

[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 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.
2. The method of claim 1, wherein the barcode oligonucleotide further comprises a 5' sequence identical or complementary to a PCR reverse primer sequence.
3. The method of claim 2, further comprising performing a PCR reaction to amplify the barcode oligonucleotide.
4. The method of claim 1, wherein the solid support is a bead or a surface.
5. The method of claim 1, wherein the capture moiety is streptavidin.
6. The method of claim 1, wherein the capture moiety comprises a carboxyl group, epoxy group, or hydroxyl group.
7. The method of claim 1, wherein the capture moiety comprises gold to capture thiolated oligonucleotides.
8. The method of claim 1, wherein the barcode oligonucleotide further comprises a universal priming sequence and a binding site.
9. The method of claim 8, wherein the barcode oligonucleotide comprises an RNAP promoter selected from the group consisting of T7, T3, and SP6.
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 8, wherein the 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.
21. The solid support of claim 18, wherein the binding site is one or more G nucleotides.
22. The method of claim 1, wherein the bound oligonucleotide comprises a 3' sequence complementary to a 3' sequence of the barcode oligonucleotide.
23. The method of claim 3, further comprising using a fluorophore-labeled reverse primer.
24. The method of claim 1, 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 barcode sequence are bound to the solid support.
25. The method of claim 1, wherein at most or on average one barcode oligonucleotide is present per hydrophilic compartment.