CLAIMS

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

(a) providing a plurality of nuclei or cells in a first plurality of compartments,

wherein each compartment comprises a subset of nuclei or cells;

(b) labeling newly synthesized RNA in the subsets of cells or nuclei obtained from the cells;

(c) processing RNA molecules in each subset of nuclei or cells to generate indexed nuclei or cells,

wherein the processing comprises adding to RNA nucleic acids present in each subset of nuclei or cells a first compartment specific index sequence to result in indexed DNA nucleic acids present in indexed nuclei or cells,

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

(d) combining the indexed nuclei or cells to generate pooled indexed nuclei or cells.

2. The method of claim 1, wherein the processing comprises:

contacting subsets with reverse transcriptase and a primer that anneals to RNA nucleic acids, resulting in double stranded DNA nucleic acids comprising the primer and the corresponding DNA nucleotide sequence of the template RNA molecules, optionally

a) wherein the primer comprises a poly-T nucleotide sequence that anneals to a mRNA poly(A) tail, optionally wherein the processing further comprises contacting subsets with a second primer, wherein the second primer comprises a sequence that anneals to a predetermined DNA nucleic acid, optionally wherein the second primer comprises a compartment specific index,

b) wherein the primer comprises a sequence that anneals to a predetermined RNA nucleic acid, optionally wherein the method comprises primers in different compartments that anneal to different nucleotides of the same predetermined RNA nucleic acid, or

c) wherein the primer comprises a template-switch primer.

3. The method of claim 1, 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 RNA nucleic acids to result in DNA nucleic acids, and then adding the first compartment specific index sequence to the DNA nucleic acids.

4. The method of claim 2 wherein the predetermined RNA nucleic acid is a mRNA.

5. The method of claim 1,

a) where pre-existing RNA nucleic acids and newly synthesized RNA nucleic acids are labeled with the same index in the same compartment,

b) wherein the labeling comprises incubating the plurality of nuclei or cells in a composition comprising a nucleotide label, wherein the nucleotide label is incorporated into the newly synthesized RNA, optionally

i) wherein the nucleotide label comprises a nucleotide analog, a hapten-labeled nucleotide, mutagenic nucleotide, or a nucleotide that can be modified by a chemical reaction, or

ii) wherein more than one nucleotide label is incorporated into the newly synthesized RNA, optionally wherein the ratio of the nucleotide label or labels is different for different compartments or time points,

c) further comprising exposing subsets of nuclei or cells to a predetermined condition before the labeling, optionally wherein the predetermined condition comprises exposure to an agent, optionally

i) wherein the agent comprises a protein, a non-ribosomal protein, a polyketide, an organic molecule, an inorganic molecule, an RNA or RNAi molecule, a carbohydrate, a glycoprotein, a nucleic acid, or a combination thereof, or

ii) wherein the agent comprises a therapeutic drug;

- wherein the predetermined condition of two or more compartments is different,

- wherein the exposing and the labeling occur at the same time or the exposing occurs before the labeling.

6. The method of claim 1, further comprising:

distributing subsets of the pooled indexed nuclei or cells into a second plurality of compartments and adding to indexed nucleic acids present in subsets of nuclei or cells a second index sequence to generate dual-indexed nuclei or cells comprising dual-indexed nucleic acid fragments, wherein the adding comprises ligation, primer extension, hybridization, amplification, or transposition;

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

7. The method of claim 6, further comprising:

distributing subsets of the pooled dual-indexed nuclei or cells into a third plurality of compartments and adding to indexed nucleic acids present in subsets of nuclei or cells a third index sequence to generate triple-indexed nuclei or cells comprising triple-indexed nucleic acid fragments, wherein the adding comprises ligation, hybridization, primer extension, amplification, or transposition;

combining the triple-indexed nuclei or cells to generate pooled triple-indexed nuclei or cells, optionally

a) wherein distributing comprises dilution,

b) wherein distributing comprises sorting,

c) wherein the adding comprises contacting subsets with a hairpin ligation duplex under conditions suitable for ligation of the hairpin ligation duplex to the end of nucleic acid fragments comprising one or two index sequences,

d) wherein the adding comprises contacting nucleic acid fragments comprising one or more index sequence with a transposome complex, wherein the transposome complex in compartments comprises a transposase and a universal sequence, wherein the contacting further comprises conditions suitable for fragmentation of the nucleic acid fragments and incorporation of the universal sequence into nucleic acid fragments.

8. The method of claim 1, wherein the adding comprises ligation of the first compartment specific index sequence, further comprising adding a second index sequence to generate dual-indexed nuclei or cells comprising dual-indexed nucleic acid fragments, wherein the adding comprises transposition.

9. The method of claim 6, wherein the adding comprises ligation of the second compartment specific index sequence, further comprising adding a third index sequence to generate dual-indexed nuclei or cells comprising triple-indexed nucleic acid fragments, wherein the adding comprises transposition.

10. The method of any one of claims 1, 6, or 7,

a) wherein the compartment comprises a well or a droplet,

b) wherein compartments of the first plurality of compartments comprise from 50 to 100,000,000 nuclei or cells.

11. The method of claims 10 and 6,

i) wherein compartments of the second plurality of compartments comprise from 50 to 100,000,000 nuclei or cells,

ii) further comprising obtaining the dual-indexed nucleic acids from the pooled dual-indexed nuclei or cells, thereby producing a sequencing library from the plurality of nuclei or cells.

12. The method of claims 10 and 7,

i) wherein compartments of the third plurality of compartments comprise from 50 to 100,000,000 nuclei or cells,

ii) further comprising obtaining the triple-indexed nucleic acids from the pooled triple -indexed nuclei or cells, thereby producing a sequencing library from the plurality of nuclei or cells.

13. The method of claim 1, further comprising obtaining the indexed nucleic acids from the pooled indexed nuclei or cells, thereby producing a sequencing library from the plurality of nuclei or cells.

14. The method of any one of claims 1-9, 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 nucleic acid fragments comprising one, two, or three index sequences 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.

15. The method of claim 6 or claim 7, wherein the adding of the compartment specific index sequence comprises a two-step process of adding a nucleotide sequence comprising a universal sequence to the nucleic acids, and then adding the compartment specific index sequence to the nucleic acids.

16. A kit when used in preparing a sequencing library in accordance with the method of claim 1, the kit comprising the nucleotide label of claim 5 and at least one enzyme that mediates ligation, primer extension, or amplification.

17. A kit when used in preparing a sequencing library in accordance with the method of claim 1, the kit comprising the primer of claim 2 that anneals to a predetermined nucleic acid and at least one enzyme that mediates ligation, primer extension, or amplification.