CLAIMS

What is claimed is:

1. A method for preparing a sequencing library comprising nucleic acids from a plurality of single nuclei or cells, the method comprising:

providing a plurality of isolated nuclei or cells in a first plurality of compartments,

wherein each compartment comprises a subset of isolated nuclei or cells, and

wherein nuclei or cells comprise nucleic acid fragments;

introducing a linear amplification mediator to the cells or nuclei;

amplifying the nucleic acid fragments by linear amplification;

processing each subset of nuclei or cells to generate indexed nuclei or cells,

wherein the processing comprises adding to nucleic acid fragments present in the isolated nuclei or cells a first compartment specific index sequence to result in indexed nucleic acids present in isolated nuclei or cells,

wherein the processing comprises ligation, primer extension, hybridization, amplification, or transposition;

combining the indexed nuclei or cells to generate pooled indexed nuclei or cells, thereby producing a sequencing library from the plurality of nuclei or cells.

2. The method of claim 1 wherein the amplifying occurs before the processing.

3. The method of claim 1 wherein the processing occurs before the amplifying.

4. A method for preparing a sequencing library comprising nucleic acids from a plurality of single nuclei or cells, the method comprising:

providing a plurality of isolated nuclei or cells,

wherein nuclei or cells comprise nucleic acid fragments;

introducing a linear amplification mediator to the isolated nuclei or cells;

distributing the isolated nuclei or cells into a first plurality of compartments,

wherein each compartment comprises a subset of isolated nuclei or cells;

amplifying the nucleic acid fragments by linear amplification;

processing each subset of isolated nuclei or cells to generate indexed nuclei or cells,

wherein the processing comprises adding to nucleic acid fragments present in the isolated nuclei or cells a first compartment specific index sequence to result in indexed nucleic acids present in isolated nuclei or cells,

wherein the processing comprises ligation, primer extension, amplification, or transposition;

combining the indexed nuclei to generate pooled indexed nuclei or cells, thereby producing a sequencing library from the plurality of nuclei or cells.

5. A method for preparing a sequencing library comprising nucleic acids from a plurality of single nuclei or cells, the method comprising:

providing a plurality of isolated nuclei or cells in a first plurality of compartments,

wherein each compartment comprises a subset of isolated nuclei or cells, and

wherein nuclei or cells comprise nucleic acid fragments;

processing each subset of nuclei or cells to generate indexed nuclei or cells,

wherein the processing comprises adding to nucleic acid fragments present in the isolated nuclei or cells (i) a first compartment specific index sequence to result in indexed nucleic acids present in isolated nuclei or cells and (ii) a nucleotide sequence recognized by a linear amplification mediator,

wherein the processing comprises ligation, primer extension, hybridization, amplification, or transposition;

introducing a linear amplification mediator to the cells or nuclei;

amplifying the nucleic acid fragments by linear amplification;

combining the indexed nuclei or cells to generate pooled indexed nuclei or cells, thereby producing a sequencing library from the plurality of nuclei or cells.

6. The method of any one of claims 1, 4, or 5, wherein the linear amplification mediator comprises a phage RNA polymerase or a linear amplification primer.

7. The method of claim 6, wherein the nucleic acid fragments comprise a T7 promoter and the phage RNA polymerase comprises a T7 RNA polymerase.

8. The method of claim 6, wherein introducing the linear amplification mediator comprises adding to nucleic acid fragments present in the isolated nuclei or cells the linear amplification mediator.

9. The method of claim 1 or 5, further comprising exposing the plurality of isolated nuclei or cells of each compartment to a predetermined condition.

10. The method of claim 9, further comprising isolating nuclei from the plurality of cells after the exposing.

11. The method of claim 4, further comprising exposing the plurality of isolated nuclei or cells to a predetermined condition.

12. The method of any one of claims 1, 4, or 5, further comprising subjecting the isolated nuclei to conditions to generate nucleosome-depleted nuclei while maintaining integrity of the isolated nuclei.

13. The method of any one of claims 1, 4, or 5, wherein the processing comprises:

contacting each subset with a transposome complex,

wherein the transposome complex in each compartment comprises the first index sequence that is different from first index sequences in the other compartments; and

fragmenting nucleic acids in the subsets into a plurality of nucleic acids and incorporating the first index sequences into at least one strand of the nucleic acids to generate the indexed nuclei or cells comprising the indexed nucleic acids.

14. The method of any one of claims 1, 4, or 5, wherein the processing comprises:

contacting each subset with reverse transcriptase and a primer that anneals to RNA molecules in the isolated nuclei, wherein the primer in each compartment comprises the

first index sequence that is different from first index sequences in the other compartments to generate the indexed nuclei or cells comprising the indexed nucleic acids.

15. The method of claim 14, wherein the contacting further comprises a target specific primer that anneals to a specific nucleotide sequence.

16. The method of any one of claims 1, 4, or 5, wherein the processing to add the first compartment specific index sequence comprises a two step process of adding a nucleotide sequence comprising a universal sequence to the nucleic acid fragments and then adding the first compartment specific index sequence to the nucleic acid fragments.

17. The method of claim 16, wherein the adding comprises a transposome complex that comprises the universal sequence.

18. The method of any one of claims 1, 4, or 5, wherein the processing comprises adding a first index to DNA nucleic acids present in the isolated nuclei or cells, a first index to RNA nucleic acids present in the isolated nuclei or cells, or a combination thereof.

19. The method of claim 18, wherein the adding a first index sequence to RNA nucleic acids comprises:

contacting each subset with a reverse transcriptase and a primer that anneals to RNA molecules in the isolated nuclei or cells,

wherein the primer in each compartment comprises the first compartment specific index sequence to generate the indexed nuclei or cells comprising the indexed nucleic acids.

20. The method of claim 18, wherein the adding a first index sequence to DNA nucleic acids comprises:

contacting each subset with a transposome complex,

wherein the transposome complex in each compartment comprises the first compartment specific index sequence; and

fragmenting nucleic acids in the subsets into a plurality of nucleic acids and incorporating the first compartment specific index sequences into at least one strand of the nucleic acids to generate the indexed nuclei or cells comprising the indexed nucleic acids.

21. The method of claim 19, wherein the first index sequence added to DNA nucleic acids and the first index sequence added to RNA nucleic acids in each compartment are identical.

22. The method of claim 19, wherein the first index sequence added to DNA nucleic acids and the first index sequence added to RNA nucleic acids in each compartment are not identical.

23. The method of any one of claims 1, 4, or 5, further comprising an exponential amplification of the nucleic acid fragments, wherein the exponential amplification comprises a target specific primer that anneals to a specific nucleotide sequence.

24. The method of any one of claims 1, 4, or 5, further comprising after the combining:

distributing subsets of the pooled indexed nuclei or cells into a second plurality of compartments; and

introducing a second compartment specific index sequence to indexed nucleic acids to generate dual-indexed nuclei or cells comprising dual-indexed nucleic acids,

wherein the introducing comprises ligation, primer extension, amplification, or transposition.

25. The method of claim 24, further comprising

combining the dual-indexed nuclei to generate pooled dual-indexed nuclei or cells,

distributing subsets of the pooled dual-indexed nuclei or cells into a third plurality of compartments; and

introducing a third compartment specific index sequence to indexed nucleic acids to generate triple-indexed nuclei or cells comprising triple-indexed nucleic acids,

wherein the introducing comprises ligation, primer extension, amplification, or transposition.

26. The method of any one of claims 1, 4, or 5, further comprising treating the indexed nuclei or cells for methylation analysis to generate nucleic acid fragments suitable for methylation analysis.

27. The method of any one of claims 1, 4, or 5, further comprising subjecting the indexed nuclei or cells to proximity ligation to generate nucleic acid fragments suitable for analysis of chromatin conformation.

28. The method of any one of claims 1, 4, or 5, further comprising amplifying the nucleic acid fragments of the sequencing library to produce DNA nanoballs.

29. The method of any one of claims 1-28, wherein the compartment comprises a well or a droplet.

30. The method of any one of claims 1-29, wherein each compartment of the first plurality of compartments comprises from 50 to 100,000,000 nuclei or cells.

31. The method of any one of claims 1-29, wherein each compartment of the second plurality of compartments comprises from 50 to 100,000,000 nuclei or cells.

32. The method of any one of claims 1-31, 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 indexed fragments under conditions suitable to produce a plurality of amplification sites that each comprise a clonal population of amplicons from an individual fragment comprising a plurality of indexes.

33. 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;

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

(e) distributing subsets of the pooled indexed nuclei into a second plurality of compartments and contacting each subset with a hairpin ligation duplex under conditions suitable for ligation of the hairpin ligation duplex to one or both ends of indexed nucleic acid fragments to result in dual indexed nucleic acid fragments, wherein the hairpin ligation duplex comprises a second index sequence that is different from second index sequences in the other compartments;

(f) combining the dual indexed nuclei to generate pooled indexed nuclei;

(g) distributing subsets of the pooled dual indexed nuclei into a third plurality of compartments;

~~(h) lysing the dual-indexed nuclei;~~

~~(i) processing the dual-indexed nucleic fragments to include a third index sequence that is different from third index sequences in the other compartments; and~~

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

Claims

1. A method for preparing a sequencing library comprising nucleic acids from a plurality of single nuclei or cells, the method comprising:

providing a plurality of isolated nuclei or cells in a first plurality of compartments,

wherein each compartment comprises a subset of isolated nuclei or cells, and

wherein nuclei or cells comprise nucleic acid fragments;

introducing a linear amplification mediator to the cells or nuclei;

amplifying the nucleic acid fragments by linear amplification;

processing each subset of nuclei or cells to generate indexed nuclei or cells,

wherein the processing comprises adding to nucleic acid fragments present in the isolated nuclei or cells a first compartment specific index sequence to result in indexed nucleic acids present in isolated nuclei or cells,

wherein the processing comprises ligation, primer extension, hybridization, amplification, or transposition;

combining the indexed nuclei or cells to generate pooled indexed nuclei or cells, thereby producing a sequencing library from the plurality of nuclei or cells.

2. The method of claim 1 wherein the amplifying by linear amplification occurs before the processing.

3. The method of claim 1 wherein the processing occurs before the amplifying by linear amplification.

4. A method for preparing a sequencing library comprising nucleic acids from a plurality of single nuclei or cells, the method comprising:

providing a plurality of isolated nuclei or cells,

wherein nuclei or cells comprise nucleic acid fragments;

introducing a linear amplification mediator to the isolated nuclei or cells;

distributing the isolated nuclei or cells into a first plurality of compartments,

wherein each compartment comprises a subset of isolated nuclei or cells;

amplifying the nucleic acid fragments by linear amplification;

processing each subset of isolated nuclei or cells to generate indexed nuclei or cells,

wherein the processing comprises adding to nucleic acid fragments present in the isolated nuclei or cells a first compartment specific index sequence to result in indexed nucleic acids present in isolated nuclei or cells,

wherein the processing comprises ligation, primer extension, amplification, or transposition;

combining the indexed nuclei to generate pooled indexed nuclei or cells, thereby producing a sequencing library from the plurality of nuclei or cells.

5. A method for preparing a sequencing library comprising nucleic acids from a plurality of single nuclei or cells, the method comprising:

providing a plurality of isolated nuclei or cells in a first plurality of compartments,

wherein each compartment comprises a subset of isolated nuclei or cells, and

wherein nuclei or cells comprise nucleic acid fragments;

processing each subset of nuclei or cells to generate indexed nuclei or cells,

wherein the processing comprises adding to nucleic acid fragments present in the isolated nuclei or cells (i) a first compartment specific index sequence to result in indexed nucleic acids present in isolated nuclei or cells, and (ii) a nucleotide sequence recognized by a linear amplification mediator,

wherein the processing comprises ligation, primer extension, hybridization, amplification, or transposition;

introducing a linear amplification mediator to the cells or nuclei;

amplifying the nucleic acid fragments by linear amplification;

combining the indexed nuclei or cells to generate pooled indexed nuclei or cells, thereby producing a sequencing library from the plurality of nuclei or cells.

6. The method of any one of claims 1, 4, or 5, wherein the linear amplification mediator comprises a phage RNA polymerase or a linear amplification primer, optionally: a) wherein the nucleic acid fragments comprise a T7 promoter and the phage RNA polymerase comprises a T7 RNA polymerase; or b) wherein introducing the linear amplification mediator comprises adding to nucleic acid fragments present in the isolated nuclei or cells the linear amplification mediator.

7. The method of claim 1 or 5, further comprising exposing the plurality of isolated nuclei or the plurality of cells of each compartment to an agent or perturbation, optionally further comprising isolating nuclei from the plurality of cells after the exposing.

8. The method of claim 4, further comprising exposing the plurality of isolated nuclei or cells to an agent or perturbation.

9. The method of any one of claims 1, 4, or 5, further comprising subjecting the isolated nuclei to conditions to generate nucleosome-depleted nuclei while maintaining integrity of the isolated nuclei.

10. The method of any one of claims 1, 4, or 5, wherein the processing comprises:

contacting each subset with a transposome complex, wherein the transposome complex in each compartment comprises the first index sequence that is different from first index sequences in the other compartments; and

fragmenting nucleic acids in the subsets into a plurality of nucleic acids and incorporating the first index sequences into at least one strand of the nucleic acids to generate the indexed nuclei or cells comprising the indexed nucleic acids.

11. The method of any one of claims 1, 4, or 5, wherein the processing comprises:

contacting each subset with reverse transcriptase and a primer that anneals to RNA molecules in the isolated nuclei, wherein the primer in each compartment comprises the first index sequence that is different from first index sequences in the

other compartments to generate the indexed nuclei or cells comprising the indexed nucleic acids, optionally wherein the contacting further comprises a target specific primer that anneals to a specific nucleotide sequence.

12. The method of any one of claims 1, 4, or 5, wherein the processing to add the first compartment specific index sequence comprises a two step process of adding a nucleotide sequence comprising a universal sequence to the nucleic acid fragments and then adding the first compartment specific index sequence to the nucleic acid fragments, optionally wherein the adding comprises a transposome complex that comprises the universal sequence.

13. The method of any one of claims 1, 4, or 5, wherein the processing comprises adding a first index to DNA nucleic acids present in the isolated nuclei or cells, a first index to RNA nucleic acids present in the isolated nuclei or cells, or a combination thereof, optionally: a) wherein the adding a first index sequence to RNA nucleic acids comprises:

contacting each subset with a reverse transcriptase and a primer that anneals to RNA molecules in the isolated nuclei or cells,

wherein the primer in each compartment comprises the first compartment specific index sequence to generate the indexed nuclei or cells comprising the indexed nucleic acids; or b) wherein the adding a first index sequence to DNA nucleic acids comprises:

contacting each subset with a transposome complex,

wherein the transposome complex in each compartment comprises the first compartment specific index sequence; and

fragmenting nucleic acids in the subsets into a plurality of nucleic acids and incorporating the first compartment specific index sequences into at least one strand of the nucleic acids to generate the indexed nuclei or cells comprising the indexed nucleic acids.

14. The method of claim 13, wherein in optional step a) the first index sequence added to DNA nucleic acids and the first index sequence added to RNA nucleic acids in

each compartment are identical, or wherein the first index sequence added to DNA nucleic acids and the first index sequence added to RNA nucleic acids in each compartment are not identical.

15. The method of any one of claims 1, 4, or 5, further comprising an exponential amplification of the nucleic acid fragments, wherein the exponential amplification comprises a target specific primer that anneals to a specific nucleotide sequence.

16. The method of any one of claims 1, 4, or 5, further comprising after the combining:

distributing subsets of the pooled indexed nuclei or cells into a second plurality of compartments; and

introducing a second compartment specific index sequence to indexed nucleic acids to generate dual-indexed nuclei or cells comprising dual-indexed nucleic acids, wherein the introducing comprises ligation, primer extension, amplification, or transposition, optionally further comprising

combining the dual-indexed nuclei to generate pooled dual-indexed nuclei or cells,

distributing subsets of the pooled dual-indexed nuclei or cells into a third plurality of compartments; and

introducing a third compartment specific index sequence to indexed nucleic acids to generate triple-indexed nuclei or cells comprising triple-indexed nucleic acids, wherein the introducing comprises ligation, primer extension, amplification, or transposition.

17. The method of any one of claims 1, 4, or 5, further comprising: (i) treating the indexed nuclei or cells for methylation analysis to generate nucleic acid fragments suitable for methylation analysis; or (ii) subjecting the indexed nuclei or cells to proximity ligation to generate nucleic acid fragments suitable for analysis of chromatin conformation; or (iii) amplifying the nucleic acid fragments of the sequencing library to produce DNA nanoballs.

18. The method of any one of claims 1, 4, or 5, wherein the compartment comprises a well or a droplet, optionally wherein each compartment of the first plurality of compartments comprises from 50 to 100,000,000 nuclei or cells.

19. The method of claim 16, wherein the compartment comprises a well or a droplet, optionally wherein each compartment of the second plurality of compartments comprises from 50 to 100,000,000 nuclei or cells.

20. The method of any one of claims 1, 4, or 5, 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 indexed nucleic acids under conditions suitable to produce a plurality of amplification sites that each comprise a clonal population of amplicons from an individual fragment comprising a plurality of indexes.

21. 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 the nucleosome-depleted nuclei into a first plurality of compartments, wherein each compartment comprises a subset of isolated nuclei, 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 and contacting each subset with a hairpin ligation duplex under conditions suitable for ligation of the hairpin ligation duplex to one or both ends of indexed nucleic acid fragments to result in dual-indexed nucleic acid fragments, wherein the hairpin ligation duplex comprises a second index sequence that is different from second index sequences in the other compartments;

(g) combining the dual-indexed nuclei to generate pooled indexed nuclei;

(h) distributing subsets of the pooled dual-indexed nuclei into a third plurality of compartments;

(i) lysing the dual-indexed nuclei;

(j) processing the dual-indexed nucleic fragments to include a third index sequence that is different from third index sequences in the other compartments to generate triple-indexed fragments; and

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

22. The method of any one of claims 1, 4, or 5, wherein the nucleic acid fragments comprise a phage promoter at one or both ends and wherein the linear amplification mediator comprises a phage RNA polymerase.

23. The method of any one of claims 1, 4, or 5, wherein the linear amplification primer comprises either one primer or two primers with one in excess.