

strandedness of the library. Accordingly, methods that capture single-stranded RNA (e.g., for library preparation), without requiring second strand synthesis would be useful for processing and analyzing nucleic acid containing RNA.

5 Summary

Provided in some examples are methods for producing a nucleic acid library, comprising combining (i) a nucleic acid composition comprising single-stranded nucleic acid (ssNA), (ii) a first oligonucleotide, and (iii) a plurality of first scaffold polynucleotide species, where (a) each polynucleotide in the plurality of first scaffold polynucleotide species comprises an ssNA hybridization region and a first oligonucleotide hybridization region; and (b) the nucleic acid composition, the first oligonucleotide, and the plurality of first scaffold polynucleotide species are combined under conditions in which a molecule of the first scaffold polynucleotide species is hybridized to (i) a first ssNA terminal region and (ii) a molecule of the first oligonucleotide, thereby forming hybridization products in which an end of the molecule of the first oligonucleotide is adjacent to an end of the first ssNA terminal region. In some examples, a method comprises prior to the combining, contacting the first oligonucleotide and/or the plurality of first scaffold polynucleotide species with an agent comprising a phosphatase activity under conditions in which the first oligonucleotide and/or the plurality of first scaffold polynucleotide species is/are dephosphorylated, thereby generating a dephosphorylated first oligonucleotide and/or dephosphorylated first scaffold polynucleotide species. In some examples, a method is an SSB-free method for producing a library from ssNA.

In one aspect, the present disclosure refers to a method of producing a nucleic acid library, comprising:

combining (i) a nucleic acid composition comprising single-stranded nucleic acid (ssNA), (ii) a first oligonucleotide, and (iii) a plurality of first scaffold polynucleotide species, wherein:

(a) each polynucleotide in the plurality of first scaffold polynucleotide species comprises an ssNA hybridization region and a first oligonucleotide hybridization region;

(b) the nucleic acid composition, the first oligonucleotide, and the plurality of first scaffold polynucleotide species are combined under conditions in which a molecule of the first scaffold polynucleotide species is hybridized to (i) a first ssNA terminal region and (ii) a molecule of the first oligonucleotide, thereby forming hybridization products in which an end of the molecule of the first oligonucleotide is adjacent to an end of the first ssNA terminal region;

(c) the ssNA is not combined with a single-stranded nucleic acid binding protein (SSB) prior to the combining or during the combining; and

(d) wherein the ssNA is not modified prior to the combining.

Also provided in some examples are compositions comprising a nucleic acid composition comprising single-stranded nucleic acid (ssNA); a first oligonucleotide; and a plurality of first scaffold polynucleotide species each comprising an ssNA hybridization region and a first oligonucleotide hybridization region.

Also provided in some examples are kits comprising a first oligonucleotide; a plurality of first scaffold polynucleotide species each comprising an ssNA hybridization region and a first oligonucleotide hybridization region; and instructions for using the first oligonucleotide and the plurality of first scaffold polynucleotide species to produce a nucleic acid library.

What is claimed is:

1. A method of producing a nucleic acid library, comprising:
combining (i) a nucleic acid composition comprising single-stranded nucleic acid (ssNA), (ii) a first oligonucleotide, and (iii) a plurality of first scaffold polynucleotide species, wherein:
 - (a) each polynucleotide in the plurality of first scaffold polynucleotide species comprises an ssNA hybridization region and a first oligonucleotide hybridization region;
 - (b) the nucleic acid composition, the first oligonucleotide, and the plurality of first scaffold polynucleotide species are combined under conditions in which a molecule of the first scaffold polynucleotide species is hybridized to (i) a first ssNA terminal region and (ii) a molecule of the first oligonucleotide, thereby forming hybridization products in which an end of the molecule of the first oligonucleotide is adjacent to an end of the first ssNA terminal region;
 - (c) the ssNA is not combined with a single-stranded nucleic acid binding protein (SSB) prior to the combining or during the combining; and
 - (d) wherein the ssNA is not modified prior to the combining.
2. The method of claim 1, further comprising prior to the combining,
contacting the ssNA with an agent comprising a phosphatase activity under conditions in which the ssNA is dephosphorylated, thereby generating dephosphorylated ssNA; and
covalently linking a second oligonucleotide to the 5' end of the ssNA, wherein (i) the second oligonucleotide comprises a phosphate at the 3' end, and (ii) the covalently linking of the second oligonucleotide comprises contacting the ssNA and the second oligonucleotide with an agent comprising a single-stranded ligase activity under conditions in which the 5' end of the ssNA is covalently linked to the 3' end of the second oligonucleotide.
3. The method of claim 1, further comprising combining the nucleic acid composition with (iv) a second oligonucleotide, and (v) a plurality of second scaffold polynucleotide species, wherein:

(e) each polynucleotide in the plurality of second scaffold polynucleotide species comprises an ssNA hybridization region and a second oligonucleotide hybridization region; and

(f) the nucleic acid composition, the second oligonucleotide, and the plurality of second scaffold polynucleotide species are combined under conditions in which a molecule of the second scaffold polynucleotide species is hybridized to (i) a second ssNA terminal region and (ii) a molecule of the second oligonucleotide, thereby forming hybridization products in which an end of the molecule of the second oligonucleotide is adjacent to an end of the second ssNA terminal region.

4. The method of claim 3, further comprising prior to the combining:

contacting the first oligonucleotide and/or the plurality of first scaffold polynucleotide species with an agent comprising a phosphatase activity under conditions in which the first oligonucleotide and/or the plurality of first scaffold polynucleotide species is/are dephosphorylated, thereby generating a dephosphorylated first oligonucleotide and/or dephosphorylated first scaffold polynucleotide species; and

contacting the second oligonucleotide and/or the plurality of second scaffold polynucleotide species with an agent comprising a phosphatase activity under conditions in which the second oligonucleotide and/or the plurality of second scaffold polynucleotide species is/are dephosphorylated, thereby generating a dephosphorylated second oligonucleotide and/or dephosphorylated second scaffold polynucleotide species.

5. The method of claim 3 or 4, wherein prior to the combining, each of the first scaffold polynucleotide species is hybridized to a first oligonucleotide to form a plurality of first scaffold duplex species, and each of the second scaffold polynucleotide species is hybridized to a second oligonucleotide to form a plurality of second scaffold duplex species.

6. The method of any one of claims 3-5, further comprising covalently linking the adjacent ends of the first oligonucleotide and the first ssNA terminal region, and covalently linking the adjacent ends of the second oligonucleotide and the second ssNA terminal region, thereby generating covalently linked hybridization products.

7. The method of claim 6, wherein the covalently linking comprises contacting the hybridization products with an agent comprising a ligase activity under conditions in which an end of the first ssNA terminal region is covalently linked to an end of the first oligonucleotide and an end of the second ssNA terminal region is covalently linked to an end of the second oligonucleotide.
8. The method of claim 5, wherein some or all of the duplexes in the plurality of first scaffold duplex species comprise an adenylation modification at the 5' end of the first oligonucleotide, and the plurality of first scaffold duplex species are combined with and covalently linked to the ssNA in the absence of ATP, thereby forming intermediate covalently linked hybridization products.
9. The method of claim 8, wherein the intermediate covalently linked hybridization products are combined with and covalently linked to the plurality of second scaffold duplex species and ATP, thereby forming covalently linked hybridization products.
10. The method of any one of claims 3-9, wherein the ssNA hybridization region of each of the first polynucleotide species is different than the ssNA hybridization region in other first polynucleotide species in the plurality of first polynucleotide species, and the ssNA hybridization region of each of the second polynucleotide species is different than the ssNA hybridization region in other second polynucleotide species in the plurality of second polynucleotide species.
11. The method of any one of claims 1-10, wherein the ssNA hybridization region comprises a random sequence.
12. The method of any one of claims 1-11, wherein the ssNA hybridization region comprises one or more universal bases.
13. The method of any one of claims 3-10, wherein:
- a) the first oligonucleotide comprises one or more of
 - (i) a first primer binding domain,
 - (ii) a first sequencing adapter, or part thereof,
 - (iii) a unique molecular identifier (UMI), and

- (iv) an index;
- b) the first oligonucleotide hybridization region comprises one or more of
 - (i) a polynucleotide complementary to the first primer binding domain,
 - (ii) a polynucleotide complementary to the first sequencing adapter, or part thereof,
 - (iii) a polynucleotide complementary to the unique molecular identifier (UMI), and
 - (iv) a polynucleotide complementary to the index;
- c) the second oligonucleotide comprises one or more of
 - (i) a second primer binding domain,
 - (ii) a second sequencing adapter, or part thereof,
 - (iii) a unique molecular identifier (UMI), and
 - (iv) an index; and
- d) the second oligonucleotide hybridization region comprises one or more of
 - (i) a polynucleotide complementary to the second primer binding domain,
 - (ii) a polynucleotide complementary to the second sequencing adapter, or part thereof,
 - (iii) a polynucleotide complementary to the unique molecular identifier (UMI), and
 - (iv) a polynucleotide complementary to the index.

14. The method of any one of claims 3-13, wherein:

- (a) the first oligonucleotide comprises one or more modified nucleotides, or some or all of the first scaffold polynucleotide species comprise one or more modified nucleotides, or the first oligonucleotide comprises one or more modified nucleotides and some or all of the first scaffold polynucleotide species comprise one or more modified nucleotides; and/or
- (b) the second oligonucleotide comprises one or more modified nucleotides, some or all of the second scaffold polynucleotide species comprise one or more modified nucleotides, or the second oligonucleotide comprises one or more modified nucleotides and some or all of the second scaffold polynucleotide species comprise one or more modified nucleotides.

15. The method of claim 14, wherein the one or more modified nucleotides are capable of blocking covalent linkage of the oligonucleotide to another oligonucleotide, polynucleotide, or nucleic acid molecule, and wherein the oligonucleotide comprises the one or more modified nucleotides at an end not adjacent to the ssNA.

16. The method of claim 14 or 15, wherein the one or more modified nucleotides are capable of blocking covalent linkage of the scaffold polynucleotide to another oligonucleotide, polynucleotide, or nucleic acid molecule, and wherein the scaffold polynucleotide comprises the one or more modified nucleotides at one or both ends of the polynucleotide.

17. The method of any one of claims 14-16, wherein the one or more modified nucleotides comprise a ligation-blocking modification.

18. The method of any one of claims 6-10 and 13-17, further comprising denaturing the covalently linked hybridization products, thereby generating single-stranded ligation products.

19. The method of claim 18, further comprising:

(a) combining the single-stranded ligation products with a third oligonucleotide under conditions in which the third oligonucleotide is hybridized to a dimer of the first oligonucleotide and the second oligonucleotide, thereby forming an oligonucleotide dimer hybridization product, wherein the oligonucleotide dimer hybridization product comprises a cleavage site; and

(b) contacting the oligonucleotide dimer hybridization product with a cleavage agent.

20. The method of any one of claims 1-19, wherein the nucleic acid composition comprises single-stranded DNA (ssDNA), single-stranded RNA (ssRNA), or ssDNA and ssRNA.

21. The method of any one of claims 1-20, wherein one or both native ends of the ssNA are present when the ssNA is combined with the first oligonucleotide and the plurality of first scaffold polynucleotide species.

22. The method of any one of claims 1-21, wherein the ssNA is from cell-free nucleic acid.