

METHODS AND SYSTEMS FOR BARCODING NUCLEIC ACID MOLECULES FROM INDIVIDUAL CELLS OR CELL POPULATIONS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/410,326, filed October 19, 2016, and U.S. Provisional Patent Application No. 62/490,546, filed April 26, 2017, each of which is incorporated herein by reference in its entirety for all purposes.

BACKGROUND

[0002] Significant advances in analyzing and characterizing biological and biochemical materials and systems have led to unprecedented advances in understanding the mechanisms of life, health, disease and treatment. Among these advances, technologies that target and characterize the genomic make up of biological systems have yielded some of the most groundbreaking results, including advances in the use and exploitation of genetic amplification technologies, and nucleic acid sequencing technologies.

[0003] Nucleic acid sequencing can be used to obtain information in a wide variety of biomedical contexts, including diagnostics, prognostics, biotechnology, and forensic biology. Sequencing may involve methods including Maxam-Gilbert sequencing and chain-termination methods, or de novo sequencing methods including shotgun sequencing and bridge PCR, or next-generation methods including polony sequencing, 454 pyrosequencing, Illumina sequencing, SOLiD sequencing, Ion Torrent semiconductor sequencing, HeliScope single molecule sequencing, SMRT® sequencing, and others. Nucleic acid sequencing technologies, including next-generation DNA sequencing, have been useful for genomic and proteomic analysis of cell populations.

SUMMARY

[0003a] In one aspect, provided herein is a method for nucleic acid sequencing, comprising: (a) providing a plurality of partitions, wherein a partition of the plurality of partitions comprises: (i) a plurality of ribonucleic acid (RNA) molecules from a single cell, wherein the plurality of RNA molecules comprises at least one immune receptor; (ii) nucleic acid barcode molecules each comprising a barcode sequence and a sequence that hybridizes to individual RNA molecules of the plurality of RNA molecules; (b) hybridizing the nucleic acid barcode molecules to the RNA molecules in the partition; (c) extending the nucleic acid barcode molecules using a reverse transcriptase having terminal transferase activity to generate barcoded cDNA molecules each comprising a 3' terminal sequence added by the reverse transcriptase;

(d) hybridizing each 3' terminal sequence of the barcoded cDNA molecules to individual hybridization regions of template switching oligonucleotides (TSOs);

(e) using the TSOs as a template to extend the barcoded cDNA molecules, thereby generating a plurality of barcoded nucleic acid molecules;

(f) amplifying, outside the partition, a subset of the plurality of barcoded nucleic acid molecules generated in (e), to generate an unenriched sequencing library; and

(g) amplifying, outside the partition, a different subset of the plurality of barcoded nucleic acid molecules generated in (e), with a target specific primer to generate a target enriched sequencing library comprising amplicons of the immune receptor.

[0004] Recognized herein is the need for methods, compositions and systems for analyzing genomic and proteomic information from individual cells or a small population of cells. Such cells include, but are not limited to, cancer cells, fetal cells, and immune cells involved in immune responses. Provided herein are methods, compositions and systems for analyzing individual cells or a small population of cells, including the analysis and attribution of nucleic acids from and to these individual cells or cell populations.

[0005] In an aspect, the present disclosure provides a method for nucleic acid sequencing, comprising (a) providing a plurality of droplets, wherein a droplet of the plurality of droplets comprises (i) a ribonucleic acid (RNA) molecule comprising a nucleic acid sequence, and (ii) a bead comprising a nucleic acid barcode molecule coupled thereto, wherein the nucleic acid