What is claimed is:

Claim 1. A method of preparing a sequencing library for determining the methylation status of nucleic acids from a plurality of single cells, the method comprising:

(a) providing isolated nuclei from a plurality of cells;

(b) subjecting the isolated nuclei to a chemical treatment to generating nucleosome-depleted nuclei, while maintaining integrity of the isolated nuclei;

(c) distributing subsets of the nucleosome-depleted nuclei into a first plurality of compartments comprising a transposome complex, wherein the transposome complex in each compartment comprises a first index sequence that is different from first index sequences in the other compartments;

(d) fragmenting nucleic acids in the subsets of nucleosome-depleted nuclei into a plurality of nucleic acid fragments and incorporating the first index sequences into at least one strand of the nucleic acid fragments to generate indexed nuclei;

(e) combining the indexed nuclei to generate pooled indexed nuclei;

(f) distributing subsets of the pooled indexed nuclei into a second plurality of compartments and subjecting the indexed nuclei to treatment to identify methylated nucleotides and generate treated nucleic acid fragments;

(g) amplifying the treated nucleic acid fragments in each compartment by linear amplification with a plurality of primers comprising a universal nucleotide sequence at the 5' end and a random nucleotide sequence at the 3' end to generate amplified fragment-adapter molecules;

(h) incorporating a second index sequence into the amplified fragment-adapter molecules to generate dual-index fragment-adapter molecules, wherein the second index sequence in each compartment is different from second index sequences in the other compartments; and

(i) combining the dual-index fragment-adapter molecules, thereby producing a sequencing library for determining the methylation status of nucleic acids from the plurality of single cells.

Claim 2. A method of preparing a sequencing library for determining the methylation status of nucleic acids from a plurality of single cells, the method comprising:

(a) providing isolated nuclei from a plurality of cells;

(b) subjecting the isolated nuclei to a chemical treatment to generate nucleosome-depleted nuclei, while maintaining integrity of the isolated nuclei;

(c) distributing subsets of the nucleosome-depleted nuclei into a first plurality of compartments comprising a transposome complex, wherein the transposome complex in each compartment comprises a first index sequence that is different from first index sequences in the other compartments;

(d) fragmenting nucleic acids in the subsets of nucleosome-depleted nuclei into a plurality of nucleic acid fragments and incorporating the first index sequences into at least one strand of the nucleic acid fragments to generate indexed nuclei;

(e) combining the indexed nuclei to generate pooled indexed nuclei;

(f) distributing subsets of the pooled indexed nuclei into a second plurality of compartments and subjecting the indexed nuclei to treatment to identify methylated nucleotides and generate treated nucleic acid fragments;

(g) ligating the treated nucleic acid fragments in each compartment to a universal adapter to generate ligated fragment-adapter molecules;

(h) incorporating a second index sequence into the ligated fragment-adapter molecules to generate dual-index fragment-adapter molecules, wherein the second index sequence in each compartment is different from second index sequences in the other compartments; and

(i) combining the dual-index fragment-adapter molecules, thereby producing a sequencing library for determining the methylation status of nucleic acids from the plurality of single cells.

Claim 3. The method of claim 1 or 2, wherein the chemical treatment comprises:

(i) a treatment with a chaotropic agent capable of disrupting nucleic acid-protein interactions, wherein optionally the chaotropic agent comprises lithium diiodosalicylate; or

(ii) a treatment with a detergent capable of disrupting nucleic acid-protein interactions, wherein optionally the detergent comprises sodium dodecyl sulfate (SDS).

Claim 4. The method of claim 1 or 2, wherein the cells are treated with a cross-linking agent prior to step (a), wherein the cross-linking agent is optionally formaldehyde.

Claim 5. The method of claim 1 or 2, wherein the distributing in steps (c) and (f) is performed by fluorescence-activated nuclei sorting.

Claim 6. The method of claim 1 or 2, wherein the subsets of the nucleosome-depleted nuclei comprise approximately equal numbers of nuclei, wherein optionally the subsets of the nucleosome-depleted nuclei comprise from 1 to 2000 nuclei.

Claim 7. The method of claim 1 or 2, wherein the first plurality of compartments is a multi-well plate, wherein preferably the multi-well plate is a 96-well plate or a 384-well plate.

Claim 8. The method of claim 1 or 2, wherein the subsets of the pooled indexed nuclei comprise:

approximately equal numbers of nuclei, wherein optionally the subsets of the pooled indexed nuclei comprise

(i) from 1 to 25 nuclei;

(ii) at least 10 times fewer nuclei than the subsets of the nucleosome-depleted nuclei; or

(iii) at least 100 times fewer nuclei than the subsets of the nucleosome-depleted nuclei.

Claim 9. The method of claim 1 or 2, wherein the second plurality of compartments is a multi-well plate, wherein preferably the multi-well plate is a 96-well plate or a 384-well plate.

Claim 10. The method of claim 1 or 2, wherein each of the transposome complexes comprises transposases and transposons, each of the transposons comprising a transferred strand, wherein optionally,

(i) the transferred strand does not comprise a cytosine residue; and/or

(ii) the transferred strand comprises the first index sequence, wherein the transferred strand preferably further comprises a first universal sequence and a first sequencing primer sequence.

Claim 11. The method of claim 1 or 2, wherein the treatment identifies methylated CpG dinucleotide sequences.

Claim 12. The method of claim 1, wherein the linear amplification of the treated nucleic acid fragments comprises 1 to 10 cycles.

Claim 13. The method of claim 1, wherein the universal nucleotide sequence at the 5' end of the primers in step (g) comprises a second sequencing primer sequence.

Claim 14. The method of claim 1, wherein the random nucleotide sequence at the 3' end of the primers in step (g) consists of 9 random nucleotides.

Claim 15. The method of claim 1 or 2, wherein the incorporation of the second index sequence in step (h) comprises contacting the amplified fragment-adapter molecules in each compartment with a first universal primer and a second universal primer, each comprising an index sequence, and performing an exponential amplification reaction, wherein the index sequence of the first universal primer is optionally:

(i) the reverse complement of the index sequence of the second universal primer; or

(ii) different from the reverse complement of the index sequence of the second universal primer; wherein the first universal primer preferably further comprises

a) a first capture sequence and a first anchor sequence complementary to a universal sequence at the 3’ end of the amplified fragment-adapter molecules, wherein the first capture sequence optionally comprises the P5 primer sequence; or

b) a second capture sequence and a second anchor sequence complementary to a universal sequence at the 5’ end of the amplified fragment-adapter molecules, wherein the second capture sequence optionally comprises the reverse complement of the P7 primer sequence.

Claim 16. The method of claim 15, wherein the exponential amplification reaction comprises a polymerase chain reaction (PCR), wherein optionally the PCR comprises 15 to 30 cycles.

Claim 17. The method of claim 1 or 2, further comprising an enrichment of target nucleic acids using a plurality of capture oligonucleotides having specificity for the target nucleic acids, wherein the capture oligonucleotides optionally

(i) are immobilized on a surface of a solid substrate; or

(ii) comprise a first member of a universal binding pair, and wherein a second member of the binding pair is immobilized on a surface of a solid substrate.

Claim 18. The method of claim 1 or 2, further comprising selection of the dual-index fragment-adapter molecules that fall within a predetermined size range.

Claim 19. The method of claim 1 or 2, further comprising sequencing of the dual-index fragment-adapter molecules to determine the methylation status of nucleic acids from the plurality of single cells.

Claim 20. The method of claim 2, further comprising adding one or more nucleotides to the 3’ ends of the treated nucleic acid fragments to create a 3’ overhang prior to the ligation of the universal adapter, wherein optionally

(i) the addition of one or more nucleotides is performed using a terminal transferase; or

(ii) the universal adapter comprises an overhang that is reverse complementary to the 3’ overhang in the treated nucleic acid fragments.

Claim 21. The method of claim 19, further comprising:

providing a surface comprising a plurality of amplification sites,

wherein the amplification sites comprise at least two populations of attached single stranded nucleic acids having a free 3' end, and

contacting the surface comprising amplification sites with the sequencing library under conditions suitable to produce a plurality of amplification sites that each comprise a clonal population of amplicons from an individual dual-index fragment-adapter molecule.

Claim 22. The method of claim 21, wherein the number of the dual-index fragment-adapter molecules exceed the number of amplification sites, wherein the dual-index fragment-adapter molecules have fluidic access to the amplification sites, and wherein each of the amplification sites comprises a capacity for several dual-index fragment-adapter molecules in the sequencing library.

Claim 23. The method of claim 21, wherein the contacting comprises simultaneously (i) transporting the dual-index fragment-adapter molecules to the amplification sites at an average transport rate, and (ii) amplifying the dual-index fragment-adapter molecules that are at the amplification sites at an average amplification rate, wherein the average amplification rate exceeds the average transport rate.

Claim 24. The method of claim 1 or 2 wherein the treatment to identify methylated nucleotides comprises bisulfite or hydrazine.

Claim 25. A composition comprising subsets of single cell whole genome libraries comprising nucleic acid fragments chemically treated to comprise methylation status-dependent chemical modification of CpG sequences, wherein the subsets of single cell whole genome libraries comprising nucleic acid fragments comprise a first index sequence and a second index sequence, and wherein the second index sequence is different for each of the subsets of single cell whole genome libraries.

Claim 26. The composition of claim 25, wherein the nucleic acid fragments further comprise a universal sequence.

Claim 27. The composition of claim 25, wherein the nucleic acid fragments further comprise a first sequencing primer sequence.

Claim 28. The composition of any one of claims 25-27, wherein the first index, the universal sequence, or the first sequencing primer sequence is cytosine-depleted.

Claim 29. The composition of claim 25, wherein the nucleosome-depleted nuclei further comprise transposome complexes.

Claim 30. The sequencing library produced by the method of claim 1 or 2.