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 plurality of target polynucleotides comprises single-stranded polynucleotides, wherein the first tailing reaction further comprises hybridizing to the first tail an overhang within one strand of a first double-stranded adapter;

b. in a first ligation reaction, ligating the other 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 at the end opposite to the first primer.

2. The method of claim 1, wherein the second adaptor is double-stranded, wherein step d of the method is performed by:

d-1 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 further comprises hybridizing to the second tail an overhang within one strand of the second adaptor; and

d-2 in the second tailing reaction, ligating the other strand of the second adaptor to the second tail.

3. The method of claim 1, wherein the second adaptor is double-stranded, wherein (i) the second ligation reaction does not comprise a second tailing reaction; and (ii) the second ligation reaction comprises hybridizing to the amplified target polynucleotides an overhang within one strand of the second adaptor and directly ligating the other strand of the second adaptor to the amplified target polynucleotides.

4. The method of any one of claims 1-3, 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.

5. The method of any one of claims 1-4, wherein the target polynucleotides are treated prior to step (b) of claim 4 to differentially modify methylated cytosines or unmethylated cytosines.

6. The method of claim 5, wherein the differentially modifying comprises treating the target polynucleotides with bisulfite.

7. The method of any one of claims 1-6, 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.

8. The method of claim 7, 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.

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

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

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

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