

MASSIVELY PARALLEL SINGLE CELL ANALYSIS

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/012,237, filed on June 13, 2014, U.S. Provisional Application No. 61/952,036, filed on March 12, 2014, and U.S. Provisional Application No. 61/871,232, filed on August 28, 2013, each of which applications are incorporated herein by reference in their entirety.

BACKGROUND

[0002] Multicellular masses, such as tissues and tumors, may comprise a heterogeneous cellular milieu. These complex cellular environments may often display multiple phenotypes, which may be indicative of multiple genotypes. Distilling multicellular complexity down to single cell variability is an important facet of understanding multicellular heterogeneity. This understanding may be important in the development of therapeutic regimens to combat diseases with multiple resistance genotypes.

SUMMARY OF THE INVENTION

[0002a] In one aspect, there is provided a method comprising: associating a single bead with a single cell, wherein said single bead comprises a plurality of oligonucleotides, wherein each of said plurality of oligonucleotides comprises an identical cellular label sequence, a molecular label sequence, and a target-binding region, and wherein at least 100 of said plurality of oligonucleotides comprise different molecular label sequences.

[0003] One aspect provided is a method, comprising obtaining a sample comprising a plurality of cells; labeling at least a portion of two or more polynucleotide molecules, complements thereof, or reaction products therefrom, from a first cell of the plurality and a second cell of the plurality with a first same cell label specific to the first cell and a second same cell label specific to the second cell; and a molecular label specific to each of the two or more polynucleotide molecules, complements thereof, or reaction products therefrom, wherein each molecular label of the two or more polynucleotide molecules, complements thereof, or reaction products therefrom, from the first cell are unique with respect to each other, and wherein each molecular label of the two or more polynucleotide molecules, complements thereof, or reaction products therefrom, from the second cell are unique with respect to each other. In some embodiments, the method further comprises sequencing the at least a portion of two or more polynucleotide molecules, complements thereof, or reaction

products therefrom. In some embodiments, the method further comprises analyzing sequence data from the sequencing to identify a number of individual molecules of the polynucleotides in a specific one of the cells. In some embodiments, the cells are cancer cells. In some embodiments, the cells are infected with viral polynucleotides. In some embodiments, the cells are bacteria or fungi. In some embodiments, the sequencing comprises sequencing with

CLAIMS

WHAT IS CLAIMED IS:

1. A method comprising:
associating a single bead with a single cell,
wherein said single bead comprises a plurality of oligonucleotides,
wherein each of said plurality of oligonucleotides comprises an identical cellular label sequence, a molecular label sequence, and a target-binding region, and
wherein at least 100 of said plurality of oligonucleotides comprise different molecular label sequences.
2. The method of claim 1, wherein said single cell is selected from the group consisting of a rare cell, a tumor cell, a cell from a human, a cell from a tissue, a cell from a tumor, a cell infected with viral polynucleotides, and any combination thereof.
3. The method of claim 1, further comprising lysing said single cell after associating said single bead with said single cell.
4. The method of claim 1, further comprising lysing said single cell before associating said single bead with said single cell.
5. The method of claim 1, wherein said single bead is magnetic.
6. The method of claim 1, wherein said plurality of oligonucleotides comprises at least 100,000 oligonucleotides.
7. The method of claim 1, wherein said plurality of oligonucleotides each comprise a universal label sequence.
8. The method of claim 1, wherein said target-binding region comprises a sequence selected from the group consisting of an oligo-dT sequence, a gene-specific sequence, and a random multimer sequence.
9. The method of claim 1, further comprising stochastically labelling target nucleic acid molecules from said single cell.
10. The method of claim 9, wherein said labelling comprises hybridizing said target nucleic acid molecules to the plurality of oligonucleotides on said single bead.
11. The method of claim 1, wherein at least 1,000 of said plurality of oligonucleotides comprise different molecular label sequences.
12. The method of claim 1, wherein at least 10,000 of said plurality of oligonucleotides comprise different molecular label sequences.
13. The method of claim 1, wherein said single bead and said single cell are in the same well.

14. The method of claim 1, wherein said single bead and said single cell are in the same droplet.
15. The method of claim 1, wherein said single bead comprises silica gel, controlled pore glass, Dynabead, Wang resin, Merrifield resin, Sephadex/Sepharose bead, cellulose bead, polystyrene bead, or any combination thereof.
16. The method of claim 10, wherein said target nucleic acid molecules comprise mRNA molecules.
17. The method of claim 16, further comprising reverse transcribing said target nucleic acid molecules, thereby generating labelled target cDNAs.
18. The method of claim 17, wherein said reverse transcribing is done while said plurality of oligonucleotides is on said single bead.
19. The method of claim 17, further comprising performing second strand synthesis on said labelled target cDNAs, thereby generating double-stranded labelled target polynucleotides.
20. The method of claim 19, further comprising amplifying said double-stranded labelled target polynucleotides, thereby generating labelled target amplicons.
21. The method of claim 20, wherein said amplifying is performed with a universal primer and a random multimer primer.
22. The method of claim 20, wherein said amplifying is performed with a universal primer or a gene-specific primer.
23. The method of claim 20, wherein said amplifying comprises conducting a nested PCR reaction.
24. The method of claim 20, wherein said amplifying comprises amplifying the whole transcriptome of said single cell.
25. The method of claim 20, further comprising estimating the number of said target nucleic acid molecules.
26. The method of claim 25, wherein said estimating comprises determining the sequence of at least a portion said double-stranded labelled target amplicons.
27. The method of claim 25, wherein said estimating comprises determining the sequence of at least a portion said double-stranded labelled target amplicons and at least a portion of said oligonucleotide.
28. The method of claim 25, wherein said estimating comprises counting the number of unique oligonucleotides associated with a distinct target polynucleotide of said single cell.