

methods that utilize a programmable microfluidic device to isolate single cells and perform scATAC-seq in nanoliter reaction chambers (see, *e.g.*, Buenrostro, *et al.*, Nature, 2015 Jul 23;523(7561):486-90) are limited by the throughput of the assay and may not generate personal epigenomic profiles on a timescale compatible with clinical decision-making.

[0010a] In one aspect, the present disclosure provides a method for processing a nucleic acid molecule, comprising: (a) providing a partition comprising: (i) a biological particle comprising (I) a tagged fragment of a deoxyribonucleic acid (DNA) molecule, and (II) a ribonucleic acid (RNA) molecule (ii) a first nucleic acid barcode molecule comprising a first barcode sequence, (iii) a second nucleic acid barcode molecule comprising a second barcode sequence, and (iv) a splint ~~sequence molecule~~, wherein said first nucleic acid barcode molecule comprises an overhang sequence ~~of said tagged fragment~~, and wherein said splint molecule comprises a first sequence complementary to a sequence of said tagged fragment and a second sequence complementary to said overhang sequence; and (b) within said partition: (i) ~~using hybridizing said sequence of~~ said tagged fragment to said splint molecule, and hybridizing said overhang sequence of, said first nucleic acid barcode molecule, ~~and to~~ said splint molecule to generate a first barcoded nucleic acid product comprising: (I) said first barcode sequence or a complement thereof and (II) a sequence of said DNA molecule; and (ii) using said RNA molecule and said second nucleic acid barcode molecule to generate a second barcoded nucleic acid product comprising: (I) said second barcode sequence or a complement thereof and (II) a complementary DNA (cDNA) sequence of said RNA molecule, wherein said second nucleic acid barcode molecule comprises a capture sequence complementary to said RNA molecule and wherein (b)(ii) comprises hybridizing said capture sequence to said RNA molecule and reverse transcribing said RNA molecule to generate said second barcoded nucleic acid product.

[0011] In an example, the present disclosure provides a method for processing a nucleic acid sample, comprising: (a) providing a biological particle comprising a deoxyribonucleic acid (DNA) molecule and a ribonucleic acid (RNA) molecule within a partition among a plurality of partitions, wherein the partition comprises (i) a first nucleic acid barcode molecule, (ii) a second nucleic acid barcode molecule, and (iii) a splint sequence, wherein the first nucleic acid barcode molecule and the second nucleic acid barcode molecule comprise a common barcode sequence, wherein the first nucleic acid barcode molecule comprises an overhang sequence, and wherein the splint sequence comprises a sequence complementary to the DNA molecule and a sequence complementary to the overhang sequence; (b) within the partition, using the DNA molecule, the first nucleic acid barcode molecule, and the splint sequence to generate a first barcoded nucleic acid product corresponding to the DNA molecule and using the RNA molecule and the second nucleic acid barcode molecule to generate a second barcoded nucleic acid product corresponding to the RNA molecule, wherein the first barcoded nucleic acid product and the second barcoded nucleic acid product comprise the common barcode sequence, or a complement thereof; and (c)

CLAIMS

WHAT IS CLAIMED IS:

1. A method for processing a nucleic acid molecule, comprising:
 - (a) providing a partition comprising:
 - (i) a biological particle comprising: (I) a tagmented fragment of a deoxyribonucleic acid (DNA) molecule, and (II) a ribonucleic acid (RNA) molecule,
 - (ii) a first nucleic acid barcode molecule comprising a first barcode sequence,
 - (iii) a second nucleic acid barcode molecule comprising a second barcode sequence, and
 - (iv) a splint ~~sequence~~molecule, wherein said first nucleic acid barcode molecule comprises an overhang sequence ~~of said tagmented fragment~~, and wherein said splint molecule comprises a first sequence complementary to a sequence of said tagmented fragment and a second sequence complementary to said overhang sequence; and
 - (b) within said partition:
 - (i) ~~using hybridizing said sequence of~~ said tagmented fragment to said splint molecule, and hybridizing said overhang sequence of, said first nucleic acid barcode molecule, ~~and to~~ said splint molecule to generate a first barcoded nucleic acid product comprising: (I) said first barcode sequence or a complement thereof and (II) a sequence of said DNA molecule; and
 - (ii) using said RNA molecule and said second nucleic acid barcode molecule to generate a second barcoded nucleic acid product comprising: (I) said second barcode sequence or a complement thereof and (II) a complementary DNA (cDNA) sequence of said RNA molecule, wherein said second nucleic acid barcode molecule comprises a capture sequence complementary to said RNA molecule and wherein (b)(ii) comprises hybridizing said capture sequence to said RNA molecule and reverse transcribing said RNA molecule to generate said second barcoded nucleic acid product.
2. The method of claim 1, wherein said biological particle is a cell, cell bead, or cell nucleus.
3. The method of claim 1, further comprising lysing or permeabilizing said biological particle within said partition to provide access to said tagmented fragment and said RNA molecule.

4. The method of any one of claims 1-3, further comprising, prior to (a), contacting an open chromatin structure of said biological particle with a transposase-nucleic acid complex to yield said biological particle comprising said tagmented fragment.
5. The method of claim 4, wherein said transposase-nucleic acid complex comprises a first ~~adapter~~transposon end oligonucleotide molecule and a second transposon end oligonucleotide molecule~~adapter~~ and wherein said tagmented fragment comprises a sequence of said DNA molecule flanked by said first transposon end oligonucleotide molecule~~adapter~~ and said second transposon end oligonucleotide molecule~~adapter~~.
6. The method of claim 5, wherein (b)(i) comprises hybridizing said splint molecule to (A) said first transposon end oligonucleotide molecule~~adapter~~ or said second transposon end oligonucleotide molecule~~adapter~~ of said tagmented fragment and (B) said first nucleic acid barcode molecule, and ligating said first nucleic acid barcode molecule and said tagmented fragment to generate said first barcoded nucleic acid product.
7. The method of claim 5, wherein said first transposon end oligonucleotide molecule~~adapter~~ comprises a first transposon end sequence and a first primer sequence and wherein said second transposon end oligonucleotide molecule~~adapter~~ comprises a second transposon end sequence and a second primer sequence.
8. The method of claim 7, wherein (b)(i) comprises:
hybridizing said splint molecule to (A) said first primer sequence or said second primer sequence of said tagmented fragment and (B) said first nucleic acid barcode molecule, and
ligating said first nucleic acid barcode molecule and said tagmented fragment to generate said first barcoded nucleic acid product.
9. The method of claim 8, further comprising, prior to said ligating, using a kinase to phosphorylate said tagmented fragment~~or said first nucleic acid barcode molecule~~.
10. The method of claim 9, wherein said kinase is a polynucleotide kinase (PNK).
11. The method of claim 7, wherein said first primer sequence or said second primer sequence is single stranded.
12. The method of claim 1, further comprising subjecting said first barcoded nucleic acid product to one or more nucleic acid extension reactions.
13. The method of claim 12, wherein said one or more nucleic acid extension reactions comprise attaching one or more functional sequences to said first barcoded nucleic acid product.

14. The method of claim 13, wherein said one or more functional sequences comprise one or more flow cell adapter sequences, one or more sequencing primer sequences, one or more sequencing primer binding sequences, or one or more sample index sequences.
- ~~15. The method of claim 1, wherein said second nucleic acid barcode molecule comprises a capture sequence complementary to said RNA molecule and wherein (b)(ii) comprises hybridizing said capture sequence to said RNA molecule and reverse transcribing said RNA molecule to generate said second barcoded nucleic acid product.~~
- ~~16.~~15. The method of claim ~~15~~, wherein said capture sequence comprises a polythymine (poly-T) sequence.
- ~~17.~~16. The method of claim 1, wherein said partition further comprises a primer comprising a sequence complementary to said RNA molecule and wherein (b)(ii) comprises hybridizing said primer to said RNA molecule, reverse transcribing said RNA molecule to generate a cDNA molecule, performing a template switching reaction onto said second nucleic acid barcode molecule, and extending said cDNA molecule to generate said second barcoded nucleic acid product.
- ~~18.~~17. The method of claim ~~16~~7, wherein said sequence complementary to said RNA molecule comprises a polythymine (poly-T) sequence.
- ~~19.~~18. The method of claim ~~16~~7, wherein said reverse transcribing comprises use of an enzyme with terminal transferase activity that appends a polynucleotide sequence to a 3' end of said cDNA molecule and wherein said second nucleic acid barcode molecule comprises a sequence complementary to said polynucleotide sequence to facilitate said template switching reaction.
- ~~20.~~19. The method of claim ~~18~~9, wherein said polynucleotide sequence comprises a polycytosine (polyC) sequence and wherein said sequence complementary to said polynucleotide sequence comprises a polyguanine (polyG) sequence.
- ~~21.~~20. The method of claim ~~19~~20, wherein said polyG sequence comprises a ribonucleotide.
- ~~22.~~21. The method of claim 1, wherein said partition is a droplet.
- ~~23.~~22. The method of claim 1, wherein said partition is a well.
- ~~24.~~23. The method of claim 1, wherein said first barcode sequence and said second barcode sequence are a same sequence.
- ~~25.~~24. The method of claim 1, wherein said first barcode sequence and said second barcode sequence are different.
- ~~26.~~25. The method of claim 1, wherein said tagmented fragment ~~or said first nucleic acid barcode molecule~~ comprises a 5' phosphate.

~~27~~26. The method of any one of claims 1-2~~5~~6, wherein said first nucleic acid barcode molecule and said second nucleic acid barcode molecule are coupled to a bead.

~~28~~27. The method of claim 2~~6~~7, wherein said bead is a gel bead.

~~29~~28. The method of claim ~~26~~ or ~~27~~ or ~~28~~, wherein said first nucleic acid barcode molecule and said second nucleic acid barcode molecule are releasable from said bead upon application of a stimulus.

~~30~~29. The method of claim 2~~6~~7, wherein said first nucleic acid barcode molecule and said second nucleic acid barcode molecule are each coupled to said bead via a labile bond.