cancer, infectious and normal/healthy donor libraries can be generated for personalized medicine to address fundamental unmet biological needs.

SUMMARY

[0003a] In one aspect, there is provided a method comprising:

(a) forming a plurality of vessels each comprising a single cell from a sample comprising a plurality of cells, a plurality of molecular-barcoded oligonucleotide molecules each comprising a unique molecular barcode, and a vessel-barcoded oligonucleotide comprising a vessel barcode;

(b) producing within one or more vessels of the plurality

(i) a first complementary polynucleotide that is complementary to a first cell polynucleotide from the single cell, and (ii) a second complementary polynucleotide that is complementary to a second cell polynucleotide from the single cell;

(c) forming a first molecular barcoded polynucleotide and a second molecular barcoded polynucleotide; and

(d) forming a first vessel-molecular-barcoded polynucleotide and a second vessel-molecular-barcoded polynucleotide.

[0003b] In one aspect, there is provided a method of barcoding polynucleotides comprising:

(a) hybridizing a molecular-barcoded oligonucleotide molecule to each of a plurality of complement polynucleotides that are complementary to a polynucleotide from a single cell, wherein the hybridized molecular-barcoded oligonucleotide molecule is from a plurality of uniquely barcoded molecular-barcoded oligonucleotide molecules within a vessel comprising the single cell;

(b) extending the complement polynucleotides hybridized to a molecular-barcoded oligonucleotide molecule;

(c) hybridizing a vessel-barcoded oligonucleotide or complement thereof to the extended complementary polynucleotides from (b), wherein each of the hybridized vessel-barcoded oligonucleotides comprises a vessel barcode that is unique to a single vessel of a plurality of vessels;

(d) extending the extended complementary polynucleotides from (c) to form polynucleotides comprising a molecular barcode and a vessel barcode; and

(e) sequencing polynucleotides comprising a molecular barcode and a vessel barcode from (d) or amplified products thereof.

[0003c] In one aspect, there is provided a composition comprising a plurality of vessels each comprising:

(a) a single cell from a sample comprising a plurality of cells;

(b) a plurality of molecular-barcoded oligonucleotide molecules;

(c) a vessel-barcoded oligonucleotide;

(d) a first polynucleotide comprising (i) a first molecular barcode sequence from a first molecular-barcoded oligonucleotide molecule of the plurality or a complement thereof, (ii) a

vessel barcode sequence from the vessel barcoded oligonucleotide or a complement thereof, and (iii) a region complementary to a first cell polynucleotide from the single cell; and

(e) a second polynucleotide comprising (i) a second molecular barcode sequence from a second molecular-barcoded oligonucleotide molecule of the plurality or a complement thereof, (ii) the vessel barcode sequence from the vessel barcoded oligonucleotide or a complement thereof, and (iii) a region complementary to a second cell polynucleotide from the single cell.

[0004] In one aspect, provided herein is a method comprising: forming a plurality of vessels each comprising a single cell from a sample comprising a plurality of cells, a plurality of molecular barcoded polynucleotides, and a vessel barcoded polynucleotide; producing: a first complementary polynucleotide that is complementary to a first cell polynucleotide from the single cell, and a second complementary polynucleotide that is complementary to a second cell polynucleotide from the single cell; attaching: a first molecular barcoded polynucleotide of the plurality to the first complementary polynucleotide, and a second molecular barcoded polynucleotide to the second complementary polynucleotide, thereby forming a first and a second single cell single-barcoded polynucleotide; and attaching the vessel barcoded polynucleotide, or an amplified product thereof to the first single cell single-barcoded polynucleotide, and the second single cell single-barcoded polynucleotide, thereby forming a first and a second single cell dual-barcoded sequences.

[0005] In one aspect, provided herein is a composition comprising: a plurality of vessels each comprising a single cell from a sample comprising a plurality of cells, a plurality of molecular barcoded polynucleotides, a vessel barcoded polynucleotide; a first complementary polynucleotide that is complementary to a first cell polynucleotide from the single cell, and a second complementary polynucleotide that is complementary to a second cell polynucleotide from the single cell; wherein the first complementary polynucleotide comprises a first molecular barcode of the plurality of molecular barcoded polynucleotides and the vessel barcode of the vessel barcoded polynucleotide or an amplified product of the vessel barcoded polynucleotide, and wherein the second complementary polynucleotide comprises a second molecular barcode of the plurality of molecular barcoded polynucleotides and the vessel barcode of the vessel barcoded polynucleotide or an amplified product of the vessel barcoded polynucleotide.

[0006] In one aspect, provided herein is a method comprising: (a) forming a plurality of vessels each comprising a single cell from a sample comprising a plurality of cells, a plurality of molecular barcoded polynucleotides, and a vessel barcoded polynucleotide; (b) producing: a first complementary polynucleotide that is complementary to a first cell polynucleotide from the single cell, and a second complementary polynucleotide that is complementary to a second cell polynucleotide from the single cell; (c) attaching: a first molecular barcoded polynucleotide of the plurality to the first complementary polynucleotide, and a second molecular barcoded polynucleotide to the second complementary polynucleotide, thereby forming a first and a second single cell single-

CLAIMS

WHAT IS CLAIMED IS:

1. A method comprising:

(a) forming a plurality of vessels each comprising a single cell from a sample comprising a plurality of cells, a plurality of molecular-barcoded oligonucleotide molecules each comprising a unique molecular barcode, and a vessel-barcoded oligonucleotide comprising a vessel barcode;

(b) producing within one or more vessels of the plurality

(i) a first complementary polynucleotide that is complementary to a first cell polynucleotide from the single cell, and (ii) a second complementary polynucleotide that is complementary to a second cell polynucleotide from the single cell;

(c) forming a first molecular barcoded polynucleotide and a second molecular barcoded polynucleotide; and

(d) forming a first vessel-molecular-barcoded polynucleotide and a second vessel-molecular-barcoded polynucleotide.

2. The method of claim 1, wherein the first cell polynucleotide is a heavy chain immunoglobulin (IgH), a T-cell receptor alpha (TCRα), or a T-cell receptor gamma (TCRγ) polynucleotide; and wherein the second cell polynucleotide is a light chain immunoglobulin (IgL), a T-cell receptor beta (TCRβ), or a T-cell receptor delta (TCRδ) polynucleotide.

3. The method of any one of claims 1-2, further comprising amplifying the first and the second vessel-molecular-barcoded polynucleotides.

4. The method of any one of claims 1-3, further comprising sequencing the first and the second vessel-molecular-barcoded polynucleotide or amplified products thereof.

5. The method of any one of claims 1-4, wherein the single cell is B-cell or a T-cell.

6. The method of any one of claims 1-5, wherein the first and the second vessel-molecular-barcoded polynucleotides in a single vessel of the plurality comprise a different molecular barcode.

7. The method of any one of claims 1-6, wherein the molecular-barcoded oligonucleotide molecules of the plurality are not amplified products.

8. The method of any one of claims 1-7, wherein the molecular barcode of a molecular-barcoded oligonucleotide molecule of the plurality in a first vessel of the plurality is different than the molecular barcode of a molecular-barcoded oligonucleotide molecule of the plurality in a second vessel of the plurality.

9. The method of any one of claims 1-8, wherein the vessel barcode of a vessel-barcoded oligonucleotide in a first vessel of the plurality is different from the vessel barcode of a vessel-barcoded oligonucleotide in a second vessel of the plurality.

10. The method of any one of claims 1-9, wherein the vessel-barcoded oligonucleotide in each vessel of the plurality in step (a) is present as a single molecule.

11. The method of any one of claims 1-10, wherein the vessel barcode of the first and the second vessel-molecular-barcoded polynucleotides in a single vessel of the plurality are the same.
12. The method of any one of claims 1-11, wherein the vessel-barcoded oligonucleotides in two or more different vessels of the plurality comprise a first common-vessel sequence upstream of their vessel barcode, a second common-vessel sequence downstream of their vessel barcode, or both.
13. The method of any one of claims 1-12, wherein each of the molecular-barcoded oligonucleotide molecules of the plurality in a same vessel of the plurality comprises a first common-molecular sequence upstream of their molecular barcode, a second common-molecular sequence downstream of their molecular barcode, or both.
14. The method of any one of claims 1-13, wherein each of the molecular-barcoded oligonucleotide molecules of the plurality in each vessel of the plurality comprises a first common-molecular sequence upstream of the molecular barcode, a second common-molecular sequence downstream of the molecular barcode, or both.
15. The method of any one of claims 1-14, wherein the vessel-barcoded oligonucleotide or a complement thereof comprises a sequence complementary to a region of a complement of each molecular-barcoded oligonucleotide molecule of the plurality.
16. The method of any one of claims 1-15, wherein the vessel-barcoded oligonucleotide or a complement thereof comprises a sequence complementary to a 3' end region of the first and the second molecular-barcoded polynucleotides.
17. The method of any one of claims 1-16, wherein one or more of the following are not fused together:
    (i) a first and a second molecular-barcoded oligonucleotide molecule of the plurality,
    (ii) the first and the second molecular-barcoded polynucleotides, and
    (iii) the first and the second vessel-molecular-barcoded polynucleotides.
18. The method of any one of claims 1-17, wherein (b) comprises extending a first target primer hybridized to a common-sequence of the first cell polynucleotides and extending a second target primer hybridized to a common-sequence of the second cell polynucleotides, wherein the first and the second target primers are extended by a reverse transcriptase comprising a non-template terminal transferase activity, wherein 3 or more identical non-template nucleotides are added to the 3' end of the first and the second complementary polynucleotides.
19. The method of any one of claims 1-18, wherein the forming of (c) comprises hybridizing a 3' end region of the first and the second molecular-barcoded oligonucleotide molecule of the plurality to 3 or more non-template nucleotides present on a 3' end of the first and the second complementary polynucleotides, respectively.
20. The method of claim 19, wherein the forming of (c) further comprises extending the 3' end of the first and the second complementary polynucleotides.

21. The method of any one of claims 1-20, wherein the forming of (d) comprises hybridizing a region of the vessel-barcoded oligonucleotide or a complement thereof to (i) a 3' end of the first molecular-barcoded polynucleotide and (ii) a 3' end of the second molecular-barcoded polynucleotide.
22. The method of claim 21, wherein the forming of (d) further comprises extending the 3' end of the first molecular-barcoded polynucleotide and extending the 3' end of the second molecular-barcoded polynucleotide.
23. The method of any one of claims 1-22, wherein the vessels of the plurality are emulsions.
24. The method of any one of claims 1-23, wherein the single cell is lysed before (b).
25. The method of any one of claims 1-24, wherein a first target primer, a second target primer, the vessel-barcoded oligonucleotide, the plurality of molecular-barcoded oligonucleotide molecules, or any combination thereof, is not attached to a solid support.
26. The method of any one of claims 1-25, wherein (b)-(f) are performed in a same single vessel of the plurality.
27. The method of any one of claims 1-26, wherein the first and the second cell polynucleotides are RNA.
28. The method of any one of claims 1-27, further comprising determining the first and the second cell polynucleotide to be from a same cell based on the vessel barcode.
29. The method of any one of claims 1-28, further comprising determining a germ line sequence of the first or second cell polynucleotide, or both.
30. The method of claim 29, further comprising determining a variance of a sequence of the first or second cell polynucleotide from the respective germ line sequence, or both.
31. The method of any one of claims 1-30, further comprising determining at least one of:
    (a) a total number of unique first cell polynucleotide sequences;
    (b) a total number of unique second cell polynucleotide sequences;
    (c) a total number of unique paired first and second cell polynucleotide sequences;
    (d) a frequency of a sequence of a first cell polynucleotide sequence;
    (e) a frequency of a sequence of a second cell polynucleotide; and
    (f) a frequency of sequence of a first cell polynucleotide and a sequence of a second cell polynucleotide that are paired.
32. The method of any one of claims 1-31, further comprising determining a number of starting molecules with a sequence of the first or second cell polynucleotide, or both, based on the molecular barcode.
33. The method of any one of claims 1-32, wherein the first molecular barcoded polynucleotide comprises a first molecular-barcoded oligonucleotide molecule of the plurality or an amplified product thereof and the first complementary polynucleotide or an amplified product thereof, and the second molecular barcoded polynucleotide comprises a second molecular-barcoded oligonucleotide molecule of the plurality or an amplified product thereof and the second complementary polynucleotide or an amplified product thereof.

34. The method of any one of claims 1-33, wherein the first vessel molecular barcoded polynucleotide comprises the vessel barcoded oligonucleotide or an amplified product thereof and the first molecular-barcoded polynucleotide or an amplified product thereof, and the second vessel molecular barcoded polynucleotide comprises the vessel barcoded oligonucleotide or an amplified product thereof and the second molecular-barcoded polynucleotide or an amplified product thereof.
35. The method of any one of claims 1-34, wherein (b)-(d) are performed in a plurality of vessels simultaneously.
36. The method of any one of claims 1-35, wherein the forming of (c) comprises attaching the first molecular-barcoded oligonucleotide molecule of the plurality to the first complementary polynucleotide and attaching the second molecular-barcoded oligonucleotide molecule of the plurality to the second complementary polynucleotide.
37. The method of any one of claims 1-36, wherein the forming of (d) comprises attaching the first vessel-barcoded oligonucleotide molecule of the plurality to the first molecular barcoded polynucleotide and attaching the second vessel-barcoded oligonucleotide molecule of the plurality to the second molecular barcoded polynucleotide.
38. The method of any one of claims 1-37, wherein the method further comprises amplifying the vessel-barcoded oligonucleotide to form a plurality of identical vessel-barcoded oligonucleotides prior to (d).
39. A method of barcoding polynucleotides comprising:
    (a) hybridizing a molecular-barcoded oligonucleotide molecule to each of a plurality of complement polynucleotides that are complementary to a polynucleotide from a single cell, wherein the hybridized molecular-barcoded oligonucleotide molecule is from a plurality of uniquely barcoded molecular-barcoded oligonucleotide molecules within a vessel comprising the single cell;
    (b) extending the complement polynucleotides hybridized to a molecular-barcoded oligonucleotide molecule;
    (c) hybridizing a vessel-barcoded oligonucleotide or complement thereof to the extended complementary polynucleotides from (b), wherein each of the hybridized vessel-barcoded oligonucleotides comprises a vessel barcode that is unique to a single vessel of a plurality of vessels;
    (d) extending the extended complementary polynucleotides from (c) to form polynucleotides comprising a molecular barcode and a vessel barcode; and
    (e) sequencing polynucleotides comprising a molecular barcode and a vessel barcode from (d) or amplified products thereof.
40. A composition comprising a plurality of vessels each comprising:
    (a) a single cell from a sample comprising a plurality of cells;
    (b) a plurality of molecular-barcoded oligonucleotide molecules;
    (c) a vessel-barcoded oligonucleotide;

(d) a first polynucleotide comprising (i) a first molecular barcode sequence from a first molecular-barcoded oligonucleotide molecule of the plurality or a complement thereof, (ii) a vessel barcode sequence from the vessel barcoded oligonucleotide or a complement thereof, and (iii) a region complementary to a first cell polynucleotide from the single cell; and

(e) a second polynucleotide comprising (i) a second molecular barcode sequence from a second molecular-barcoded oligonucleotide molecule of the plurality or a complement thereof, (ii) the vessel barcode sequence from the vessel barcoded oligonucleotide or a complement thereof, and (iii) a region complementary to a second cell polynucleotide from the single cell.