

Claims:

1. A method of producing a double-stranded nucleic acid library wherein each fragment in the library comprises a unique molecular identifier (UMI) wherein the method comprises:
 - a. applying a sample comprising double-stranded target nucleic acids to a first transposome complex comprising:
 - i. a first transposase,
 - ii. a first transposon comprising a first 3' end transposon end sequence, a first adapter sequence, and a first UMI, and
 - iii. a second transposon comprising a sequence all or partially complementary to the first 3' end transposon end sequence;
 - b. tagging the double-stranded target nucleic acids with the first transposome complex to produce tagged double-stranded target nucleic acid fragments, wherein each tagged double-stranded target nucleic acid fragment comprises the first adapter sequence and the first UMI,
 - c. releasing the tagged double-stranded target nucleic acid fragments from the first transposome complex,
 - d. optionally extending the tagged double-stranded target nucleic acid fragments,
 - e. optionally ligating the first transposon with the tagged double-stranded target nucleic acid fragments or with the extended, tagged double-stranded target nucleic acid fragments,
 - f. producing tagged double-stranded target nucleic acid fragments, and
 - g. amplifying the tagged double-stranded target nucleic acid fragments.
2. The method of claim 1, wherein the first UMI in the first transposon is located between the first adapter sequence and the first 3' transposon end sequence.
3. The method of claim 1 or 2, wherein the first adapter sequence in the first transposon is located between the first UMI and the first 3' transposon end sequence.
4. The method of any one of claims 1-3, further comprising a second transposome complex comprising:
 - a. a second transposase,

- b. a third transposon comprising a second adapter sequence and a second 3' transposon end sequence, and
 - c. a fourth transposon comprising a sequence all or partially complementary to the second 3' end transposon end sequence.
- 5. The method of claim 4, wherein the tagmenting step produces tagmented double-stranded target nucleic acid fragments comprising:
 - a. a first strand comprising the first adapter sequence and the first UMI, and
 - b. a second strand comprising the second adapter sequence.
- 6. The method of claim 4 or 5, wherein
 - a. the third transposon further comprises a second UMI, and
 - b. the second adapter sequence is located between the second UMI and the second 3' transposon end sequence.
- 7. The method of claim 6, wherein the tagmenting step produces double-stranded target nucleic acid fragments comprising:
 - a. a first strand comprising the first adapter sequence and the first UMI, and
 - b. a second strand comprising the second adapter sequence and the second UMI.
- 8. A method of producing a double-stranded nucleic acid library wherein each fragment in the library comprises a UMI wherein the method comprises:
 - a. applying a sample comprising double-stranded target nucleic acids to a transposome complex comprising:
 - i. a transposase,
 - ii. a first transposon comprising a first 3' end transposon end sequence and a first adapter sequence, and
 - iii. a second transposon comprising a sequence all or partially complementary to the first 3' end transposon end sequence;
 - b. tagmenting a first strand of the double-stranded target nucleic acids with the transposome complex to produce tagmented double-stranded target nucleic acid fragments, wherein each tagmented double-stranded target nucleic acid fragment comprises the first adapter sequence,

- c. releasing the tagmented double-stranded target nucleic acid fragments from the transposome complex,
 - d. hybridizing a polynucleotide comprising a second adapter sequence, a UMI, and a sequence all or partially complementary to the first 3' end transposon sequence,
 - e. optionally extending a second strand of the tagmented double-stranded target nucleic acid fragments,
 - f. optionally ligating the polynucleotide with the tagmented double-stranded target nucleic acid fragments or with the extended tagmented double-stranded target nucleic acid fragments,
 - g. producing tagmented double-stranded target nucleic acid fragments comprising the UMI, wherein the UMI is located directly adjacent to the 3' end of an insert DNA, and
 - h. amplifying the tagmented double-stranded target nucleic acid fragments comprising the UMI.
9. A method of producing a double-stranded nucleic acid library wherein each fragment in the library comprises a UMI wherein the method comprises:
- a. applying a sample comprising double-stranded target nucleic acids to a transposome complex comprising:
 - i. a transposase,
 - ii. a first transposon comprising a first 3' end transposon end sequence and a first adapter sequence, and
 - iii. a second transposon comprising a sequence all or partially complementary to the first 3' end transposon end sequence;
 - b. tagmenting a first strand of the double-stranded target nucleic acids with the transposome complex to produce tagmented double-stranded target nucleic acid fragments, wherein each tagmented double-stranded target nucleic acid fragment comprises the first adapter sequence,
 - c. releasing the tagmented double stranded target nucleic acid fragments from transposome complex,
 - d. hybridizing a first polynucleotide comprising a UMI, and a second adapter sequence,

- e. optionally extending a second strand of the double-stranded target nucleic acid fragments, and copying the first polynucleotide,
 - f. optionally adding a second polynucleotide comprising regions complementary to the first polynucleotide to produce a double-stranded adapter,
 - g. optionally extending a second strand of the tagmented double-stranded target nucleic acid fragments,
 - h. optionally ligating the second polynucleotide with the second strand of the extended tagmented double-stranded target nucleic acid fragments,
 - i. producing tagmented double stranded target nucleic acid fragments comprising the UMI, wherein the UMI is located between the double-stranded target nucleic acid fragments and the second adapter sequence, and
 - j. amplifying the tagmented double-stranded target nucleic acid fragments comprising the UMI.
10. A method of producing a double-stranded nucleic acid library wherein each fragment in the library comprises two different UMIs wherein the method comprises
- a. applying a sample comprising double-stranded target nucleic acids to:
 - i. a first transposome complex comprising:
 - 1. a first transposase and
 - 2. a first forked adapter comprising (a) a first transposon on a first strand of the double-stranded target nucleic acid fragments, and (b) a second transposon, wherein
 - the first transposon comprises a first 3' end transposon end sequence, a first copy of a first adapter sequence, and a first UMI, and
 - the second transposon comprises a first copy of a second adapter sequence, and a sequence all or partially complementary to the first 3' end transposon end sequence and the first UMI;
 - further wherein the first copy of the first adapter sequence is single-stranded and the first copy of the second adapter sequence includes a double-stranded portion; and
 - ii. a second transposome complex comprising:

1. a second transposase and
 2. a second forked adapter comprising (a) a third transposon on a second strand of the double-stranded target nucleic acid fragments, and (b) a fourth transposon, wherein
 - the third transposon comprises a second 3' end transposon end sequence, a second copy of the first adapter sequence, and a second UMI, and
 - the third transposon comprises a second copy of the second adapter sequence, and a sequence all or partially complementary to the second 3' end transposon end sequence and the second UMI;
 - further wherein the second copy of the first adapter sequence is single-stranded and the second copy of the second adapter sequence includes a double-stranded portion;
 - b. fragmenting the double-stranded target nucleic acids with the forked adapters to produce fragmented double-stranded target nucleic acid fragments, wherein each fragmented double-stranded target nucleic acid fragment comprises the first and second copies of the first adapter sequence, the first UMI, the first and second copies of the second adapter sequence, and the second UMI,
 - c. releasing the fragmented double-stranded target nucleic acid fragments from the transposome complexes,
 - d. optionally extending the fragmented double-stranded target nucleic acid fragments,
 - e. ligating the second and fourth transposons with the double-stranded target nucleic acid fragments or with the extended fragmented double-stranded target nucleic acid fragments,
 - f. producing fragmented double-stranded target nucleic acid fragments, and
 - g. amplifying the fragmented double-stranded target nucleic acid fragments.
11. A method of producing a double-stranded nucleic acid library wherein each fragment in the library comprises four different UMIs wherein the method comprises
- a. applying a sample comprising double-stranded target nucleic acids to:
 - i. a first transposome complex comprising:
 1. a first transposase and

2. a first forked adapter comprising (a) a first transposon on a first strand of the double-stranded target nucleic acid fragments, and (b) a second transposon, wherein

the first transposon comprises a first 3' end transposon end sequence, a first copy of a first adapter sequence, a first copy of a first UMI, and a first copy of a second adapter sequence, and

the second transposon comprises a sequence all or partially complementary to the first 3' end transposon end sequence, a first copy of a third adapter sequence, a first copy of a second UMI, and a fourth adapter sequence;

further wherein the first copies of the first, second, and third adapter sequences are single-stranded and the fourth adapter sequence includes a double-stranded portion; and

- ii. a second transposome complex comprising:

1. a second transposase and

2. a second forked adapter comprising (a) a third transposon on a second strand of the double-stranded target nucleic acid fragments, and (b) a fourth transposon, wherein

the third transposon comprises a second 3' end transposon end sequence, a first copy of a fifth adapter sequence, a first copy of a third UMI, and a first copy of a sixth adapter sequence;

the fourth transposon comprises a sequence all or partially complementary to the second 3' end transposon end sequence, a first copy of a seventh adapter sequence, a first copy of a fourth UMI, and an eighth adapter sequence;

further wherein the first copies of the fifth, sixth, and seventh adapter sequences are single-stranded and the eighth adapter sequence includes a double-stranded portion;

- b. tagging the double-stranded target nucleic acids with the forked adapters to produce tagmented double-stranded target nucleic acid fragments, wherein each tagmented double-stranded target nucleic acid fragment comprises the first copies of the

- first, second, third, fifth, sixth, and seventh adapter sequences; the first copies of the first, second, third, and fourth UMIs; the sixth adapter sequence; and the eighth adapter sequence,
- c. releasing the tagmented double-stranded target nucleic acid fragments from the transposome complexes,
 - d. optionally extending the tagmented double-stranded target nucleic acid fragments,
 - e. ligating the second and fourth transposons with the double-stranded target nucleic acid fragments or with the extended tagmented double-stranded target nucleic acid fragments,
 - f. producing tagmented double-stranded target nucleic acid fragments, and
 - g. amplifying the tagmented double-stranded target nucleic acid fragments.
12. The method of any one of claims 6, 7, 10 or 11, wherein the first, second, third, and fourth UMIs may be complementary or different sequences.
13. The method of any one of claims 1-12, wherein the double-stranded target nucleic acids are double-stranded DNA, ctDNA, cfDN, RNA, cDNA or DNA:RNA duplexes are generated from RNA.
14. The method of any one of claims 1-13, wherein:
- a. the first adapter sequence is a 5' first-read sequencing adapter sequence;
 - b. the second adapter sequence is a 5' second-read sequencing adapter sequence; and/or
 - c. the 5' first-read and 5' second-read sequencing adapter sequences comprise unique primer binding sites.
15. The method of any one of claims 1, 2, 4-8, or 12-14, wherein the first UMI is on the first strand of the tagmented double-stranded target nucleic acid fragments.
16. The method of any one of claims 1, 3, 5-7, 12-14, wherein a first copy of the first UMI is on the first strand and a second copy of the first UMI is on the second strand of the tagmented double-stranded target nucleic acid fragments.

17. The method of any one of claims 1-7, 12-14, wherein the first UMI is on the first strand of the tagmented double-stranded target nucleic acid fragments, the second UMI is on the second strand of the tagmented double-stranded target nucleic acid fragments.
18. The method of any one of claims 1-17, wherein the first, second, third, or fourth transposon further comprises:
- a biotin tag;
 - a first unique primer binding sequence, optionally wherein the first unique primer binding sequence comprises A2, A14, and/or B15; and/or
 - a second unique primer binding sequence, optionally wherein the second unique primer binding sequence comprises A2, A14, and/or B15.
19. The method of any one of claims 8-9 or 13-14, wherein the hybridizing step generates a forked adapter.
20. The method of any one of claims 1-19, further comprising extending from a 3' end of the double-stranded target nucleic acid fragments to a 5' end of the transposons.
21. The method of any one of claims 1-7 or 10-20, wherein the ligating step comprises ligating a 3' end of the tagmented double-stranded target nucleic acid fragments or a 3' end of the extended tagmented double-stranded target nucleic acid fragments with a 5' end of the first, second, or fourth transposon.
22. The method of any one of claims 8, 13-14, 18-21, wherein the polynucleotide comprises a 3' adapter comprising:
- a hairpin UMI,
 - a hairpin UMI and a universal hybridizing tail, optionally wherein the universal hybridizing tail comprises nucleotides that can bind to any DNA nucleotide.,
 - a splint ligation adapter, or
 - a 3' template switch oligonucleotide.
23. The method of claim 22, wherein the hairpin UMI is stable during the extending step and/or the ligating step, but not during the amplifying step, optionally wherein the hairpin UMI comprises a 3 or 4 base pair stem.
24. The method of claim 22, wherein

- a. the polynucleotide comprises a 3' adapter comprising a hairpin UMI,
 - b. the extending step comprises extending from a 3' end of the second strand of the tagmented double-stranded target nucleic acid fragments to a 5' end of the hairpin UMI, and optionally,
 - c. wherein the ligating step comprises ligating the 3' end of second strand of the extended tagmented double-stranded target nucleic acid fragments with the 5' end of the hairpin UMI.
25. The method of claim 22, wherein
- a. the polynucleotide comprises a splint ligation adapter, and
 - b. the extending step comprises extending from a 3' end of the second strand of the tagmented double-stranded target nucleic acid fragments to a 5' end of the splint ligation adapter.
26. The method of claim 22, wherein
- a. the polynucleotide comprises a template switch oligonucleotide, and
 - b. the extending step comprises extending from a 3' end of the second strand of the tagmented double-stranded target nucleic acid fragments to a junction in the template switch oligonucleotide by copying the first strand of the tagmented double-stranded target nucleic acid fragments,
 - c. switching templates from the first strand to an unpaired region of the 3' template switch oligonucleotide, and
 - d. copying the unpaired region of the 3' template switch oligonucleotide from the junction to a 5' end of the unpaired region of the 3' template switch oligonucleotide.
27. The method of claim 26, wherein the extending, switching, and copying are performed by a polymerase capable of DNA-directed template-switching, optionally wherein the polymerase capable of DNA-directed template-switching comprises MMLV reverse transcriptase.
28. The method of any one of claims 1-27, wherein the amplifying step comprises:
- a. adding oligonucleotides to one or both ends of the tagmented double-stranded target nucleic acid fragments for attaching the library to a solid support;
 - b. adding at least a first-read sequencing oligonucleotide and/or a second-read sequencing oligonucleotide;

- c. adding at least a P5 oligonucleotide and a P7 oligonucleotide;and/or
- d. adding at least a plurality of i5 oligonucleotides and a plurality of i7 oligonucleotides.

29. A method of sequencing a double-stranded nucleic acid library produced by the method of any one of claims 1-28, wherein the UMIs are sequenced to provide increased sensitivity in DNA sequencing.