CLAIMS

1. A method of preparing a sequencing library comprising 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 and contacting each subset with a transposome complex, wherein the transposome complex in each compartment comprises a transposase and 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 comprising indexed nucleic acid fragments, wherein the indexed nucleic acid fragments remain attached to the transposases;

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

(f) distributing subsets of the pooled indexed nuclei into a second plurality of compartments;

(g) incorporating into the indexed nucleic acid fragments in each compartment a second index sequence to generate dual-index fragments, wherein the second index sequence in each compartment is different from second index sequences in the other compartments;

(h) combining the dual-index fragments, thereby producing a sequencing library comprising whole genome nucleic acids from the plurality of single cells.

2. The method of claim 1, wherein the chemical treatment comprises a treatment with a chaotropic agent capable of disrupting nucleic acid-protein interactions.

3. The method of claim 2 wherein the chaotropic agent comprises lithium 3, 5-diiodosalicylic acid.

4. The method of claim 3, wherein the chemical treatment comprises a treatment with a detergent capable of disrupting nucleic acid-protein interactions.

5. The method of claim 4, wherein the detergent comprises Sodium dodecyl sulfate (SDS), optionally wherein the nuclei are treated with a cross-linking agent prior to step (b) wherein the cross-linking agent is formaldehyde.

6. The method of claim 5 wherein the cross-linking by formaldehyde is reversed after step (f) and prior to step (g), optionally

wherein the transposases are disassociated from the indexed nucleic acid fragments prior to the reversal of the cross-linking.

7. The method of claim 1, wherein a) the nuclei are treated with a restriction enzyme prior to step (d), or

b) the distributing in steps (c) and (f) is performed by fluorescence-activated nuclei sorting, or

c) the subsets of the nucleosome-depleted nuclei comprise approximately equal numbers of nuclei.

8. The method of claim 1, wherein the first plurality of compartments is a multi-well plate.

9. The method of claim 1, wherein the subsets of the pooled indexed nuclei comprise approximately equal numbers of nuclei optionally wherein the subsets of the pooled indexed nuclei comprise from 1 to about 25 nuclei.

10. The method of claim 1, wherein (i) the subsets of the pooled indexed nuclei include at least 10 times fewer nuclei than the subsets of the nucleosome-depleted nuclei, or

(ii) the subsets of the pooled indexed nuclei include at least 100 times fewer nuclei than the subsets of the nucleosome-depleted nuclei

11. The method of claim 1, wherein step (c) comprises adding the transposome complex to the compartments after the subsets of nucleosome-depleted nuclei are distributed.

12. The method of claim 1, wherein each of the transposome complexes comprises a transposon, each of the transposons comprising a transferred strand.

13. The method of claim 1, further comprising an enrichment of dual-index fragments using a plurality of capture oligonucleotides having specificity for the dual-index fragments, optionally wherein the capture oligonucleotides are immobilized on a surface of a solid substrate.

14. The method of claim 1, further comprising sequencing of the dual-index fragments to determine the nucleotide sequence of nucleic acids from the plurality of single cells.

15. The method of claim 14, further comprising:

providing a surface comprising a plurality of amplification sites, wherein the amplification sites comprise at least two populations of attached single stranded capture oligonucleotides having a free 3' end, and

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

16. A composition comprising chemically treated nucleosome-depleted isolated nuclei, wherein the isolated nuclei comprise indexed nucleic acid fragments, optionally wherein the isolated nuclei comprise non-natural cross-links.

17. A multi-well plate, wherein a well of the multi-well plate comprises the composition of claim 16.