

CLAIMS

WHAT IS CLAIMED IS:

1. A method of barcoding nucleic acid analytes comprising:

- (a) providing
 - (i) a sample comprising a biological particle that comprises a nucleic acid molecule, wherein said nucleic acid molecule comprises a first target region and a second target region, wherein said first target region and said second target region are disposed on a same strand of said nucleic acid molecule,
 - (ii) a first probe comprising a first probe sequence and a first adapter sequence, wherein said first probe sequence of said first probe is complementary to said first target region of said nucleic acid molecule,
 - (iii) a second probe comprising a second probe sequence, wherein said second probe sequence of said second probe is complementary to said second target region of said nucleic acid molecule,
 - (iv) a barcode oligonucleotide comprising a barcode sequence segment and a second adapter sequence, and
 - (v) a splint oligonucleotide comprising a first sequence complementary to at least a portion of said first adapter sequence and a second sequence complementary to at least a portion of said second adapter sequence;
- (b) subjecting said biological particle to conditions sufficient to (i) hybridize said first probe sequence of said first probe to said first target region of said nucleic acid molecule, and (ii) hybridize said second probe sequence of said second probe to said second target region of said nucleic acid molecule to yield a probe-associated nucleic acid molecule;
- (c) subjecting said probe-associated nucleic acid molecule to conditions sufficient to yield a probe-linked nucleic acid molecule comprising said first probe linked to said second probe;
- (d) subjecting said probe-linked nucleic acid molecule to conditions sufficient to (i) hybridize said first sequence to said first adapter sequence, and (ii) said second sequence to said second adapter sequence to yield an adapter-bound probe-linked nucleic acid molecule; and
- (e) subjecting said adapter-bound probe-linked nucleic acid molecule to conditions sufficient to yield a barcoded nucleic acid molecule, wherein said barcoded nucleic acid molecule comprises said barcode sequence segment or a complement thereof.

2. The method of claim 1, wherein said barcode oligonucleotide further comprises a third adapter sequence and (a) further comprises providing:

(vi) a second barcode oligonucleotide comprising a second barcode sequence segment and a fourth adapter sequence, and

(vii) a second splint oligonucleotide comprising a third sequence complementary to at least a portion of said third adapter sequence and a fourth sequence complementary to at least a portion of said fourth adapter sequence, and

wherein said method further comprises

(f) subjecting said barcoded nucleic acid molecule to conditions sufficient to (i) hybridize said third sequence to said third adapter sequence, and (ii) said fourth sequence to said fourth adapter sequence to yield an adapter-bound barcoded nucleic acid molecule; and

(g) subjecting said adapter-bound barcoded nucleic acid molecule to conditions sufficient to yield a second barcoded nucleic acid molecule, wherein said second barcoded nucleic acid molecule comprises said barcode sequence segment or a complement thereof and said second barcode sequence segment or a complement thereof.

3. The method of claim 2, wherein said second barcode oligonucleotide further comprises a fifth adapter sequence and (a) further comprises providing

(viii) a third barcode oligonucleotide comprising a third barcode sequence segment and a sixth adapter sequence, and

(ix) a third splint oligonucleotide comprising a fifth sequence complementary to at least a portion of said fifth adapter sequence and a sixth sequence complementary to at least a portion of said sixth adapter sequence, and

wherein said method further comprises

(h) subjecting said second barcoded nucleic acid molecule to conditions sufficient to (i) hybridize said fifth sequence to said fifth adapter sequence, and (ii) said sixth sequence to said sixth adapter sequence to yield an adapter-bound second barcoded nucleic acid molecule; and

(i) subjecting said adapter-bound second barcoded nucleic acid molecule to conditions sufficient to yield a third barcoded nucleic acid molecule, wherein said third barcoded nucleic acid molecule comprises said barcode sequence segment or a complement thereof, said second barcode sequence segment or a complement thereof, and said third barcode sequence segment or a complement thereof.

4. The method of any one of claims 1 to 3, wherein (c) comprises subjecting said probe-associated nucleic acid molecule to conditions sufficient to ligate said first probe to said second probe to yield said probe-linked nucleic acid molecule.

5. The method of any one of claims 1 to 4, wherein (e) comprises subjecting said adapter-bound probe-linked nucleic acid molecule to conditions sufficient to ligate said first adapter sequence to said second adapter sequence to yield said barcoded nucleic acid molecule.
6. The method of claim 2, wherein (g) comprises subjecting said adapter-bound barcoded nucleic acid molecule to conditions sufficient to ligate said third adapter sequence to said fourth adapter sequence to yield said second barcoded nucleic acid molecule.
7. The method of claim 3, wherein (i) comprises subjecting said adapter-bound second barcoded nucleic acid molecule to conditions sufficient to ligate said fifth adapter sequence to said sixth adapter sequence to yield said third barcoded nucleic acid molecule.
8. The method of any one of claims 1 to 7, wherein said biological particle comprises a member selected from the group consisting of a cell, an organelle, and a cell bead.
9. The method of any one of claims 1 to 8, wherein said biological particle is a cell.
10. The method of claim 9, further comprising, subsequent to (a), lysing or permeabilizing said cell, thereby providing access to said nucleic acid molecule.
11. The method of claim 9 or claim 10, wherein said cell is a fixed cell.
12. The method of any one of claims 9 to 11, wherein said cell is a permeabilized cell.
13. The method of any one of claims 9 to 12, wherein said cell is a fixed and permeabilized cell.
14. The method of any one of claims 9 to 13, wherein said cell is selected from the group consisting of a prokaryotic cell, a eukaryotic cell, and a human cell.
15. The method of any one of claims 1 to 14, wherein said biological particle is provided in a partition.
16. The method of claim 15, wherein said partition is a microwell.

17. The method of claim 16, wherein said biological particle is a cell, wherein said cell is a fixed cell.

18. The method of claim 17, wherein said fixed cell is a permeabilized cell.

19. The method of claim 18, wherein said microwell is part of a plurality of microwells and said cell is part of a plurality of cells.

20. The method of claim 19, wherein said plurality of cells comprise a plurality of barcoded nucleic acid molecules generated in (e).

21. The method of claim 20, wherein said plurality of cells from said plurality of wells are pooled following (e) to provide a pooled mixture of cells comprising barcoded nucleic acid molecules.

22. The method of claim 21, wherein said pooled mixture of cells is partitioned into additional partitions, wherein a single partition comprises one or more cells of said pooled mixture of cells and a plurality of additional nucleic acid barcode molecules.

23. The method of claim 22, in said single partition, further comprising barcoding said barcoded nucleic acid molecule of (e) with said plurality of additional nucleic acid barcode molecules.

24. The method of claim 23, wherein said single partition comprises two or more cells from said pooled mixture of cells.

25. The method of any one of claims 22 to 24, wherein said additional partitions are droplets.

26. The method of any one of claims 22 to 24, wherein said additional partitions are wells.