

[0017] Furthermore, if the nucleic acids are attached to a solid surface, they will not be as free to move about in comparison to nucleic acids in solution. Solid phase kinetics are much slower than aqueous phase kinetics for nucleic acid complementary base pairing, and may result in much less efficient addition of barcodes to nucleic acids of interest. Preferably, nucleic acid barcodes should exist in the aqueous phase before participating in the barcoding reaction.

[0018] This current invention improves upon a previous invention (PCT/US2012/000221) to attach unique barcodes to each sample, where each sample is usually a single cell, but is generalizable to any type of sample. The current invention enables delivery of unique barcodes to any type of reaction container, and is also suitable for nanoliter-sized reaction containers and does not require keeping unique nucleic acid barcodes in separate storage containers. It is amendable to but does not require manually pipetting a unique barcode into each reaction container. It delivers one or more copies of a unique barcode or unique barcode set into each reaction container and the barcode is attached to nucleic acids of interest in a reaction that occurs in the aqueous phase with rapid aqueous phase kinetics. As the reaction attaches barcodes to all nucleic acids of interest in a cell, i.e. all reverse transcribed RNA in a cell, the current invention enables single cell transcriptomics analysis, and is not limited to associating immunoglobulin variable genes to specific samples. Furthermore, the amplification reaction can occur at a sufficiently low temperature that it is compatible with mesophilic enzymes (that are otherwise inactivated at high temperatures) to add barcodes to nucleic acids of interest.

SUMMARY OF THE INVENTION

[0018a] Disclosed herein is a method of attaching a polynucleotide to a solid support, wherein the polynucleotide contains an RNA polymerase (RNAP) promoter and a barcode sequence, the method comprising the steps of: a) generating a hydrophilic compartment of an inverse emulsion, the hydrophilic compartment containing: a solid support, a barcode oligonucleotide comprising the RNAP promoter and the barcode sequence, and an oligonucleotide bound to a surface of the solid support via a capture moiety; and b) performing a polymerase extension reaction to incorporate the RNAP promoter and barcode sequence into the bound oligonucleotide on the solid support.

[0018b] Disclosed herein is a solid support attached to a polynucleotide, wherein the polynucleotide contains an RNAP promoter and a barcode sequence.

[0019] Disclosed herein are methods and compositions for analyzing nucleic acids associated with single cells using nucleic acid barcodes. One method disclosed herein for producing one or more polynucleotides of interest comprises obtaining a plurality of nucleic acids associated with one or more samples, wherein the samples are obtained from one or more subjects, and the nucleic acids associated with a sample are present in a separate reaction volume. The nucleic acids can be RNA or DNA molecules (for example, cDNA molecules). In some embodiments, an adapter molecule is added to the nucleic acids associated with the sample. In some embodiments, the adapter molecule is generated using an enzymatic reaction and comprises a universal priming sequence, a barcode sequence, and a binding site. In some embodiments, the barcode sequence is incorporated into one or more polynucleotides associated with the sample,

WHAT IS CLAIMED IS:

~~1. — A method for producing one or more polynucleotides of interest, the method comprising:~~

~~obtaining a plurality of RNAs associated with one or more samples, wherein~~

~~the samples are obtained from one or more subjects, and~~

~~the RNAs associated with a sample are present in a separate reaction volume;~~

~~adding an adapter molecule to the RNAs associated with the sample, wherein the adapter molecule is generated using an enzymatic reaction and comprises a universal priming sequence, a barcode sequence, and a binding site; and~~

~~incorporating the barcode sequence into one or more polynucleotides associated with the sample,~~

~~thereby producing the one or more polynucleotides of interest.~~

~~2. — The method of claim 1, further comprising generating the adapter molecule using the enzymatic reaction.~~

~~3. — The method of claim 1 or 2, wherein the adapter molecule is generated by contacting a template molecule with one or more enzymes.~~

~~4. — The method of claim 3, wherein the template molecule is a DNA molecule comprising an RNA polymerase (RNAP) promoter, and the one or more enzymes include an RNA polymerase.~~

~~5. — The method of claim 4, wherein the RNAP promoter is selected from the group consisting of T7, T3, and SP6.~~

~~6. — The method of claim 3, wherein the template molecule is a DNA molecule comprising a nicking endonuclease restriction site, and the one or more enzymes include a nicking endonuclease and a strand displacing DNA polymerase.~~

~~7. — The method of claim 6, wherein the nicking endonuclease restriction site is selected from the group consisting of Nt.BbvCI, Nt.BspQI, Nt.BsmAI, Nt.BstNBI, Nt.AlwI, and Nt.BsmAI.~~

~~8. — The method of claim 6, wherein the strand-displacing DNA polymerase is selected from the group consisting of Klenow exo-, Bst Large Fragment and engineered variants of Bst Large Fragment.~~

~~9. — The method of claim 3, wherein:~~

~~the template molecule is bound to a solid support,~~

~~the solid support is contacted with an aqueous solution, and~~

~~the adapter molecule is released into the aqueous solution as it is generated.~~

~~10. — The method of claim 9, wherein adding the adapter molecule to the RNAs associated with one sample comprises combining the aqueous solution with the reaction volume in which the RNAs are present.~~

~~11. — The method of claim 9, wherein the aqueous solution is present in the same reaction volume as the RNAs associated with one sample.~~

~~12. — The method of claim 9, wherein:~~

~~the template molecule comprises an endonuclease restriction site,~~

~~the one or more enzymes comprise a restriction endonuclease, and~~

~~the adapter molecule comprises a portion of the template molecule, said portion being generated and released into the aqueous solution upon contacting the template molecule with the restriction endonuclease.~~

~~13. — The method of claim 9, wherein the solid support is a bead or a surface.~~

~~14. — The method of claim 1, wherein the adapter molecule is free in solution prior to adding the adapter molecule to the RNAs associated with one sample.~~

~~15. — The method of claim 1, wherein the adapter molecule is generated in a compartment, and adding the adapter molecule to the RNAs associated with one sample comprises combining the compartment with the reaction volume in which the RNAs are present.~~

~~16. — The method of claim 1, wherein the adapter molecule is generated in the reaction volume in which the RNAs to which the adapter molecule is added are present.~~

~~17. — The method of claim 1, wherein the adapter molecule is not generated in the reaction volume in which the RNAs to which the adapter molecule is added are present.~~

~~18. — The method of claim 1, wherein the enzymatic reaction is an isothermal reaction.~~

~~19. — The method of claim 1, wherein the adapter molecule further comprises a unique molecular identifier (UMI) sequence.~~

~~20. — The method of claim 1, wherein the adapter molecule is an RNA molecule.~~

~~21. — The method of claim 20, wherein the adapter molecule is generated using RNAP.~~

~~22. — The method of claim 1, wherein the adapter molecule is a DNA molecule.~~

~~23. — The method of claim 22, wherein the adapter molecule is generated using DNAP.~~

~~24. — The method of claim 22, wherein:~~

~~producing the one or more polynucleotides of interest comprises reverse transcribing the RNAs associated with the sample, thereby synthesizing a plurality of first-strand cDNAs,~~

~~at least some of the RNAs associated with the sample comprise a sequence region complementary to the binding site of the adapter molecule, and~~

~~the adapter molecule is used as a primer for reverse transcription, such that the barcode sequence is incorporated into first-strand cDNAs associated with the sample.~~

~~25. — The method of claim 24, wherein the binding site comprises a poly-T tract.~~

~~26. — The method of claim 24, wherein the binding site comprises a random tract.~~

~~27. — The method of claim 24, wherein the binding site occurs at the 3' end of the adapter molecule.~~

~~28. — The method of claim 24, wherein the adapter molecule is generated in a compartment, and reverse transcribing the RNAs associated with the sample occurs upon combining the compartment with the reaction volume in which the RNAs are present.~~

~~29. — The method of claim 24, wherein reverse transcribing the RNAs associated with the sample occurs in the same reaction volume where the adapter molecule added to the RNAs is generated.~~

~~30. — The method of claim 1, further comprising reverse transcribing the RNAs associated with the sample to obtain a plurality of cDNAs, wherein reverse transcribing an RNA comprises synthesizing a first strand of cDNA using a reverse transcriptase and a first strand primer.~~

~~31. — The method of claim 30, wherein the reverse transcriptase is MMLV H⁻ reverse transcriptase.~~

~~32. — The method of claim 30, wherein the adapter molecule is generated in a compartment, and adding the adapter molecule to the RNAs associated with one sample comprises combining the compartment with the reaction volume in which the RNAs are present.~~

~~33. — The method of claim 32, wherein first stands of cDNA are synthesized prior to combining the compartment with the reaction volume.~~

~~34. — The method of claim 32, wherein first stands of cDNA are synthesized subsequent to combining the compartment with the reaction volume.~~

~~35. — The method of claim 30, wherein reverse transcribing the RNAs associated with the sample occurs in the same reaction volume where the adapter molecule added to the RNAs is generated.~~

~~36. — The method of claim 35, wherein a buffer in the reaction volume comprises at least one of Tris, potassium ions, chloride ions, sulphate ions, ammonium ions, acetic acid ions, or magnesium ions at a pH range from pH 8.0 to pH 8.8.~~

~~37. — The method of claim 30, wherein:
the reverse transcriptase has template switching activity,
at least some first strands of cDNA associated with the sample comprise a 3' overhang,
the binding site of the adapter molecule comprises a 3' portion complementary to the 3' overhang, and~~

~~the adapter molecule serves as a template for the reverse transcriptase, such that the barcode sequence is incorporated into first strands of cDNAs associated with the sample.~~

~~38. — The method of claim 37, wherein the 3' overhang comprises one or more C nucleotides and the 3' portion of the binding site comprises one or more G nucleotides.~~

~~39. — The method of claim 37, wherein the first strand primer comprises a poly-T tract.~~

~~40. — The method of claim 37, wherein the first strand primer comprises a random sequence.~~

~~41. — The method of claim 30, wherein producing polynucleotides of interest comprises amplifying the first strands of cDNA for the sample using a first primer and a second primer, the second primer having the same sequence as at least a portion of the first strand primer, wherein the first primer or the second primer is the adapter molecule.~~

~~42. — The method of claim 41, wherein the first primer is the adapter molecule.~~

~~43. — The method of claim 41, wherein the second primer is the adapter molecule.~~

~~44. — The method of claim 41, wherein the first strand primer comprises a poly-T tract.~~

- ~~45. — The method of claim 41, wherein the first strand primer comprises a random sequence.~~
- ~~46. — The method of claim 1, wherein the sample comprises a cell.~~
- ~~47. — The method of claim 46, wherein the cell is a blood cell, an immune cell, a tissue cell, or a tumor cell.~~
- ~~48. — The method of claim 47, wherein the cell is a B cell or a T cell.~~
- ~~49. — The method of claim 46, wherein the RNAs associated with the sample comprise mRNAs.~~
- ~~50. — The method of claim 49, wherein the RNAs associated with the sample comprise at least 1, 3, 10, 30, 100, 300, 1,000, 3,000, 10,000, 30,000, 100,000, 300,000, or 1,000,000 mRNAs.~~
- ~~51. — The method of claim 49, wherein the RNAs associated with the sample comprise at least a partial transcriptome of a cell.~~
- ~~52. — The method of claim 46, wherein the RNAs associated with the sample comprise the total RNA of a cell.~~
- ~~53. — The method of claim 46, wherein at least 10, 30, 100, 300, 1,000, 3,000, 10,000, 30,000, 100,000, 300,000, or 1,000,000 polynucleotides of interest are produced per sample.~~
- ~~54. — The method of claim 46, wherein the one or more samples comprise at least 10, 30, 100, 300, 1,000, 3,000, 10,000, 30,000, 100,000, 300,000, or 1,000,000 cells.~~
- ~~55. — The method of claim 46, wherein the one or more samples are obtained from the same subject.~~
- ~~56. — The method of claim 46, further comprising contacting the samples with a lysis buffer.~~
- ~~57. — The method of claim 46, further comprising:~~

~~contacting the samples with a nucleic acid marker, thereby allowing the nucleic acid marker to bind to a subset of the samples; and~~

~~washing the samples, thereby removing the nucleic acid marker from samples to which the nucleic acid marker does not bind,~~

~~wherein, for samples within the subset, the adapter molecule added to the RNAs associated with the sample is also added to the nucleic acid marker, and one or more polynucleotides of interest are produced using the nucleic acid marker.~~

~~58. The method of claim 57, wherein the nucleic acid marker comprises a nucleic acid coupled to a molecular label.~~

~~59. The method of claim 58, wherein the molecular label is an antibody, antigen, or protein.~~

~~60. The method of claim 58, wherein the molecular label has an affinity for one or more cell surface moieties.~~

~~61. The method of claim 58, wherein the nucleic acid is an RNA.~~

~~62. The method of claim 58, wherein the nucleic acid is a DNA.~~

~~63. The method of claim 62, wherein the DNA comprises an RNAP promoter.~~

~~64. The method of any one of claims 58-63, wherein:
the samples are contacted with a first nucleic acid marker and a second nucleic acid marker,~~

~~the first nucleic acid marker comprises a first nucleic acid coupled to a first molecular label,~~

~~the second nucleic acid marker comprises a second nucleic acid coupled to a second molecular label,~~

~~the first nucleic acid and second nucleic acid comprise different sequence regions,
and~~

~~the first and second molecular labels are different.~~

~~65. — The method of claim 1, wherein the one or more samples are obtained from the same subject.~~

~~66. — The method of claim 1, wherein the one or more samples are obtained from at least 3, 10, 30, or 100 different subjects.~~

~~67. — An adapter construct comprising an RNAP promoter sequence, a universal priming sequence, a barcode sequence, and a binding site.~~

~~68. — The adapter construct of claim 67, wherein the RNAP promoter is selected from the group consisting of T7, T3, and SP6.~~

~~69. — An adapter construct comprising a nicking endonuclease restriction site, a universal priming sequence, a barcode sequence, and a binding site.~~

~~70. — The adapter construct of claim 69, wherein the nicking endonuclease restriction site is selected from the group consisting of Nt.BbvCI, Nt.BspQI, Nt.BsmAI, Nt.BstNBI, Nt.AlwI, and Nt.BsmAI.~~

~~71. — The adapter construct of claim 67 or 69, further comprising a unique molecular identifier (UMI) sequence.~~

~~72. — A solid support comprising the adapter construct of claim 67, 69, or 71.~~

~~73. — The solid support of claim 72, wherein the adapter construct is bound to the solid support via a covalent bond.~~

~~74. — The solid support of claim 72, wherein multiple copies of the adapter construct are bound to the solid support.~~

~~75. — The solid support of claim 74, wherein at least 10, 30, 100, 300, 1,000, 3,000, 10,000, 30,000, 100,000, 300,000, or 1,000,000 copies of the adapter construct are bound to the solid support.~~

~~76. — The solid support of claim 74, wherein each copy of the adapter construct comprises the same barcode sequence.~~

~~77.~~—An adapter template library comprising a plurality of the solid supports of claim ~~72~~.

~~78.~~—The adapter template library of claim ~~77~~, wherein the plurality comprises at least 10, 30, 100, 300, 1,000, 3,000, 10,000, 30,000, 100,000, 300,000, or 1,000,000 solid supports.

~~79.~~—A nucleic acid marker comprising a nucleic acid coupled to a molecular label, wherein the molecular label is an antibody, antigen, or protein.

~~80.~~—The nucleic acid marker of claim ~~79~~, wherein the molecular label has an affinity for one or more cell surface moieties.

~~81.~~—The nucleic acid marker of claim ~~79~~, wherein the nucleic acid is an RNA.

~~82.~~—The nucleic acid marker of claim ~~79~~, wherein the nucleic acid is a DNA.

~~83.~~—The nucleic acid marker of claim ~~82~~, wherein the DNA comprises an RNAP promoter.

~~84.~~—A plurality of nucleic acid markers of claims ~~79–83~~, wherein at least one nucleic acid marker comprises a first nucleic acid coupled to a first molecular label, and at least one nucleic acid marker comprises a second nucleic acid coupled to a second molecular label, wherein the first nucleic acid and second nucleic acid comprise different sequence regions, and the first and second molecular labels are different.

~~85.~~—A kit comprising the adapter construct of claims ~~67, 69, or 71~~, the solid support of claim ~~72~~, the adapter template library of claim ~~77~~, or the nucleic acid marker of any one of claims ~~79–83~~.

~~86.~~—The kit of claim ~~85~~, further comprising enzymes for generating an adapter molecule comprising a universal priming sequence, a barcode sequence, and a binding site from the adapter construct of claims ~~67 or 69~~.

~~87.~~—The kit of claims ~~85 or 86~~, further comprising a cell suspension buffer comprising an osmoprotectant.

~~88. — The kit of claim 87, wherein the osmoprotectant is a betaine or a close structural analog thereof.~~

~~89. — The kit of claim 88, wherein the osmoprotectant is a glycine betaine.~~

~~90. — The kit of claim 87, wherein the osmoprotectant is a sugar or polyol.~~

~~91. — The kit of claim 90, wherein the osmoprotectant is trehalose.~~

~~92. — The kit of claim 87, wherein the osmoprotectant is an amino acid.~~

~~93. — The kit of claim 92, wherein the osmoprotectant is proline.~~

~~94. — The kit of claim 87, wherein the osmolarity of the buffer is about 250-350 mOsm/L.~~

~~95. — The kit of claim 87, wherein the osmoprotectant contributes up to 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the osmolarity of the buffer.~~

~~96. — The kit of claim 87, wherein the buffer comprises about 230-330 mM betaine and about 10 mM NaCl.~~

97. — 1. A method of attaching a polynucleotide to a solid support, wherein the polynucleotide contains an RNA polymerase (RNAP) promoter and a barcode sequence, the method comprising the steps of:

a) generating a hydrophilic compartment of an inverse emulsion, the hydrophilic compartment containing: a solid support, a barcode oligonucleotide comprising the RNAP promoter and the barcode sequence, and an oligonucleotide bound to a surface of the solid support via a capture moiety; and

~~a solid support,~~

~~a barcode oligonucleotide comprising a barcode sequence, and~~

~~an oligonucleotide bound to a surface of the solid support via a capture moiety,~~

~~wherein the bound oligonucleotide comprises a 3' sequence complementary to a 3' sequence of the barcode oligonucleotide; and~~

b) performing a polymerase extension reaction to incorporate the [RNAP promoter and](#) barcode sequence into the bound oligonucleotide on the solid support.

~~98.~~—[2.](#) The method of claim [97](#)[1](#), wherein the barcode oligonucleotide further comprises a ~~5'~~[5'](#) sequence identical or complementary to a PCR reverse primer sequence.

~~99.~~—[3.](#) The method of claim [98](#)[2](#), further comprising performing a PCR reaction ~~using a fluorophore-labeled reverse primer~~[to amplify the barcode oligonucleotide.](#)

~~100.~~—[4.](#) The method of claim [97](#)[1](#), wherein the solid support is a bead or a surface.

~~101.~~—[5.](#) The method of claim [97](#)[1](#), wherein the capture moiety is streptavidin.

~~102.~~—[6.](#) The method of claim [97](#)[1](#), wherein the capture moiety comprises a carboxyl group, epoxy group, or hydroxyl group.

~~103.~~—[7.](#) The method of claim [97](#)[1](#), wherein the capture moiety comprises gold to capture thiolated oligonucleotides.

~~104.~~—[8.](#) The method of claim [97](#)[1](#), wherein the barcode oligonucleotide further comprises a universal priming sequence and a binding site.

~~105.~~—[9.](#) The method of claim ~~104~~[8](#), wherein the barcode oligonucleotide ~~further~~ comprises an RNAP promoter selected from the group consisting of T7, T3, and SP6.

~~106.~~—[10.](#) The method of claim [8](#), wherein the barcode oligonucleotide further comprises a [nicking endonuclease restriction site selected from the group consisting of Nt.BbvCI, Nt.BspQI, Nt.BsmAI, Nt.BstNBI, Nt.AlwI, and Nt.BsmAI.](#)

[11.](#) The method of claim ~~104~~[8](#), wherein the ~~barcode oligonucleotide~~[binding site is one or more G nucleotides.](#)

[12.](#) A solid support attached to a polynucleotide, wherein the polynucleotide contains an RNAP promoter and a barcode sequence.

[13.](#) The solid support of claim [12](#), wherein the solid support is a bead or a surface.

[14.](#) The solid support of claim [12](#), wherein the polynucleotide is attached to a surface of the solid support via a capture moiety.

[15.](#) The solid support of claim [14](#), wherein the capture moiety is streptavidin.

[16.](#) The solid support of claim [14](#), wherein the capture moiety comprises a carboxyl group, epoxy group, or hydroxyl group.

17. The solid support of claim 14, wherein the capture moiety comprises gold to capture thiolated oligonucleotides.

18. The solid support of claim 12, wherein the polynucleotide further comprises a universal priming sequence and a binding site.

19. The solid support of claim 12, wherein the polynucleotide comprises an RNAP promoter selected from the group consisting of T7, T3, and SP6.

20. The solid support of claim 18, wherein the polynucleotide further comprises a nicking endonuclease restriction site selected from the group consisting of Nt.BbvCI, Nt.BspQI, Nt.BsmAI, Nt.BstNBI, Nt.AlwI, and Nt.BsmAI.

~~107.~~ 21. The ~~method~~ solid support of claim ~~104~~ 18, wherein the binding site is one or more G nucleotides.

~~108.~~—A method of attaching a polynucleotide to a solid support, wherein the polynucleotide contains a barcode sequence, the method comprising the steps of:

a) ~~providing:~~

~~a solid support,~~

~~a first barcode oligonucleotide comprising a W sequence, and~~

~~an oligonucleotide bound to a surface of the solid support via a capture moiety~~ 22. The method of claim 1, wherein the bound oligonucleotide comprises (i) ~~an S1_x sequence and~~ (ii) aa 3' sequence complementary to a 3'3' sequence of the ~~first~~ barcode oligonucleotide~~;~~.

~~b) performing a polymerase extension reaction or a ligation reaction to incorporate the W sequence into the bound oligonucleotide;~~

~~c) providing a second barcode oligonucleotide comprising an (i) S2_y sequence and (ii) a 3' sequence complementary to the 3' end of the bound oligonucleotide resulting from step b); and~~

~~d) performing a polymerase extension reaction or ligation reaction to incorporate the S2_y sequence into the bound oligonucleotide,~~

~~thereby attaching a polynucleotide to the solid support, wherein the polynucleotide contains a barcode sequence, and the barcode sequence comprises the S1_x, W, and S2_y sequences.~~

~~109.~~—The method of claim ~~108~~, wherein the solid support is a bead or a surface.

~~110.~~—23. The method of claim ~~108~~, wherein the capture moiety is streptavidin3, further comprising using a fluorophore-labeled reverse primer.

~~111.~~—24. The method of claim ~~108~~, wherein the capture moiety comprises a carboxyl group, epoxy group, or hydroxyl group.

~~112.~~—The method of claim ~~108~~, wherein the capture moiety comprises gold to capture thiolated oligonucleotides.

~~113.~~—The method of claim ~~108~~, wherein the first or second barcode oligonucleotide further comprises a universal priming sequence and a binding site.

~~114.~~—The method of claim ~~113~~, wherein the first or second barcode oligonucleotide further comprises an RNAP promoter selected from the group consisting of T7, T3, and SP6.

~~115.~~—The method of claim ~~113~~, wherein the first or second barcode oligonucleotide further comprises a nicking endonuclease restriction site selected from the group consisting of Nt.BbvCI, Nt.BspQI, Nt.BsmAI, Nt.BstNBI, Nt.AIwI, and Nt.BsmAI.

~~116.~~—The method of claim ~~113~~, wherein the binding site is one or more G nucleotides.

~~117.~~—A solid support prepared by the method of any one of claims ~~97-116~~, wherein the solid support is attached to a polynucleotide and the polynucleotide contains a barcode sequence.

~~118.~~—A barcode library comprising a plurality of the solid supports of claim ~~117~~.

~~119.~~—A microfluidic droplet device for encapsulating cells, barcode adapter templates, and reagents for producing polynucleotides of interest, the device comprising (a) three independently controlled pressure sources, (b) three microfluidic pathways, (c) three flow sensors, (d) two sample loops, (e) a microfluidic droplet chip, and (f) a sample collection container, wherein:

each pressure source is coupled to and drives fluid through one of the microfluidic pathways,

one of the flow sensors is disposed along each microfluidic pathway downstream of the respective pressure source,

a first microfluidic pathway passes through a first sample loop,

a second microfluidic pathway passes through a second sample loop,
the first and second sample loops being in contact with a thermal cooling unit,
the first and second microfluidic pathways merge at a first junction to form a combined pathway,

the combined pathway and third microfluidic pathway merge at a second junction to form a sample pathway, the second junction occurring within the microfluidic droplet chip and downstream of the first junction, and

the sample pathway passes into the sample collection container downstream of the second junction,

such that (a)-(f) are fluidly connected.

120.—The device of claim **119**, wherein each pressure source comprises a pressure pump.

121.—The device of claim **119**, wherein each pressure source comprises a syringe pump.

122.—The device of claim **119**, wherein the first sample loop is configured to meter the flow of an aqueous solution toward the microfluidic droplet chip, and wherein the aqueous solution comprises cells and barcode adapter templates.

123.—The device of claim **119**, wherein the second sample loop is configured to meter the flow of a reaction mixture toward the microfluidic droplet chip, and wherein the reaction mixture comprises reagents for cell lysis and reagents for producing polynucleotides of interest.

124.—The device of claim **119**, wherein the third microfluidic pathway is configured to deliver an oil/surfactant mix to the microfluidic droplet chip.

125.—The device of claim **119**, wherein the thermal cooling unit comprises a Peltier device.

126.—The device of claim **119**, wherein the thermal cooling unit comprises an ice bin.

~~127.~~—The device of claim ~~119~~, wherein the first junction occurs within the droplet chip.

~~128.~~—The device of claim ~~119~~, wherein the third microfluidic pathway is split into two subpathways upstream of the microfluidic droplet chip, the two subpathways merge with the combined pathway at the second junction, and the second junction has flow-focusing geometry.

~~129.~~—The device of claim ~~119~~, wherein the second junction has a t-junction geometry.~~130.~~ The device of claim ~~119~~, wherein the first microfluidic pathway is configured to accommodate cells, and the second microfluidic pathway is configured to accommodate barcode adapter templates bound to solid supports.

~~131.~~—The adapter template library of claim ~~77~~1, wherein at least ~~two of the solid supports comprise adapter constructs with different barcode sequences or UMI sequences~~10, 30, 100, 300, 1,000, 3,000, 10,000, 30,000, 100,000, 300,000, or 1,000,000 copies of the barcode sequence are bound to the solid support.

~~132.~~—25. The ~~adapter template library~~method of claim ~~131~~1, wherein ~~every solid support of the plurality of solid supports comprises an adapter construct with a different~~at most or on average one barcode ~~sequence or a different UMI sequence.~~oligonucleotide is present per hydrophilic compartment.