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

~~3. The method of claim 2,~~ a) wherein the primer comprises a poly-T nucleotide sequence that anneals to a mRNA poly(A) tail~~.~~

~~4. The method of claim 3~~, 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~~.~~

~~5. The method of claim 4~~, optionally wherein the second primer comprises a compartment specific index~~.~~,

~~6. The method of claim 2,~~ b) wherein the primer comprises a sequence that anneals to a predetermined RNA nucleic acid~~.~~

~~7. The method of claim 6~~, optionally wherein the method comprises primers in different compartments that anneal to different nucleotides of the same predetermined RNA nucleic acid~~.~~, or

~~8. The method of claim 2,~~ c) wherein the primer comprises a template-switch primer.

~~9~~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.

~~10. 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) contacting each subset with reverse transcriptase and a primer that anneals to a predetermined RNA nucleic acid, resulting in double stranded DNA nucleic acids comprising the primer and the corresponding DNA nucleotide sequence of the template RNA nucleic acids;~~

~~(c) processing DNA molecules in each subset of nuclei or cells to generate indexed nuclei or cells, wherein the processing comprises adding to DNA nucleic acids present in each subset of nuclei or cells a first compartment specific index sequence to result in indexed 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.~~

~~11. The method of claim 10, wherein the primer comprises the first compartment specific index sequence.~~

~~12. The method of claim 10, further comprising, prior to the contacting, labeling newly synthesized RNA in the subsets of cells or nuclei obtained from the cells.~~

~~13. The method of claim 10, 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 acids and then adding the first compartment specific index sequence to the nucleic acids.~~

~~1~~4. The method of claim ~~6 or 10,~~ 2 wherein the predetermined RNA nucleic acid is a mRNA.

~~1~~5. The method of claim 1 ~~or 12~~,

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

~~16. The method of claim 1 or 12,~~ 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~~.~~

~~17. The method of claim 16~~, 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

~~18. The method of claim 16,~~ ii) wherein more than one nucleotide label is incorporated into the newly synthesized RNA~~.~~

~~19. The method of claim 18~~, optionally wherein the ratio of the nucleotide label or labels is different for different compartments or time points~~.~~,

~~20. The method of claim 1 or 12,~~ c) further comprising exposing subsets of nuclei or cells to a predetermined condition before the labeling~~.~~

~~21. The method of claim 20~~, optionally wherein the predetermined condition comprises exposure to an agent~~.~~

~~22. The method of claim 21~~, 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

~~23. The method of claim 21,~~ ii) wherein the agent comprises a therapeutic drug~~.~~;

~~24. The method of claim 20,~~ - wherein the predetermined condition of two or more compartments is different~~.~~,

~~25. The method of claim 20,~~ - wherein the exposing and the labeling occur at the same time or the exposing occurs before the labeling.

~~2~~6. The method of claim 1 ~~or 10~~, 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.

~~2~~27. The method of claim ~~2~~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

~~28. The method of claim 26 or 27,~~ a) wherein distributing comprises dilution~~.~~,

~~29. The method of claim 26 or 27,~~ b) wherein distributing comprises sorting~~.~~,

~~30. The method of claim 26 or 27,~~ 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~~.~~,

~~31. The method of claim 26 or 27,~~ 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.

~~32~~8. The method of claim 1~~ or 10~~, 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.

~~33~~9. The method of claim ~~2~~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.

~~34~~10. The method of any one of claims 1, ~~2~~6, or ~~2~~7,

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

~~35. The method of any one of claims 1, 26, or 27,~~ b) wherein compartments of the first plurality of compartments comprise from 50 to 100,000,000 nuclei or cells.

~~36~~11. The method of ~~any one of~~ claims ~~26~~ 10 and 6,

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

~~37. The method of any one of claims 27, wherein compartments of the third plurality of compartments comprise from 50 to 100,000,000 nuclei or cells.~~

~~38. The method of claim 1 or 10, 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.~~

~~39. The method of claim 26,~~ 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.

~~40~~12. The method of claims ~~27~~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.

~~41~~14. The method of any one of claims 1-~~33~~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.

~~42~~15. The method of ~~any one of claims 26 or 27~~ 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.

~~43. 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) contacting each subset with reverse transcriptase and a primer, resulting in double stranded DNA nucleic acids comprising the primer and the corresponding DNA nucleotide sequence of the template RNA nucleic acids;~~

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

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

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

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

~~(e) distributing the pooled indexed nuclei or cells into a second plurality of compartments,~~

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

~~(f) processing DNA molecules in each subset of nuclei or cells to generate dual-indexed nuclei or cells,~~

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

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

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

(h) distributing the pooled dual-indexed nuclei or cells into a third plurality of compartments,

wherein each compartment comprises a subset of nuclei or cells;

(i) processing DNA molecules in each subset of nuclei or cells to generate triple-indexed nuclei or cells,

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

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

(j) combining the triple-indexed nuclei or cells to generate pooled triple-indexed nuclei or cells.

44. 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;

(b) contacting the plurality of nuclei or cells with reverse transcriptase and a primer, resulting in double stranded DNA nucleic acids comprising the primer and the corresponding DNA nucleotide sequence of the template RNA nucleic acids;

(c) distributing the nuclei or cells into a first plurality of compartments,

wherein each compartment comprises a subset of nuclei or cells;

(d) processing DNA molecules in each subset of nuclei or cells to generate indexed nuclei or cells,

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

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

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

(f) distributing the pooled indexed nuclei or cells into a second plurality of compartments,

wherein each compartment comprises a subset of nuclei or cells;

(g) processing DNA molecules in each subset of nuclei or cells to generate dual-indexed nuclei or cells,

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

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

(h) combining the dual-indexed nuclei or cells to generate pooled dual-indexed nuclei or cells;

(i) distributing the pooled dual-indexed nuclei or cells into a third plurality of compartments,

wherein each compartment comprises a subset of nuclei or cells;

(j) processing DNA molecules in each subset of nuclei or cells to generate triple-indexed nuclei or cells,

wherein the processing comprises adding to DNA nucleic acids present in subsets of nuclei or cells a third compartment specific index sequence to result in triple-indexed nucleic acids present in indexed nuclei or cells,

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

(k) combining the triple-indexed nuclei or cells to generate pooled triple-indexed nuclei or cells.

45. The method of claim 43 or 44, wherein the primer 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.

~~46. The method of claim 45, wherein the primer comprises a poly-T nucleotide sequence that anneals to a mRNA poly(A) tail.~~

~~47. The method of claim 46, wherein the contacting further comprises contacting subsets with a second primer, wherein the second primer comprises a sequence that anneals to a predetermined DNA nucleic acid.~~

~~48. The method of claim 47, wherein the second primer comprises a compartment specific index.~~

~~49. The method of claim 45, wherein the primer comprises a sequence that anneals to a predetermined RNA nucleic acid.~~

~~50. The method of claim 49, wherein the predetermined RNA nucleic acid is a mRNA.~~

~~51. The method of claim 43 or 44, wherein the primer comprises a template-switch primer.~~

~~52. The method of claim 43 or 44, wherein the processing to add one or more of the first, second, or third 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 first compartment specific index sequence to the DNA nucleic acids.~~

~~53. The method of claim 43 or 44, wherein the primer comprises the first compartment specific index sequence.~~

~~54. The method of claim 43 or 44, further comprising, prior to the contacting, labeling newly synthesized RNA in the subsets of cells or nuclei obtained from the cells.~~

55. The method of claim 54, where pre-existing RNA nucleic acids and newly synthesized RNA nucleic acids are labeled with the same index in the same compartment.

56. The method of claim 54, 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.

57. The method of claim 54, 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.

58. The method of claim 54, wherein more than one nucleotide label is incorporated into the newly synthesized RNA.

59. The method of claim 58, wherein the ratio of the nucleotide label or labels is different for different compartments or time points.

60. The method of claim 54, further comprising exposing the subset of nuclei or cells of compartments to a predetermined condition before the labeling.

61. The method of claim 60, wherein the predetermined condition comprises exposure to an agent.

62. The method of claim 61, 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

63. The method of claim 61, wherein the agent comprises a therapeutic drug.

~~64. The method of claim 60, wherein the predetermined condition of two or more compartments is different.~~

~~65. The method of claim 60, wherein the exposing and the labeling occur at the same time or the exposing occurs before the labeling.~~

~~66. The method of claim 43 or 44, wherein one of more distributing comprises dilution.~~

~~67. The method of claim 43 or 44, wherein one of more distributing comprises sorting.~~

~~68. The method of claim 43 or 44, wherein adding one or more of first, second or third compartment specific index sequence 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.~~

~~69. The method of claim 43 or 44, wherein the adding one or more of first, second or third compartment specific index sequence comprises contacting nucleic acid fragments 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 a nucleotide sequence into nucleic acid fragments.~~

~~70. The method of 43 or 44, wherein the adding of the first or second compartment specific index comprises ligation, and the adding of a subsequent compartment specific index sequence comprises transposition.~~

~~71. The method of any one of claims 43 or 44, wherein the compartment comprises a well or a droplet.~~

72. The method of any one of claims 43 or 44, wherein compartments of the first plurality of compartments comprise from 50 to 100,000,000 nuclei or cells.

73. The method of any one of claims 43 or 44, wherein compartments of the second plurality of compartments comprise from 50 to 100,000,000 nuclei or cells.

74. The method of any one of claims 43 or 44, wherein compartments of the third plurality of compartments comprise from 50 to 100,000,000 nuclei or cells.

75. The method of claim 43 or 44, 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.

76. The method of any one of claims 43 or 44, 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 triple-indexed nucleic acid 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.

77. A method of preparing a sequencing library comprising nucleic acids from a plurality of single cells, the method comprising:

(a) providing nuclei from a plurality of cells;

(b) distributing subsets of the nuclei into a first plurality of compartments and contacting each subset with reverse transcriptase and a primer, wherein the primer in each compartment comprises a first index sequence that is different from first index sequences in

~~the other compartments to generate indexed nuclei comprising indexed nucleic acid fragments;~~

~~(c) combining the indexed nuclei to generate pooled indexed nuclei;~~

~~(d) 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 the end of indexed nucleic acid fragments comprising a first index sequence to generate dual-indexed nuclei comprising 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;~~

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

~~(f) distributing subsets of the pooled dual-indexed nuclei into a third plurality of compartments and subjecting the dual-indexed nucleic acid fragments to conditions for second strand synthesis;~~

~~(g) contacting the dual-indexed nucleic acid fragments with a transposome complex, wherein the transposome complex in each compartment comprises a transposase and a universal sequence, wherein the contacting comprises conditions suitable for fragmentation of the dual-indexed nucleic acid fragments and incorporation of the universal sequence into dual-indexed nucleic acid fragments to generate dual-indexed nucleic acid fragments comprising the first and the second indexes at one end and the universal sequence at the other end;~~

~~(i) incorporating into the dual-indexed nucleic acid fragments in each compartment a third index sequence to generate triple-index fragments;~~

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

~~78. The method of claim 77, wherein the primers comprise an poly-T sequence that anneals to a mRNA poly(A) tail.~~

79. The method of claim 77, wherein the primer of each compartment comprises a sequence that anneals to a predetermined mRNA.

80. The method of claim 79, wherein the method comprises primers in different compartments that anneal to different nucleotides of the same predetermined mRNA.

81. A method of preparing a transcriptome sequencing library comprising nucleic acids from a plurality of single cells, the method comprising:

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

(b) contacting the pooled nuclei with reverse transcriptase and a primer comprising an oligo-dT sequence that anneals to a mRNA poly(A) tail to generate pooled nuclei comprising nucleic acid fragments;

(c) distributing subsets of the pooled nuclei into a plurality of compartments and contacting each subset with a hairpin ligation duplex under conditions suitable for ligation of the hairpin ligation duplex to the end of nucleic acid fragments to generate indexed nuclei comprising indexed nucleic acid fragments, wherein the hairpin ligation duplex comprises an index sequence that is different from index sequences in the other compartments;

(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 subjecting the indexed nucleic acid fragments to conditions for second strand synthesis;

(f) contacting the indexed nucleic acid fragments with a transposome complex, wherein the transposome complex in each compartment comprises a transposase and a universal sequence, wherein the contacting comprises conditions suitable for fragmentation of the indexed nucleic acid fragments and incorporation of the universal sequence into indexed nucleic acid fragments to generate indexed nucleic acid fragments comprising the index at one end and the universal sequence at the other end;

~~(i) incorporating into the indexed nucleic acid fragments in each compartment a second index sequence to generate dual-index fragments;~~

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

~~82. A method for isolating nuclei, the method comprising:~~

~~(a) snap freezing a tissue in liquid nitrogen;~~

~~(b) reducing the size of the tissue to result in a processed tissue; and~~

~~(c) extracting nuclei from the processed tissue by incubation in a buffer that promotes cell lysis and retains integrity of the nuclei in the absence of one or more exogenous enzymes.~~

~~83. The method of claim 82, wherein the reducing comprises mincing the tissue, subjecting the tissue to a blunt force, or a combination thereof.~~

~~84. The method of claim 82, further comprising:~~

~~(d) exposing the extracted nuclei to a cross-linking agent to result in fixed nuclei; and~~

~~(e) washing the fixed nuclei.~~

~~85~~16. A kit ~~for use~~ when used in preparing a sequencing library in accordance with the method of claim 1, the kit comprising the nucleotide label of claim ~~16 or 56~~ 5 and at least one enzyme that mediates ligation, primer extension, or amplification.

~~86~~17. A kit ~~for use~~ when used in preparing a sequencing library in accordance with the method of claim 1, the kit comprising the primer of claim ~~4, 6, or 10~~ 2 that anneals to a predetermined nucleic acid and at least one enzyme that mediates ligation, primer extension, or amplification.