CLAIMS

1. A method of preparing a library of barcoded DNA fragments of a target nucleic acid comprising:

a. contacting a target nucleic acid with a plurality of transposome complexes, each transposome complex comprising:

transposons and transposases, wherein the transposons comprise transferred strands and non-transferred strands, wherein at least one of the transposons of the transposome complex comprise an adaptor sequence capable of hybridizing to a complementary capture sequence;

b. fragmenting the target nucleic acid into a plurality of fragments and inserting plurality of transferred strands to the 5' end of at least one strand of the fragments while maintaining the contiguity of the target nucleic acid;

c. contacting the plurality of fragments of the target nucleic acid with a plurality of solid supports, each of the solid supports in the plurality comprising a plurality of immobilized oligonucleotides, each of the oligonucleotides comprising a complementary capture sequence and a first barcode sequence, and wherein the first barcode sequence from each solid support in the plurality of the solid supports differs from the first barcode sequence from other solid supports in the plurality of solid supports;

d. transferring the barcode sequence information to the target nucleic acid fragments, thereby producing an library of double-stranded fragments wherein at least one strand is 5'-tagged with the first barcode, wherein at least two fragments of the same target nucleic acid receives identical barcode information.

2. A method for determining contiguity information of a target nucleic acid sequence, the method comprising:

a. contacting the target nucleic acid with a plurality of transposome complexes, each transposome complex comprising:

transposons and transposases, wherein the transposons comprise transferred strands and non-transferred strands, wherein at least one of the transposons of the transposome complex comprise an adaptor sequence capable of hybridizing to a complementary capture sequence;

b. fragmenting the target nucleic acid into a plurality of fragments and inserting plurality of transferred strands while maintain the contiguity of the target nucleic acid;

c. contacting the plurality of fragments of the target nucleic acid with a plurality of solid supports, each of the solid supports in the plurality comprising a plurality of immobilized oligonucleotides, each of the oligonucleotides comprising a complementary capture sequence and a first barcode sequence, and wherein the first barcode sequence from each solid support in the plurality of the solid supports differs from the first barcode sequence from other solid supports in the plurality of solid supports;

d. transferring the barcode sequence information to the target nucleic acid fragments, wherein at least two fragments of the same target nucleic acid receives identical barcode information;

e. determining the sequence of the target nucleic acid fragments and the barcode sequences;

f. determining the contiguity information of the target nucleic acid by identifying the barcode sequences.

3. A method for simultaneously determining haplotype information and methylation status of a target nucleic acid sequence, the method comprising:

a. contacting the target nucleic acid with a plurality of transposome complexes, each transposome complex comprising:

transposons and transposases, wherein the transposons comprise transferred strands and non-transferred strands, wherein at least one of the transposons of the transposome complex comprise an adaptor sequence capable of hybridizing to a complementary capture sequence;

b. fragmenting the target nucleic acid into a plurality of fragments and inserting plurality of transferred strands while maintaining the contiguity of the target nucleic acid;

c. contacting the plurality of fragments of the target nucleic acid with a plurality of solid supports, each of the solid supports in the plurality comprising a plurality of immobilized oligonucleotides, each of the oligonucleotides comprising a complementary capture sequence and a first barcode sequence, and wherein the first barcode sequence from each solid support in the plurality of the solid supports differs from the first barcode sequence from other solid supports in the plurality of solid supports;

d. transferring the barcode sequence information to the target nucleic acid fragments, wherein at least two fragments of the same target nucleic acid receives identical barcode information;

e. subjecting the target nucleic acid fragments comprising barcodes to bisulfite treatment, thereby generating bisulfite treated target nucleic acid fragments comprising barcodes;

f. determining the sequence of the bisulfite treated target nucleic acid fragments and the barcode sequences;

g. determining the contiguity information of the target nucleic acid by identifying the barcode sequences,

wherein the sequence information is indicative of the methylation status of the target nucleic acid and the contiguity information is indicative of the haplotype information.

4. The method of any one of claims 1-3, wherein a single barcode sequence is present in the plurality of immobilized oligonucleotides on each individual solid support.

5. The method of any one of claims 1-3, wherein different barcode sequences are present in the plurality of immobilized oligonucleotides on each individual solid support.

6. The method of any one of claims_1-3, wherein the transferring of the barcode sequence information to the target nucleic acid fragments is by ligation.

7. The method of any one of claims 1-3, wherein the transferring of the barcode sequence information to the target nucleic acid fragments is by polymerase extension.

8. The method of any one of claims 1-3, wherein the transferring of the barcode sequence information to the target nucleic acid fragments is by both ligation and polymerase extension.

9. The method of claim 7, wherein the polymerase extension is by extending the 3'-end of the non-ligated transposon strand with a DNA polymerase using the immobilized oligonucleotide as a template.

10. The method of any one of claims 1-3, wherein at least a portion of the adaptor sequences further comprise a second barcode sequence.

11. The method of any one of claims 1-3, wherein the transposome complexes are multimeric, and wherein the adaptor sequences of the transposons of each monomeric unit are different from the other monomeric unit in the same transposome complex.

12. The method of any one of claims 1-3, wherein the adaptor sequence further comprises a first primer binding sequence.

13. The method of claim 12, wherein the first primer binding site has no sequence homology to the capture sequence or to the complement of the capture sequence.

14. The method of any one of claims 1-3, wherein the immobilized oligonucleotides on the solid support further comprise a second primer binding sequences.

15. The method of any one of claims 1-3, wherein the transposome complexes are multimeric, and wherein the transposome monomeric units are linked to each other in the same transposome complex.

16. The method of claim 15, wherein the transposase of a transposome monomeric unit is linked to another transposase of another transposome monomeric unit of the same transposome complex.

17. The method of claim 15, wherein the transposons of a transposome monomeric unit are linked to transposons of another transposome monomeric unit of the same transposome complex.

18. The method of claim 2 or 3, wherein the contiguity information of a target nucleic acid sequence is indicative of haplotype information.

19. The method of claim 2 or 3, wherein the contiguity information of a target nucleic acid sequence is indicative of genomic variants.

20. The method of claim 19, wherein the genomic variants are selected from the group consisting of deletions, translocations, interchromosomal gene fusions, duplications, and paralogs.

21. The method of any one of claims 1-3, wherein the oligonucleotides immobilized on the solid support comprises a partially double stranded region and a partially single stranded region.

22. The method of claim 21, wherein the partially single stranded region of the oligonucleotide comprises the second barcode sequence and the second primer binding sequence.

23. The method of any one of claims 1-3, wherein target nucleic acid fragments comprising the barcodes are amplified prior to determining the sequence of the target nucleic acid fragments.

24. The method of claim 23, wherein the steps (a)-(d) and the subsequent amplification are carried out in a single reaction compartment prior to determining the sequence of the target nucleic acid fragments.

25. The method of claim 23, wherein a third barcode sequence is introduced to the target nucleic acid fragments during the amplification.

26. The method of any one of claims 1-3, further comprising:

combining the target nucleic acid fragments comprising the barcodes of step (d) from a plurality of first set of reaction compartments into a pool of target nucleic acid fragments comprising the barcodes;

redistributing the pool of target nucleic acid fragments comprising the barcodes to a plurality of second set of reaction compartments;

introducing a third barcode in to the target nucleic acid fragments by amplifying the target nucleic acid fragments in the second set of reaction compartments prior to sequencing.

27. The method of any one of claims 1-3, further comprising pre-fragmenting the target nucleic acid prior to contacting the target nucleic acid with transposome complexes.

28. The method of claim 27, wherein the pre-fragmenting the target nucleic acid is by a method selected from the group consisting of sonication and restriction digestion.

29. A method of preparing an immobilized library of tagged DNA fragments comprising:

(a) providing a plurality of solid supports having transposome complexes immobilized thereon, wherein the transposome complexes are multimeric and the transposome monomeric units of the same transposome complex are linked to each other, and wherein said transposome monomeric units comprise a transposase bound to a first polynucleotide, said first polynucleotide comprising

(i) a 3' portion comprising a transposon end sequence, and

(ii) a first adaptor comprising a first barcode,

(b) applying a target DNA to the plurality of solid supports under conditions whereby the target DNA is fragmented by the transposome complexes, and the 3' transposon end sequence of the first polynucleotide is transferred to a 5' end of at least one strand of the fragments; thereby producing an immobilized library of double-stranded fragments wherein at least one strand is 5'- tagged with the first barcode.

30. The method of claim 29, wherein the transposase of a transposome monomeric unit is linked to another transposase of another transposome monomeric unit of the same transposome complex.

31. The method of claim 29, wherein the transposons of a transposome monomeric unit is linked to transposons of another transposome monomeric unit of the same transposome complex.

32. A method of preparing a sequencing library for determining the methylation status of a target nucleic acid comprising:

a. fragmenting the target nucleic acid into two or more fragments;

b. incorporating a first common adaptor sequence to the 5'-end of the fragments of the target nucleic acid, wherein the adaptor sequence comprises a first primer binding sequence and an affinity moiety, wherein the affinity moiety is one member of a binding pair;

c. denaturing the target nucleic acid fragments;

d. immobilizing the target nucleic acid fragments on a solid support, wherein the solid support comprises another member of the binding pair and the immobilization of the target nucleic acid is by binding of the binding pair;

e. subjecting the immobilized target nucleic acid fragments to bisulfite treatment;

f. incorporating a second common adaptor sequence to the bisulfite treated immobilized target nucleic acid fragments, wherein the second common adaptor comprises a second primer binding site;

g. amplifying the bisulfite treated target nucleic acid fragments immobilized on solid support using a first and a second primer, thereby producing a sequencing library for determining the methylation status of a target nucleic acid.

33. The method of claim 32, wherein the first common adaptor sequence is incorporated to the 5'-end fragments of the target nucleic acid by one-sided transposition.

34. The method of claim 32, wherein the first common adaptor sequence is incorporated to the 5'-end fragments of the target nucleic acid by ligation.

35. The method of any one of claims 32-34, wherein incorporating the second common adaptor sequence into the bisulfite treated immobilized target nucleic acid fragments comprises

(i) extending the 3'-end of the immobilized target nucleic acid fragments using terminal transferase such that the 3'-end of the immobilized target nucleic acid fragments comprise a homopolymeric tail;

(ii) hybridizing an oligonucleotide comprising a first portion and a second portion, wherein the first portion comprises a single stranded homopolymeric portion that is complementary to the homopolymeric tail of the immobilized target nucleic acid fragments, and wherein the second portion comprising a double stranded portion comprising the second common adaptor sequence;

(iii) ligating the second common adaptor sequence to the immobilized target nucleic acid fragments, thereby incorporating the second common adaptor sequence into the bisulfite treated immobilized target nucleic acid fragments.

36. The method of any one of claims 1-3, wherein the target nucleic acid is from a single cell.

37. The method of any one of claims 1-3, wherein the target nucleic acid is from a single organelle.

38. The method of any one of claims 1-3, wherein the target nucleic acid is genomic DNA.

39. The method of any one of claims 1-3, wherein the target nucleic acid is cross-linked to other nucleic acids.

40. The method of any one of claims 1-3, wherein the target nucleic acid is cell free tumor DNA.

41. The method of claim 40, wherein the cell free tumor DNA is obtained from placental fluid.

42. The method of claim 40, wherein the cell free tumor DNA is obtained from plasma.

43. The method of claim 42, wherein the plasma is collected from whole blood using a membrane separator comprising a collection zone for the plasma.

44. The method of claim 43, wherein the collection zone for the plasma comprises transposome complexes immobilized on solid support.

45. The method of any one of claims 1-3, wherein the target nucleic acid cDNA.

46. The method of any one of claims 1-3, wherein the target nucleic acid is from formalin fixed paraffin embedded tissue sample.

47. The method of any one of claims 1-3, wherein the target nucleic acid is histone protected DNA.

48. The method of any one of claims 1-3, wherein the solid support is a bead.

49. The method of any one of claims 1-3, wherein the plurality of solid supports is plurality of beads and wherein the plurality of beads is of different sizes.

50. A method of preparing a sequencing library for determining the methylation status of a target nucleic acid comprising:

a. providing a plurality of solid support comprising immobilized transposome complexes immobilized thereon, wherein the transposome complexes comprise transposons and transposases, wherein the transposons comprise transferred strands and non-transferred strands, the transferred strand comprising

(i) a first portion at the 3'-end comprising a transposase recognition sequence, and

(ii) a second portion located 5'to the first portion comprising a first adaptor sequence and first member of a binding pair, wherein the first member of the binding pair binds to a second member of the binding pair on the solid support, thereby immobilizes the transposon to the solid support, and wherein the first adaptor comprises a first primer binding sequence;

the non-transferred strand comprising

(i) a first portion at the 5'-end comprising the transposase recognition sequence

(ii) a second portion located 3'to the first portion comprising a second adaptor sequence, wherein the terminal nucleotide at the 3'-end is blocked, and wherein the second adaptor comprises a second primer binding sequence;

b. contacting a target nucleic acid with the plurality of solid support comprising immobilized transposome complexes;

c. fragmenting the target nucleic acid into a plurality of fragments and inserting plurality of transferred strands to the 5' end of at least one strand of the fragments, thereby immobilizing the target nucleic acid fragments to the solid support;

d. extending the 3'-end of the fragmented target nucleic acid with a DNA polymerase;

e. ligating the non-transferred strand to the 3'-end of the fragmented target nucleic acid;

f. subjecting the immobilized target nucleic acid fragments to bisulfite treatment;

g. extending the 3'-end of the immobilized target nucleic acid fragments damaged during the bisulfite treatment by using a DNA polymerase such that the 3'-end of the immobilized target nucleic acid fragments comprises a homopolymeric tail;

h. incorporating a second adaptor sequence to the 3'-end of the immobilized target nucleic acid fragments damaged during the bisulfite treatment;

i. amplifying the bisulfite treated target nucleic acid fragments immobilized on solid support using a first and a second primer, thereby producing a sequencing library for determining the methylation status of a target nucleic acid.

51. A method of preparing a sequencing library for determining the methylation status of a target nucleic acid comprising:

a. contacting the target nucleic acid with transposome complexes, wherein the transposome complexes comprise transposons and transposases, wherein the transposons comprise transferred strands and non-transferred strands, the transferred strand comprising

(i) a first portion at the 3'-end comprising a transposase recognition sequence, and

(ii) a second portion located 5'to the first portion comprising a first adaptor sequence and first member of a binding pair, wherein the first member of the binding pair binds to a second member of the binding pair;

the non-transferred strand comprising

(i) a first portion at the 5'-end comprising the transposase recognition sequence

(ii) a second portion located 3'to the first portion comprising a second adaptor sequence, wherein the terminal nucleotide at the 3'-end is blocked, and wherein the second adaptor comprises a second primer binding sequence,

b. fragmenting the target nucleic acid into a plurality of fragments and inserting plurality of transferred strands to the 5' end of at least one strand of the fragments, thereby immobilizing the target nucleic acid fragments to the solid support;

c. contacting a target nucleic acid fragments comprising the transposon end with the plurality of solid support comprising second member of the binding pair, wherein binding of the first member of the binding pair to the second member of the binding pair immobilizes the target nucleic acid to the solid support;

d. extending the 3'-end of the fragmented target nucleic acid with a DNA polymerase;

e. ligating the non-transferred strand to the 3'-end of the fragmented target nucleic acid;

f. subjecting the immobilized target nucleic acid fragments to bisulfite treatment;

g. extending the 3'-end of the immobilized target nucleic acid fragments damaged during the bisulfite treatment by using a DNA polymerase such that the 3'-end of the immobilized target nucleic acid fragments comprises a homopolymeric tail;

h. incorporating a second adaptor sequence to the 3'-end of the immobilized target nucleic acid fragments damaged during the bisulfite treatment;

i. amplifying the bisulfite treated target nucleic acid fragments immobilized on solid support using a first and a second primer, thereby producing a sequencing library for determining the methylation status of a target nucleic acid.

52. The method of any one of claims 50-51, wherein the solid support is a bead.

53. The method of any one of claim 50-51, wherein the first and second members of the binding pair are biotin and streptavidin.

54. The method of any one of claims 50-51, wherein the first adaptor further comprises a barcode.

55. The method of any one of claims 50-51, wherein the second adaptor further comprises a barcode.

56. The method of any one of claims 50-51, wherein the first and second adaptors comprise first and second adaptors comprise a first and second barcodes.

57. The method of any one of claims 50-51, wherein extending the 3'-end of the immobilized target nucleic acid fragments damaged during the bisulfite treatment is by terminal transferase.

58. The method of any one of claim 50-51, wherein the terminal nucleotide at the 3'-end of the second adaptor is blocked by a member selected from the group consisting of a dideoxy nucleotide, a phosphate group, thiophosphate group, and an azido group.