~~WHAT IS CLAIMED IS~~ Claims:

1. An annealed transposon/attachment polynucleotide hybrid comprising:

(a) a first transposon comprising a 3’ transposon end sequence and a 5’ adaptor sequence;

(b) a second transposon comprising a 5’ transposon end sequence and a 3’ adaptor sequence, wherein

(i) the 5’ transposon end sequence of the second transposon is complementary to the 3’ transposon end sequence of the first transposon,

(ii) the 5’ adaptor sequence of the first transposon is non-complementary to the 3’ adaptor sequence of the second transposon; and

(c) an attachment polynucleotide comprising:

(i) an attachment adaptor sequence hybridized to one of the two adaptor sequences; and

(ii) a binding element, wherein the binding element can be used to immobilize the attachment polynucleotide to a solid support.

2. A transposome complex comprising:

(a) a transposase;

(b) a first transposon comprising a 3’ transposon end sequence and a 5’ adaptor sequence;

(c) a second transposon comprising a 5’ transposon end sequence and a 3’ adaptor sequence, wherein

(i) the 5’ transposon end sequence of the second transposon is complementary to at least a portion of the 3’ transposon end sequence of the first transposon to form a double-stranded transposon end sequence to which the transposase binds,

(ii) the 5’ adaptor sequence of the first transposon is non-complementary to the 3’ adaptor sequence of the second transposon; and

(d) an attachment polynucleotide comprising:

(i) an attachment adaptor sequence hybridized to one of the two adaptor sequences and

(ii) a binding element, wherein the binding element can be used to immobilize the attachment polynucleotide to a solid support.

3. ~~An immobilized~~ The transposome complex of claim 2, ~~comprising:~~

~~a solid support; and~~

~~a~~ wherein the ~~transposome complex~~binding element ~~immobilized~~ immobilizes the transposome complex to the solid support, and

~~wherein the transposome complex comprises:~~

~~(a) a transposase;~~

~~(b) a first transposon comprising a 3' transposon end sequence and a 5' adaptor sequence;~~

~~(c) a second transposon comprising a 5' transposon end sequence and a 3' adaptor sequence, wherein the 5' transposon end sequence is complementary to at least a portion of the 3' transposon end sequence; and~~

~~(d) an attachment polynucleotide comprising:~~

~~(i) an attachment adaptor sequence hybridized to one of the two adaptor sequences and~~

~~(ii) a binding element,~~

wherein the binding element is immobilized to the solid support.

4. ~~An immobilized~~ The transposome complex of claim 3, wherein the binding element is attached to the 5' adaptor sequence of the first transposon through a cleavable linker.~~comprising:~~

~~(a) a transposase;~~

~~(b) a first transposon comprising a 3' transposon end sequence and a 5' adaptor sequence;~~

~~(c) a second transposon comprising a 5' transposon end sequence complementary to at least a portion of the 3' transposon end sequence and a 3' adaptor sequence; and~~

~~(d) a binding element attached to the 5' adaptor sequence through a cleavable linker; wherein the binding element is immobilized to a solid support.~~

5. The transposome complex of any one of claims 2-4, wherein the attachment polynucleotide further comprises a spacer region, an anchor region, a primer sequence, or an index tag sequence, or a combination thereof, and optionally further comprises a capture sequence, a barcode sequence, a cleavage sequence, or a sequencing-related sequence, or a combination thereof.

6. The transposome complex of claim 5, wherein the first transposon further comprises a primer sequence 5' of the 5' adaptor sequence, and the attachment polynucleotide comprises (i) a portion complementary with and hybridized to the 5' adaptor sequence and (ii) a sequence complementary to the primer sequence.

7. The transposome complex of claim 5, wherein the first transposon further comprises a primer sequence 5’ of the 5’ adaptor sequence, and the attachment polynucleotide comprises an index tag sequence and a primer sequence.

8. The transposome complex of claim 5, wherein the second transposon comprises the 3’ adaptor sequence and the attachment polynucleotide comprises (i) a portion complementary to and hybridized with the 3’ adaptor sequence, (ii) an index tag sequence, and (iii) a primer sequence.

9. The transposome complex of claim 5, wherein the attachment polynucleotide comprises a spacer region or a spacer region and an anchor region.

10. The transposome complex of claim 9, wherein the first transposon comprises the 5’ adaptor sequence, and the attachment polynucleotide comprises (i) a portion complementary with and hybridized to the 5’ adaptor sequence, (ii) a spacer region, and (iii) a primer sequence.

11. The transposome complex of any one of claims 1-10, wherein the attachment polynucleotide comprises ~~an A14’ sequence~~ a sequence comprising the complement of a A14 sequence.

12. The transposome complex of any one of claims 9-11, wherein the spacer region is a non-DNA spacer.

13. The transposome complex of any one of claims 9-12, wherein the spacer region is ~~a length~~ approximately ~~equivalent to~~ 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, or 30 base pairs, optionally wherein the spacer region is ~~a length~~ approximately ~~equivalent to~~ 8 or 10 base pairs or nucleotides.

14. The transposome complex of any one of claims 9-13, wherein the spacer region comprises universal bases, optionally wherein the universal bases comprise an inosine or a nitroindole sequence.

15. The transposome complex of any one of claims 9-13, wherein the spacer region comprises one or more C18 spacers, one or more C9 spacers, or a combination thereof, wherein the one or more C18 spacers comprises an 18-atom hexa-ethylene glycol spacer, wherein the one or more C9 spacers comprises a triethyleneglycol spacer.

16. The transposome complex of claim 15, wherein the spacer region comprises a 2 x sp18 synthetic linker, two C18 spacers, a C9 spacer, or two C18 spacers and one C9 spacer.

17. The transposome complex of any one of claims 5-16, wherein the anchor region is complementary to an anchor region complement of an indexing oligonucleotide, where the indexing oligonucleotide comprises the anchor region complement and an index tag sequence (-Anchor'–Index Tag Sequence–).

18. The transposome complex of claim 17, wherein the anchor region complement is common to a plurality of indexing oligonucleotides.

19. The transposome complex of claim 17, wherein each index tag sequence of the plurality of indexing oligonucleotides is the same or different.

20. The transposome complex of any one of claims 2-19, wherein the binding element comprises biotin.

21. The transposome complex of claim 20, wherein the binding element is a 3' biotin linker.

22. The transposome complex of any one of claims 2-10, wherein the transposase is a Tn5 transposase, a wild-type Tn5 transposase, or a hyperactive Tn5 transposase, or a mutant thereof.

23. The transposome complex of any one of claims 2-22, wherein the primer sequence of the attachment polynucleotide is complementary to an index primer sequence of the indexing polynucleotide.

24. The transposome complex of any one of claims 3-23, wherein the solid support is a bead, a paramagnetic bead, a flow cell, a surface of a microfluidic device, a tube, a well of a plate, a slide, a patterned surface, or a microparticle.

25. The transposome complex of claim 24, wherein the solid support comprises a plurality of solid supports.

26. The transposome complex of claim 25, wherein the plurality of solid supports comprises a plurality of beads.

27. A kit comprising:
~~a~~ the transposome complex of any one of claims 2-26; and
an index mix comprising index sequences.

28. The kit of claim 27, wherein the index mix comprises i5 index sequences and i7 index sequences, wherein the i5 index sequences hybridize to the attachment polynucleotide, and wherein the i7 index sequences hybridize to the 3' adaptor sequence.

29. A method of generating a library of tagged nucleic acid fragments comprising contacting the immobilized transposome complex of any one of claims 3-26 with a target nucleic acid under conditions sufficient to fragment the target nucleic acid into a plurality of target fragments, and to join the 3' end of the first transposon to the 5' ends of the target fragments to produce a plurality of 5' tagged target fragments.

30. The method of claim 29, further comprising:
treating the solid support to remove unbound nucleic acids; or
treating the solid support to remove the transposase from the complex, optionally by:
(a) heating the solid support and/or
(b) washing the solid support with an enzyme denaturing agent,
wherein the enzyme denaturing agent optionally comprises sodium dodecyl sulfate (SDS), guanidine hydrochloride, urea, or proteinase.

31. The method of claim 29 or claim 30, wherein the contacting comprises mixing a biological sample with the transposome complex.

32. The method of claim 31, wherein said biological sample comprises a cell lysate or whole cells, or is selected from blood, plasma, serum, lymph, mucus, sputum, urine, semen, cerebrospinal fluid, bronchial aspirate, feces, macerated tissue, and fixed tissue FFPE.

33. The method of any one of claims 29-32, wherein the contacting further comprises hybridizing a plurality of indexing oligonucleotides to the attachment polynucleotides, wherein the plurality of indexing oligonucleotides comprise the same or different index sequences.

34. The method of any one of claims 29-33, further comprising treating the plurality of 5' tagged target fragments with a polymerase and a ligase to extend and ligate the strands to produce fully double-stranded tagged fragments.

35. The method of claim 34, wherein the treating with a polymerase and a ligase is done in the presence of a DNA secondary structure disruptor, wherein the disruptor is optionally DMSO.

36. The method of claim 34 or claim 35, wherein the treating with a polymerase and a ligase is done in the presence of an oligonucleotide that is a complement of the primer sequence, wherein the oligonucleotide is a P7' oligonucleotide, and wherein the primer sequence is a P7 sequence.

37. The method of any one of claims 34-36, wherein the polymerase comprises a T4 DNA polymerase mutant lacking exonuclease activity.

38. The method of any one of claims 29-37, further comprising removing the fully double-stranded tagged fragments from the solid support.

39. The method of claim 38, wherein the removing comprises applying heat and/or a denaturant sufficient to cleave the fully double-stranded tagged fragments from the solid support.

40. The method of any one of claims 29-39, further comprising sequencing one or more of the 5' tagged target fragments or fully double-stranded tagged fragments.

41. The method of claim 40, wherein the fragments are not quantified by PCR after the contacting and before the sequencing.

42. The method of any one of claims 29-41, wherein the nucleic acid is DNA or RNA.

43. The method of claim 29, wherein the 3' adaptor sequence is complementary to at least a portion of an index adaptor sequence.

44. The method of claim 29, wherein the primer sequence of the attachment polynucleotide is complementary to at least a portion of an index primer sequence.

45. A method of generating a library of tagged nucleic acid fragments comprising:

a) contacting the immobilized transposome complex of any one of claims 3-26 with a target nucleic acid under conditions sufficient to fragment the target nucleic acid into a plurality of target fragments, and to join the 3' end of the first transposon to the 5' ends of the target fragments to produce a plurality of 5' tagged target fragments;
b) treating the solid support to remove unbound nucleic acids; or treating the solid support to remove the transposase from the complex, optionally by (a) heating the solid support and/or (b) washing the solid support with an enzyme denaturing agent, wherein the enzyme denaturing agent optionally comprises sodium dodecyl sulfate (SDS), guanidine hydrochloride, urea, or proteinase;
c) treating the plurality of 5' tagged target fragments with a polymerase and a ligase to extend and ligate the 5' tagged target fragments to produce fully double-stranded tagged fragments, optionally wherein the treating with a polymerase and a ligase is done in the presence of a DNA secondary structure disruptor, wherein the disruptor is optionally DMSO;
d) removing the fully double-stranded tagged fragments from the solid support, optionally wherein the removing comprises applying heat and/or a denaturant sufficient to cleave the fully double-stranded tagged fragments from the solid support, optionally wherein the denaturant is NaOH; and
e) selecting the fully double-stranded tagged fragments using capture beads, optionally wherein the capture beads are magnetic beads, further optionally wherein two separate selecting steps are performed.

46. The method of claim 45, wherein the immobilized transposome complex comprises:
a solid support; and
a transposome complex immobilized to the solid support,
wherein the transposome complex comprises:

(a) a transposase;

(b) a first transposon comprising a 3' transposon end sequence and an anchor sequence (Anchor);

(c) a second transposon comprising a 5' transposon end sequence and a ~~B15'~~ sequence comprising the complement of a B15 sequence; and

(d) an attachment polynucleotide comprising:

(i) an anchor sequence complement (Anchor'), ~~an A14'~~ a sequence comprising the complement of a A14 sequence, a spacer, and a ~~P5'~~ sequence comprising the complement of a P5 sequence, and

(ii) a binding element comprising biotin, wherein the biotin is immobilized to the solid support.

47. The method of claim 45 or 46, further comprising sequencing one or more of the fully double-stranded tagged fragments.

48. A method of generating a library of tagged nucleic acid fragments comprising:

a. contacting a first immobilized transposome complex of any one of claims 3-26 and a second immobilized transposome complex of any one of claims 3-26 with a target nucleic acid under conditions sufficient to fragment the target nucleic acid into a plurality of target fragments, and to join the 3' end of each first transposon to the 5' ends of the target fragments to produce a plurality of first 5' tagged target fragments generated from the first immobilized transposome complex and a plurality of second 5' tagged target fragments generated from the second immobilized transposome complex,

~~wherein the first immobilized transposome complex comprises a solid support and a first transposome complex immobilized to the solid support, and~~ wherein the first transposome complex comprises~~:~~

~~i. a transposase;~~

~~ii. a first transposon comprising a 3' transposon end sequence and an anchor sequence;~~

~~iii. a second transposon comprising a 5' transposon end sequence; and~~

~~iv.~~ a first attachment polynucleotide comprising (i) an anchor sequence complement, ~~an A14'~~ a sequence comprising the complement of a A14 sequence, a spacer, and a ~~P5'~~ sequence comprising the complement of a P5 sequence, and (ii) a binding element comprising biotin, wherein the biotin is immobilized to the solid support,

and wherein the second immobilized transposome complex comprises ~~a solid support and a second transposome complex immobilized to the solid support, wherein the second transposome complex comprises:~~

~~i. a transposase;~~

~~ii. a first transposon comprising a 3' transposon end sequence and an anchor sequence;~~

~~iii. a second transposon comprising a 5' transposon end sequence; and~~

~~iv.~~ a second attachment polynucleotide comprising (i) an anchor sequence complement, a ~~B15'~~ sequence comprising the complement of B15, a spacer, and a ~~P7'~~ sequence comprising the complement of P7, and (ii) a binding element comprising biotin, wherein the biotin is immobilized to the solid support;

b. treating the plurality of 5' tagged target fragments with a ligase to ligate each 5' tagged target fragment to either a first indexing oligonucleotide or second indexing oligonucleotide by contacting the 5' tagged target fragments with a pool of first and second indexing oligonucleotides, wherein each first indexing oligonucleotides comprises a A14 sequence, a i5 sequence, and P5 sequence and can associate with a first 5' tagged target fragment; and wherein each second indexing oligonucleotide comprises a B15 sequence, a i7 sequence, and P7 sequence and can associate with a second 5' tagged target fragment, to produce a plurality of 5' tagged target fragments ligated to indexing oligonucleotides;

c. treating the solid support to remove the transposases from the complex, optionally by (a) heating the solid support and/or (b) washing the solid support with an enzyme denaturing agent, wherein the enzyme denaturing agent optionally comprises sodium dodecyl sulfate (SDS), guanidine hydrochloride, urea, or proteinase; and

d. treating the plurality of 5' tagged target fragments ligated to indexing oligonucleotides with a polymerase to extend and produce fully double-stranded tagged fragments.

49. The method of claim 48, wherein the contacting a first immobilized transposome complex and a second immobilized transposome complex and the treating the plurality of 5' tagged target fragments with a ligase are performed in a single reaction.

50. The method of claim 48 or claim 49, wherein the double-stranded tagged fragments are produced in solution.

51. The method of any one of claims 48-50, further comprising selecting the fully double-stranded tagged fragments using capture beads, optionally wherein the capture beads are magnetic beads, further optionally wherein two separate selecting steps are performed.

52. The method of any one of claims 48-51, further comprising sequencing one or more of the fully double-stranded tagged fragments.