blocked bead composition of claim 1 or 2.

8. The method of claim 6 or 7, wherein the plurality of the transposomes is immobilized on the plurality of beads at a density such that one or more of: (i) an average length of the plurality of polynucleotides greater than about 1 kbp; (ii) the number of transposomes immobilized on each bead of the plurality of beads is no more than about 100 transposomes; (iii) the plurality of beads comprises a total activity such that an average length of the plurality of polynucleotides greater than about 1 kbp; and (iv) the plurality of beads comprises an activity in a range from about 0.05 AU/µl to about 0.25 AU/µl.

9. The method of any one of claims 6-8, comprising one or more of:

(i) wherein the transposon adapters comprise the same sequence; optionally, wherein the transposon adapters comprise the nucleotide sequence of SEQ ID NO:01; and

(ii) wherein the transposomes of the plurality of transposomes are the same; optionally, wherein the transposomes of the plurality of transposomes are B15 transposomes

10. The method of any one of claims 6-9, wherein the step (c) comprises a mutagenesis PCR, such that mutations are introduced into amplified polynucleotides; optionally, wherein the mutagenesis PCR comprises amplifying the plurality of polynucleotides with a low bias DNA polymerase, and/or with a nucleotide analogue; optionally, wherein the nucleotide analogue comprises dPTP, and/or 8-oxo-dGTP; optionally wherein, one or more of (i) the low bias DNA polymerase is a Thermococcal polymerase, or a functional derivative thereof; optionally, wherein the Thermococcal polymerase is derived from a Thermococcal strain selected from the group consisting of T. kodakarensis, T. siculi, T. celer and T. sp KS-1; and (ii) the mutagenesis PCR comprises no more than 12 cycles, 10 cycles, 9 cycles, 8 cycles, 7 cycles, 6 cycles, 5 cycles, 4 cycles, 3 cycles, or 2 cycles.

11. The method of any one of claims 6-10, wherein a first end of a polynucleotide of the plurality of polynucleotides is capable of annealing to a second end of the polynucleotide of the plurality of polynucleotides; and/or, wherein a first end of an amplified polynucleotide is capable of annealing to a second end of the amplified polynucleotide.

12. The method of any one of claims 6-11, wherein step (c) further comprises a suppression PCR; optionally, wherein the suppression PCR comprises use of a single amplification primer, and/or the suppression PCR comprises no more than 16 cycles, 14 cycles, 10 cycles, 9 cycles, 8 cycles, 7 cycles, 6 cycles, 5 cycles, 4 cycles, 3 cycles, or 2 cycles.

13. The method of any one of claims 6-12, wherein the amplified polynucleotides have an average length greater than about 1 kbp, 2 kbp, 3 kbp, 4 kbp, 5 kbp, 10 kbp, 15 kbp, or 20 kbp.

14. The method of claim 12 or 13, further comprising enriching for target nucleic acids in the amplified polynucleotides; optionally, wherein the enriching comprises hybridizing a plurality of selection probes with the amplified polynucleotides, wherein the plurality of selection probes is capable of specifically hybridizing with the target nucleic acids; optionally wherein one or more of (i) the plurality of selection probes lacks sequences capable of hybridizing to a repetitive genomic DNA element; optionally, wherein the repetitive genomic DNA element is selected from a tandem repeat, an Alu repeat, a short interspersed nuclear element (SINE), a long interspersed nuclear element (LINE), an integrated viral sequence, a viral long terminal repeat (LTR), and a transposon; and (ii) further comprising amplifying the target nucleic acids.

15. The method of any one of claims 6-14, wherein step (d) comprises contacting the amplified polynucleotides with an additional plurality of transposomes; optionally, wherein the additional plurality of transposomes comprise transposon adapters comprising (i) indexes, and/or (iii) sequencing primer binding sites; optionally, further comprising enriching for target polynucleotides in the library of nucleic acids; optionally, wherein the enriching comprises hybridizing a plurality of selection probes with the library of nucleic acids, wherein the plurality of selection probes is capable of specifically hybridizing with the target polynucleotides; optionally, further comprising amplifying the target polynucleotides.

16. A method for determining a sequence of a target nucleic acid, comprising:

performing the method of any one of claims 6-15;

sequencing the library of nucleic acids to obtain sequence reads; and

assembling sequence reads to obtain the sequence of a target nucleic acid.

17. The method of any one of claims 6-16, wherein one or more of:

(i) one or more of steps (a)-(d) is performed in a reaction vessel, and the method further comprises adding carrier nucleic acids to the reaction vessel;

(ii) an amount of the plurality of nucleic acid fragments is less than about 100 ng, 50 ng, 30 ng, 20 ng, 10 ng, 5 ng, or 1 ng; and

(iii) the plurality of nucleic acid fragments is mammalian; optionally, wherein the plurality of nucleic acid fragments is human; optionally, wherein the plurality of nucleic acid fragments comprises genomic DNA.

18. A method of sequencing a target nucleic acid, comprising:

(a) obtaining a sample comprising the target nucleic acid and carrier nucleic acids, wherein target nucleic acid comprises an adaptor capable of hybridizing to a primer;

(b) obtaining a substrate comprising the primer;

(c) amplifying the target nucleic acid on the substrate, comprising:

(i) hybridizing the target nucleic acid to the primer, and

(ii) extending the primer; and

(d) sequencing the amplified target nucleic acid.

19. The method of claim 18, wherein one or more of:

(A) one of more of:

(i) the target nucleic acid has a concentration less than 10 nM, 100 pM, 20 pM or 5 pM;

(ii) step (a) lacks adjusting the concentration of the target nucleic acid;

(iii) the target nucleic acid comprises a single adaptor;

(iv) the adaptor comprises a nucleotide sequence selected from a P5 sequence (SEQ ID NO:05), a P7 sequence (SEQ ID NO:06), or a complement thereof;

(v) the target nucleic acid is (i) derived from a bacteriophage genome; optionally, wherein the bacteriophage genome is a PhiX genome; or (ii) is mammalian; optionally, wherein the target nucleic acid is human; and

(vi) the target nucleic acid comprises DNA; optionally, wherein the target nucleic acid is single-stranded; and

(B) one or more of:

(i) the carrier nucleic acids lack the adaptor;

(ii) the carrier nucleic acids and the target nucleic acid are each derived from a genome of an organism of a different kingdom, phylum, class, order, family, genus or species; optionally, wherein the carrier nucleic acids are derived from a genome of a fish; optionally, wherein the carrier nucleic acids comprise salmon sperm DNA, tRNA, or siRNA;

(iii) the carrier nucleic acids comprise DNA;

(iv) the carrier nucleic acids comprise single-stranded nucleic acids; and

(v) the carrier nucleic acids have an average length less than 5000 consecutive nucleotides; optionally, an average length in a range from 100 to 5000 consecutive nucleotides; optionally, an average length in a range from 100 to 1000 consecutive nucleotides.

20. The method of claim 18 or 19, wherein the amplifying comprises: (i) bridge amplification; (ii) exclusion amplification; optionally, wherein the exclusion amplification comprises one or more reagents selected from a polymerase or recombinase, a single-stranded DNA binding protein, a crowding agent, and creatine phosphate (CP); or (iii) isothermal amplification.