CLAIMS

WHAT IS CLAIMED IS:

1. A method for preparing a polynucleotide library, the method comprising:
    a. in a first tailing reaction, adding a first tail to each of a plurality of target polynucleotides by template-independent polymerization, wherein the first tailing reaction comprises a first adapter comprising an overhang that hybridizes to the first tail;
    b. in a first ligation reaction, ligating a strand of the first adapter to the first tail;
    c. amplifying target polynucleotides comprising the strand of the first adapter by extending a first primer hybridized to the strand of the first adapter;
    d. in a second tailing reaction, adding a second tail to each of a plurality of the amplified target polynucleotides by template-independent polymerization, wherein the second tailing reaction comprises a second adapter comprising an overhang that hybridizes to the second tail; and
    e. in a second ligation reaction, ligating a strand of the second adapter to the second tail.
2. The method of claim 1, wherein the method comprises one or more of: (a) fragmenting polynucleotides to produce the target polynucleotides; (b) dephosphorylation of one or both ends of the target polynucleotides; and (c) denaturing double-stranded polynucleotides to single-stranded polynucleotides to produce the target polynucleotides.
3. The method of any one of the preceding claims, wherein the plurality of target polynucleotides comprises single-stranded DNA.
4. The method of any one of the preceding claims, wherein the target polynucleotides are treated prior to step (b) of claim 2 to differentially modify methylated cytosines or unmethylated cytosines.
5. The method of claim 4, wherein the differentially modifying comprises treating the target polynucleotides with bisulfite.
6. The method of any one of the preceding claims, further comprising amplifying target polynucleotides comprising the strand of the second adapter by extending a second primer hybridized to the strand of the second adapter.
7. The method of claim 6, further comprising an amplification reaction with a third primer and a fourth primer, wherein (i) the third primer hybridizes to a complement of

at least a portion of the first primer, and (ii) the fourth primer hybridizes to a complement of at least a portion of the second primer.

8. The method of claim 7, wherein the third primer, the fourth primer, or both comprise an index sequence that identifies a sample source of the target polynucleotides.

9. The method of claim 7 or 8, further comprising sequencing amplification products of the amplification comprising the third and fourth primer.

10. The method of claim 9, further comprising grouping sequencing reads according to the index sequence.

11. The method of claim 9 or 10, wherein sequencing comprises detecting a sequence variant or a difference in nucleotide methylation, relative to a reference sequence.

12. A method for preparing a polynucleotide library, the method comprising:
    a. in a first tailing reaction, adding a first tail to each of a plurality of target polynucleotides by template-independent polymerization, wherein the first tailing reaction comprises a first adapter comprising an overhang that hybridizes to the first tail;
    b. in a first ligation reaction, ligating a strand of the first adapter to the first tail;
    c. amplifying target polynucleotides comprising the strand of the first adapter by extending a first primer hybridized to the strand of the first adapter; and
    d. in a second ligation reaction, ligating a strand of a second adapter to the amplified target polynucleotides.

13. The method of claim 12, wherein the second ligation reaction comprises, in a second tailing reaction, adding a second tail to each of a plurality of the amplified target polynucleotides by template-independent polymerization.

14. The method of claim 13, wherein the second tailing reaction comprises a second adapter comprising an overhang that hybridizes to the second tail.

15. The method of claim 14, wherein, in the second ligation reaction, ligating a strand of the second adapter to the second tail.

16. The method of claim 12, wherein the second ligation reaction comprises a second adapter comprising an overhang that hybridizes to the amplified target polynucleotides.

17. The method of any one of claims 12-16, wherein the method comprises one or more of: (a) fragmenting polynucleotides to produce the target polynucleotides; (b) dephosphorylation of one or both ends of the target polynucleotides; and (c)

denaturing double-stranded polynucleotides to single-stranded polynucleotides to produce the target polynucleotides.

18. The method of any one of claims 12-17, wherein the plurality of target polynucleotides comprises single-stranded DNA.

19. The method of any one of claims 12-18, wherein the target polynucleotides are treated prior to step (b) of claim 17 to differentially modify methylated cytosines or unmethylated cytosines.

20. The method of claim 19, wherein the differentially modifying comprises treating the target polynucleotides with bisulfite.

21. The method of any one claims 12-20, further comprising amplifying target polynucleotides comprising the strand of the second adapter by extending a second primer hybridized to the strand of the second adapter.

22. The method of claim 21, further comprising an amplification reaction with a third primer and a fourth primer, wherein (i) the third primer hybridizes to a complement of at least a portion of the first primer, and (ii) the fourth primer hybridizes to a complement of at least a portion of the second primer.

23. The method of claim 22, wherein the third primer, the fourth primer, or both comprise an index sequence that identifies a sample source of the target polynucleotides.

24. The method of claim 22 or 23, further comprising sequencing amplification products of the amplification comprising the third and fourth primer.

25. The method of claim 24, further comprising grouping sequencing reads according to the index sequence.

26. A kit for preparing a polynucleotide library, the kit comprising:
    a. a template-independent polymerase;
    b. a first pool of nucleotides that can be polymerized by the template-independent polymerase;
    c. a second pool of nucleotides that can be polymerized by the template-independent polymerase;
    d. a first adapter comprising an overhang that is hybridizable to tails formed by polymerizing the first pool of polynucleotides; and
    e. a second adapter comprising an overhang that is hybridizable to the amplified target polynucleotides.

27. The kit of claim 26, further comprising a first primer that is hybridizable to a strand of the first adapter under conditions for a primer extension reaction.

28. The kit of claim 26 or 27, further comprising a second primer that is hybridizable to a strand of the second adapter under conditions for a primer extension reaction.

29. The kit of claim 28, wherein the sequence of the first primer that is hybridizable to the strand of the first adapter is different from the sequence of the second primer that is hybridizable to the second adapter.

30. The kit of claim 28 or 29, further comprising a third primer and a fourth primer, wherein (i) the third primer is hybridizable to a complement of at least a portion of the first primer under conditions for a primer extension reaction, and (ii) the fourth primer is hybridizable to a complement of at least a portion of the second primer under conditions for a primer extension reaction.