

SYSTEMS AND METHODS FOR PROCESSING RNA FROM CELLS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/824,851, filed March 27, 2019, which is entirely incorporated herein by reference for all purposes.

BACKGROUND

[0002] A sample may be processed for various purposes, such as identification of nucleic acid molecules within the sample. The sample may be a biological sample. Biological samples may be processed, such as for detection of a disease (e.g., cancer) or identification of a particular species.

[0003] Biological samples may comprise nucleic acid molecules. Nucleic acid molecules such as DNA, RNA, etc. may be analyzed. There are various approaches for processing samples, such as polymerase chain reaction (PCR) and sequencing.

[0004] Biological samples may be processed within various reaction environments, such as partitions. Partitions may be wells or droplets. Droplets or wells may be employed to process biological samples in a manner that enables the biological samples to be partitioned and processed separately. For example, such droplets may be fluidically isolated from other droplets, enabling accurate control of respective environments in the droplets.

[0005] Biological samples in partitions may be subjected to various processes, such as chemical processes or physical processes. Samples in partitions may be subjected to heating or cooling, or chemical reactions, such as to yield species that may be qualitatively or quantitatively processed.

SUMMARY

[0005a] In one aspect, the present disclosure refers to a method for assaying ribonucleic acid (RNA) molecules, comprising:

- (a) providing a cell, nucleus, or cell bead that comprises ribonucleic acid (RNA) molecules;
- (b) depleting ribosomal RNA (rRNA) molecules from said RNA molecules, in said cell, nucleus, or cell bead, to yield a plurality of remaining RNA molecules comprising non-poly-adenylated RNA molecules;
- (c) fragmenting, in said cell, nucleus, or cell bead, said plurality of remaining RNA molecules to yield a plurality of RNA fragments comprising a non-poly-adenylated RNA fragment;

- (d) partitioning said cell, nucleus, or cell bead into a partition;
- (e) providing a plurality of nucleic acid barcode molecules to said partition, wherein nucleic acid barcode molecules of said plurality of nucleic acid barcode molecules comprise a common barcode sequence; and
- (f) covalently attaching said non-poly-adenylated RNA fragment of said plurality of RNA fragments and a nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules to generate a barcoded nucleic acid molecule; and
- (g) using said barcoded nucleic acid molecule to generate amplification products of said barcoded nucleic acid molecule.

[0006] A variety of diseases can be diagnosed using genetic tests, and personalized medicine benefits from analysis of individual genomes and transcriptomes. In analyzing transcriptomes, messenger RNA (mRNA) is commonly studied. However, other species of RNA are important in disease progression, diagnosis, and treatment. Current methods for obtaining full-length transcript information are limited. Moreover, ribosomal RNA (rRNA) is abundant and may exhaust resources when assessing total RNA from samples or introduce bias. Provided herein are methods for streamlined sample processing to obtain full-length transcript information while avoiding rRNA. These methods may provide information on underexplored RNA species, e.g., microRNA, long non-coding RNA, transfer RNA, small nucleolar RNA, circular RNA, RNA isoforms, etc.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for assaying ribonucleic acid (RNA) molecules, comprising:
 - (a) providing a cell, nucleus, or cell bead that comprises ribonucleic acid (RNA) molecules;
 - (b) depleting ribosomal RNA (rRNA) molecules from said RNA molecules, in said cell, nucleus, or cell bead, to yield a plurality of remaining RNA molecules comprising non-poly-adenylated RNA molecules;
 - (c) fragmenting, in said cell, nucleus, or cell bead, said plurality of remaining RNA molecules to yield a plurality of RNA fragments comprising a non-poly-adenylated RNA fragment;
 - (d) partitioning said cell, nucleus, or cell bead into a partition;
 - (e) providing a plurality of nucleic acid barcode molecules to said partition, wherein nucleic acid barcode molecules of said plurality of nucleic acid barcode molecules comprise a common barcode sequence; and
 - (f) covalently attaching said non-poly-adenylated RNA fragment of said plurality of RNA fragments to a nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules to generate a barcoded nucleic acid molecule; and
 - (g) using said barcoded nucleic acid molecule to generate amplification products of said barcoded nucleic acid molecule.
2. The method of claim 1, wherein (g) comprises performing a nucleic acid reaction to generate said amplification products of said barcoded nucleic acid molecule.
3. The method of claim 2, wherein said nucleic acid barcode molecule has, hybridized thereto, an RNA molecule, and wherein said nucleic acid reaction is performed using a thermostable group II intron reverse transcriptase (TGIRT).
4. The method of claim 2, further comprising, subsequent to said nucleic acid reaction, adding adaptor molecules to said amplification products of said barcoded nucleic acid molecule.
5. The method of claim 4, wherein said adaptor molecules comprises a sequence comprising a 5'-App and 3'-blocker.

6. The method of claim 4, wherein said adding comprises ligating said adaptor molecules to said amplification products of said barcoded nucleic acid molecule using a 5'-App DNA/RNA ligase.
7. The method of claim 4, wherein said adaptor molecules comprise a splint oligonucleotide.
8. The method of claim 7, wherein said splint oligonucleotide comprises a random N-mer or a 3' blocker.
9. The method of claim 7, wherein said adding comprises ligating said adaptor molecules to said amplification products of said barcoded nucleic acid molecule using a T4 DNA ligase.
10. The method of claim 2, wherein said nucleic acid reaction is performed using a T4KQ ligase.
11. The method of claim 10, further comprising, subsequent to said nucleic acid reaction, adding adaptor sequences to said amplification products of said barcoded nucleic acid molecule using a reverse transcription reaction.
12. The method of claim 11, wherein said adaptor sequences comprise a template switching oligonucleotide.
13. The method of claim 11, wherein said adaptor sequences comprise a sequencing primer sequence, a reverse transcription primer sequence, and a uridine residue.
14. The method of claim 13, further comprising, subsequent to said reverse transcription reaction, subjecting said amplification products of said barcoded nucleic acid molecule to circularization, clean up, and linearization.
15. The method of claim 14, wherein said circularization is performed using a T4 DNA ligase, a splint oligonucleotide, or a single-stranded DNA ligase.
16. The method of claim 15, wherein said circularization is performed using said single-stranded DNA ligase, wherein said single-stranded DNA ligase is Circligase.
17. The method of claim 13, further comprising, prior to said reverse transcription reaction, subjecting said amplification products of said barcoded nucleic acid molecule to (i) phosphorylation using a first enzyme, and (ii) ligation of said sequencing primer sequence using a second enzyme.
18. The method of claim 17, wherein said first enzyme is a T4 polynucleotide kinase and said second enzyme is a T4 RNA ligase.
19. The method of claim 1, wherein said cell, nucleus, or cell bead is a fixed and permeabilized cell, nucleus or cell bead.
20. The method of claim 1, wherein said fragmenting comprises a chemical fragmentation process or an enzymatic fragmentation process.

21. The method of claim 20, wherein said chemical fragmentation process comprises (i) using divalent metal cations and heating or (ii) using a reactive oxygen species.
22. The method of claim 20, further comprising, subsequent to said fragmenting, cleaving a 3' phosphate group from said non-poly-adenylated RNA fragment.
23. The method of claim 20, wherein said enzymatic fragmentation process comprises using RNase III.
24. The method of claim 1, wherein said plurality of nucleic acid barcode molecules comprises a capture sequence.
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25. The method of claim 1, wherein said non-poly-adenylated RNA molecules comprises miRNA, long non-coding RNA, short non-coding RNA, tRNA, RNA isoforms, snoRNA, small RNA, piRNA, or circular RNA.
26. The method of claim 1, wherein said partition is a droplet or a well.