

Claims

1. A method of preparing double-stranded nucleic acid fragments comprising adapters comprising:
 - a. combining a sample comprising nucleic acid with transposome complexes comprising:
 - i. a transposase;
 - ii. a first transposon comprising a modified transposon end sequence comprising a uracil, an inosine, a ribose, an 8-oxoguanine, a thymine glycol, a modified purine, and/or a modified pyrimidine; and
 - iii. a second transposon comprising a second transposon end sequence complementary to at least a portion of the first transposon end sequence;
 - b. preparing nucleic acid fragments;
 - c. combining the sample with (1) an endonuclease or (2) a combination of a DNA glycosylase and heat, basic conditions, or an endonuclease/lyase that recognizes abasic sites and cleaving the first transposon end at the uracil, inosine, ribose, 8-oxoguanine, thymine glycol, modified purine, and/or modified pyrimidine within the mosaic end sequence to remove all or part of the first transposon end from the nucleic acid fragments; and
 - d. ligating an adapter onto the 5' and/or 3' ends of the nucleic acid fragments.
2. The method of claim 1, wherein the modified purine is 3-methyladenine or 7-methylguanine and/or the modified pyrimidine is 5-methylcytosine, 5-formylcytosine, or 5-carboxycytosine.
3. The method of claim 1 or 2, wherein the nucleic acid is double-stranded DNA.
4. The method of claim 1 or 2, wherein the nucleic acid is RNA, and double-stranded cDNA or DNA:RNA duplexes are generated before combining with the transposome complexes.
5. The method of any one of claims 1-4, wherein the all or part of the first transposon end that is cleaved is partitioned away from the rest of the sample.
6. The method of any one of claims 1-5, further comprising filling in the 3' ends of the fragments and phosphorylating the 3' ends of fragments with a kinase before ligating.
7. The method of claim 6, wherein the filling in is performed with T4 DNA polymerase.

8. The method of claim 6 or 7, further comprising adding a single A overhang to the 3' end of the fragments.
9. The method of claim 8, wherein a polymerase adds the single A overhang.
10. The method of claim 9, wherein the polymerase is (i) Taq or (ii) Klenow fragment, exo-.
11. The method of any one of claims 1-10, wherein the fragments comprise 0-3 bases of the mosaic end sequence.
12. The method of any one of claims 1-11, wherein preparing fragments leads to preparation of at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% the number of fragments, as compared with preparing fragments with a transposome complex that comprises a first transposon comprising a transposon end sequence comprising a wildtype mosaic end sequence comprising SEQ ID No: 1.
13. The method of any one of claims 1-12, further comprising sequencing the fragments after ligating the adapter.
14. The method of claim 13, wherein:
 - a. the method does not require amplification of fragments before sequencing; or
 - b. fragments are amplified before sequencing.
15. The method of claim 13 or 14, further comprising enriching fragments of interest after ligating the adapter and before sequencing.
16. The method of any one of claims 1-14, wherein:
 - a. the modified transposon end sequence comprises a uracil and the combination of a DNA glycosylase and an endonuclease/lyase that recognizes abasic sites is a uracil-specific excision reagent (USER);
 - b. the modified transposon end sequence comprises an inosine and the endonuclease is endonuclease V;
 - c. the modified transposon end sequence comprises a ribose and the endonuclease is RNase HII;
 - d. the modified transposon end sequence comprises a 8-oxoguanine and the endonuclease is formamidopyrimidine-DNA glycosylase (FPG) or oxoguanine glycosylase (OGG);
 - e. the modified transposon end sequence comprises a thymine glycol and the DNA glycosylase is endonuclease EndoIII (Nth) or Endo VIII;

- f. the modified transposon end sequence comprises a modified purine and the DNA glycosylase is human 3-alkyladenine DNA glycosylase and the endonuclease is endonuclease III or VIII; or
- g. the modified transposon end sequence comprises a modified pyrimidine, and:
 - i. the DNA glycosylase is thymine-DNA glycosylase (TDG) or mammalian DNA glycosylase-methyl-CpG binding domain protein 4 (MBD4) and the endonuclease/lyase that recognizes abasic sites is the endonuclease is endonuclease III or VIII; or
 - ii. the endonuclease is DNA glycosylase/lyase ROS1 (ROS1).
- 17. The method of claim 16, wherein the USER is a mixture of uracil DNA glycosylase and endonuclease VIII or endonuclease III.
- 18. The method of claim 16, wherein the modified purine is 3-methyladenine or 7-methylguanine.
- 19. The method of claim 16, wherein the modified pyrimidine is 5-methylcytosine, 5-formylcytosine, or 5-carboxycytosine.
- 20. The method of any one of claims 1-19, wherein the first transposon comprises a modified transposon end sequence comprising more than one mutation chosen from a uracil, an inosine, a ribose, 8-oxoguanine, a thymine glycol, a modified purine, or a modified pyrimidine and the (1) an endonuclease or (2) a combination of a DNA glycosylase and heat, basic conditions, or an endonuclease/lyase that recognizes abasic sites is an enzyme mixture.
- 21. The method of claim 20, wherein the modified purine is 3-methyladenine or 7-methylguanine and/or the modified pyrimidine is 5-methylcytosine, 5-formylcytosine, or 5-carboxycytosine.
- 22. The method of any one of claims 1-21, wherein cleaving the first transposon end generates a sticky end for ligating the adapter.
- 23. The method of claim 22, wherein the sticky end is longer than one base.
- 24. The method of any one of claims 1-23, wherein
 - a. the adapter comprises a double-stranded adapter;
 - b. adapters are added to the 5' and 3' end of fragments;

c. the adapter comprises a unique molecular identifier (UMI), primer sequence, anchor sequence, universal sequence, spacer region, index sequence, capture sequence, barcode sequence, cleavage sequence, sequencing-related sequence, and combinations thereof;

d. the adapter comprises a UMI; and/or

e. the adapter is a forked adapter.

25. The method of claim 24, wherein the adapters added to the 5' and 3' end of the fragments are different.

26. The method of claim 24 or claim 25, wherein an adapter comprising a UMI is ligated to both the 3' and 5' end of fragments.

27. The method of any one of claims 1-26, wherein:

a. the ligating is performed with a DNA ligase;

b. the method is performed in a single reaction vessel;

c. the density of transposomes immobilized on the solid surface is selected to modulate fragment size and library yield of the immobilized fragments;

d. the method allows for bead-based normalization;

e. the sample comprises partially fragmented DNA;

f. the sample is formalin fixed paraffin embedded tissue or cell-free DNA; and/or

g. the library comprises fragments prepared by a single tagmentation event.