

[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.

[0018a] In one aspect, there is provided 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.

[0018b] In one aspect, there is provided an adapter construct comprising an RNAP promoter sequence, a universal priming sequence, a barcode sequence, and a binding site.

[0018c] In one aspect, there is provided a solid support comprising the adapter construct as disclosed herein.

[0018d] In one aspect, there is provided an adapter template library comprising a plurality of the solid supports as disclosed herein.

[0018e] In one aspect, there is provided a nucleic acid marker comprising a nucleic acid coupled to a molecular label, wherein the molecular label is an antibody, antigen, or protein.

[0018f] In one aspect, there is provided a plurality of nucleic acid markers as disclosed herein, 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.

[0018g] In one aspect, there is provided a kit comprising the adapter construct as disclosed herein, the solid support as disclosed herein, the adapter template library as disclosed herein, or the nucleic acid marker as disclosed herein.

[0018h] In one aspect, there is provided a method of attaching a polynucleotide to a solid support, wherein the polynucleotide contains 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 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 barcode sequence into the bound oligonucleotide on the solid support.

[0018i] In one aspect, there is provided 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, wherein the bound oligonucleotide comprises (i) an S1_x sequence and (ii) a sequence complementary to a 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.

[0018j] In one aspect, there is provided a solid support prepared by the method as disclosed herein, wherein the solid support is attached to a polynucleotide and the polynucleotide contains a barcode sequence.

[0018k] In one aspect, there is provided a barcode library comprising a plurality of the solid supports as disclosed herein.

[0018l] In one aspect, there is provided 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.

SUMMARY OF THE INVENTION

[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,