METHODS AND SYSTEMS TO MINIMIZE BARCODE EXCHANGE

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/714,476, filed August 3, 2018, which is entirely incorporated herein by reference.

BACKGROUND

[0002] A sample may be processed for various purposes, such as identification of a type of moiety 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. There are various approaches for processing samples, such as polymerase chain reaction (PCR) and sequencing.

[0003] 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.

[0004] 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.

[0005] Certain applications may benefit from the amplification or sequencing of species obtained from single cells obtained from a much larger population. In some cases, the single cells of interest may be quite rare. Thus, there is a need for sample preparation techniques that allow sequencing of nucleic acids from single cells of interest.

SUMMARY

[0005a] In one aspect, provided herein is a method for nucleic acid processing, comprising: (a) contacting, in a bulk solution, a plurality of biological particles comprising template nucleic acid molecules with a plurality of transposase complexes comprising an adapter sequence~~s~~ to generate, from said template nucleic acid molecules, a plurality of adapter-flanked fragments, wherein said plurality of biological particles is a plurality of cells or a plurality of cell nuclei; (b) partitioning said plurality of biological particles into a plurality of partitions, wherein a partition of said plurality of partitions comprises: (i) a biological particle of said plurality of biological particles, wherein said biological particle comprises an adapter-flanked fragment of said plurality of adapter-flanked fragments; (ii) a plurality of nucleic acid barcode molecules comprising a common

barcode sequence; and (iii) a nucleic acid splint molecule comprising a first sequence complementary to said adapter sequence and a second sequence complementary to a sequence of a nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules, wherein said nucleic acid splint molecule comprises a non-canonical base; and (c) using said adapter-flanked fragment, said nucleic acid barcode molecule, and said nucleic acid splint molecule to generate a barcoded nucleic acid molecule comprising a sequence of said adapter-flanked fragment and said common barcode sequence.

[0006] In an example, provided herein is a method for nucleic acid processing, comprising: (a) in a plurality of partitions, using nucleic acid barcode molecules from a plurality of nucleic acid barcode molecules and a plurality of nucleic acid molecules to generate a plurality of barcoded nucleic acid molecules; (b) recovering the plurality of barcoded nucleic acid molecules from the plurality of partitions, to yield a mixture comprising the plurality of nucleic acid barcode molecules and a remainder of the plurality of nucleic acid barcode molecules; (c) subsequent to (b), subjecting the mixture to conditions sufficient to couple a blocking oligonucleotide to the remainder of the plurality of nucleic acid barcode molecules, wherein the blocking oligonucleotide comprises (i) a complementary region comprising a sequence at least 75% complementary to a sequence in the remainder of the plurality of nucleic acid barcode molecules; and (ii) a 3’ blocking group.

[0007] In some examples, the 3’ blocking group is configured to prevent ligation of the remainder to a nucleic acid molecule. In some examples, the 3’ blocking group is configured to prevent extension of the primer sequence when hybridized to a target nucleic acid molecule. In some examples, the plurality of partitions is a plurality of droplets. In some examples, the plurality of partitions is a plurality of wells. In some examples, the blocking oligonucleotide comprises, from 5’ to 3’, a first stem sequence, a loop sequence, a second stem sequence at least 75% complementary to the first stem sequence, and the complementary region. In some examples, the 3’ blocking group is at a 3’ terminus of the blocking oligonucleotide. In some examples, the 3’ blocking group is: a 2’-3’-dideoxycytosine (ddC), a 2’-5’ linked nucleoside, a 3’ C3 spacer, an amino-modified C6, an inverted deoxythymidine (dT), a 3’ amino modified nucleotide, or a 3’ phosphate group. In some examples, the 2'-5' linked nucleoside is a 3'-deoxythymidine (3’-dT) 3'-deoxycytidine (3’-dC), 3’-deoxyadenosine (3’-dA), or a 3'-deoxyguanosine (3’-dG). In some examples, the blocking oligonucleotide is ligated to the remainder. In some examples, the blocking oligonucleotide comprises a 5’ phosphate group.

[0008] In another example, disclosed herein is a composition, comprising: a nucleic acid molecule comprising, from 5’ to 3’: (a) a first region comprising a barcode sequence and a primer sequence; and (b) a second region comprising (i) a sequence complementary to the primer sequence; and (ii) 3’ a blocking group. In some examples, the 3’ blocking group is at a 3’

CLAIMS

1. A method for nucleic acid processing, comprising:

(a) contacting, in a bulk solution, a plurality of biological particles comprising template nucleic acid molecules with a plurality of transposase complexes comprising an adapter sequences to generate, from said template nucleic acid molecules, a plurality of adapter-flanked fragments, wherein said plurality of biological particles is a plurality of cells or a plurality of cell nuclei;

(b) partitioning said plurality of biological particles into a plurality of partitions, wherein a partition of said plurality of partitions comprises:

(i) a biological particle of said plurality of biological particles, wherein said biological particle comprises an adapter-flanked fragment of said plurality of adapter-flanked fragments;

(ii) a plurality of nucleic acid barcode molecules comprising a common barcode sequence; and

(iii) a nucleic acid splint molecule comprising a first sequence complementary to said adapter sequence and a second sequence complementary to a sequence of a nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules, wherein said nucleic acid splint molecule comprises a non-canonical base; and

(c) using said adapter-flanked fragment, said nucleic acid barcode molecule, and said nucleic acid splint molecule to generate a barcoded nucleic acid molecule comprising a sequence of said adapter-flanked fragment and said common barcode sequence.

2. The method of claim 1, wherein said nucleic acid splint molecule further comprises a blocking group.

3. The method of claim 2, wherein said blocking group is a 3’ blocking group.

4. The method of claim 3, wherein said 3’ blocking group is a 2’-3’-dideoxycytosine (ddC), a 2’-5’ linked nucleoside, a 3’ C3 spacer, an amino-modified C6, an inverted deoxythymidine (dT), a 3’ amino modified nucleotide, or a 3’ phosphate group.

5. The method of claim 4, wherein said 2'-5' linked nucleoside is a 3'-deoxythymidine (3’-dT) 3'-deoxycytidine (3’-dC), 3’-deoxyadenosine (3’-dA), or a 3'-deoxyguanosine (3’-dG).

6. The method of claim 1, wherein said noncanonical base is uracil.

7. The method of claim 1, further comprising, subsequent to (c), generating an abasic site in said nucleic acid splint molecule at a position of said noncanonical base.

8. The method of claim 7, wherein said noncanonical base is uracil and wherein said abasic site is generated using a uracil-DNA-glycosylase (UDG).

9. The method of claim 1, further comprising, subsequent to (c), pooling ~~the~~ contents of said plurality of partitions to yield pooled contents, processing said pooled contents ~~of said plurality of partitions~~ to generate an abasic site at a position of said non-canonical base, and performing one or more nucleic acid reactions on said barcoded nucleic acid molecule or a derivative thereof to generate a reaction product, wherein said processing reduces barcode exchange in said reaction product.

10. The method of claim 9, wherein said noncanonical base is uracil and wherein said processing comprises using an enzyme to generate said abasic site.

11. The method of claim 10, wherein said enzyme is uracil-DNA-glycosylase (UDG).

12. The method of claim 9, wherein said one or more nucleic acid reactions comprises one or more nucleic acid amplification reactions.

13. The method of claim 1, wherein (c) comprises ligating said adapter-flanked fragment to said nucleic acid barcode molecule to generate said barcoded nucleic acid molecule.

14. The method of claim 1, wherein said adapter-flanked fragment comprises a first adapter sequence and a second adapter sequence, ~~and~~ wherein said plurality of transposase complexes comprises a plurality of transposase molecules, and wherein said adapter sequences comprise said first adapter sequence~~;~~ and said second adapter sequence.

15. The method of claim 1, wherein said plurality of partitions is a plurality of droplets in an emulsion.

16. The method of claim 1, wherein said plurality of partitions is a plurality of microwells in an array, wherein said array comprises 1,000 microwells.

17. The method of claim 1, wherein said plurality of nucleic acid barcode molecules are attached to a bead.

18. The method of claim 17, wherein said plurality of nucleic acid barcode molecules is releasable from said bead upon application of a stimulus.

19. The method of claim 17, wherein said bead is a gel bead.

20. The method of claim 19, wherein said gel bead is degradable upon application of a stimulus.

21. The method of claim 1, further comprising permeabilizing said plurality of biological particles before (a).

22. The method of claim 21, wherein said transposase complexes comprise Tn5 transposase.

23. The method of claim 22, wherein said biological particle is subjected to a condition to release said adapter-flanked fragment from said biological particle before (c).

24. The method of claim 1, further comprising amplifying said barcoded nucleic acid molecule to generate amplified products.

25. The method of claim 22, further comprising amplifying said barcoded nucleic acid molecule to generate amplified products.

26. The method of claim 24, further comprising determining sequences of said amplified products.

27. The method of claim 25, further comprising determining sequences of said amplified products.

28. The method of claim 26, further comprising analyzing the sequences of said amplified product to determine areas of accessible chromatin in the biological particle.

29. The method of claim 27, further comprising analyzing the sequences of said amplified product to determine areas of accessible chromatin in the biological particle.

30. The method of claim 9, further comprising permeabilizing said plurality of biological particles before (a).

31. The method of claim 30, wherein said transposase complexes comprise Tn5 transposase.

32. The method of claim 31, wherein said biological particle is subjected to a condition to release said adapter-flanked fragment from said biological particle before (c).

33. The method of claim 32, further comprising amplifying said barcoded nucleic acid molecule to generate amplified products.

34. The method of claim 33, further comprising determining sequences of said amplified products.

35. The method of claim 34, further comprising analyzing the sequences of said amplified product to determine areas of accessible chromatin in the biological particle.