

CLAIMS

1. A method of preparing a nucleic acid library, comprising:
 - (a) obtaining a plurality of nucleic acids, wherein the plurality of nucleic acids is single-stranded nucleic acids;
 - (b) dephosphorylating the 5' ends of the single-stranded nucleic acids;
 - (c) ligating single-stranded first adaptors to the 3' ends of the single-stranded nucleic acids in the presence of a ligase, wherein the 3' end of the single-stranded first adaptors comprises a blocking group, thereby obtaining ligated single-stranded nucleic acids and non-ligated single-stranded first adaptors;
 - (d) removing non-ligated single-stranded first adaptors by hybridizing the non-ligated single-stranded first adaptors with a capture probe, wherein the 3' end of the capture probe and the 5' end of the capture probe each comprise a blocking group;
 - (e) phosphorylating the 5' ends of the ligated single-stranded nucleic acids; and
 - (f) ligating a second adaptor to the 5' ends of the phosphorylated ligated single-stranded nucleic acids in the presence of the ligase, thereby obtaining a library of nucleic acids.
2. The method of claim 1, wherein the second adaptor is attached to a substrate.
3. The method of claim 1, wherein steps (b) – (f) are performed in a single reaction volume.
4. The method of claim 1, wherein the blocking group comprises a 3'-spacer C3 or a dideoxynucleotide.
5. The method of claim 1, wherein the ligation of the single-stranded first adaptor and/or the ligating of the second adaptor is performed in the presence of a volume excluding agent selected from the group consisting of a polyethylene glycol (PEG), dextran, hetastarch, and polyvinylpyrrolidone.
6. The method of claim 5, wherein the first ligation step and/or second ligation step is performed in a reaction volume comprising at least about 37 % (wt/vol) PEG.
7. The method of claim 1, wherein the capture probe is in solution.
8. The method of claim 1, wherein the single-stranded first adaptor and/or second adaptor comprises a sequencing primer binding site or an adaptor index.

9. The method of claim 8, wherein the sequencing primer binding site comprises a P7 sequence, a P5 sequence, or a complement of a P7 sequence, a complement of a P5 sequence, a reverse complement of a P7 sequence, or a reverse complement of a P5 sequence.
10. The method of claim 8, wherein the single-stranded first adaptor comprises a first adaptor index and the second adaptor comprises a second adaptor index.
11. The method of claim 10, wherein the first adaptor index is different from the second adaptor index.
12. The method of claim 1, wherein the average size of a nucleic acid of the plurality of nucleic acids is less than 200 nucleotides.
13. The method of claim 1, wherein step (f) is performed subsequent to step (d).
14. The method of claim 1, wherein step (f) is performed in a reaction mixture comprising the capture probe.
15. The method of claim 1, wherein steps (b) – (f) are performed sequentially in a single reaction mixture.