

CLAIMS

WHAT IS CLAIMED IS:

1. A method for nucleic acid processing, comprising:

- (a) providing a plurality of partitions, wherein a partition of said plurality of partitions comprises (i) at least two beads of a plurality of beads, ~~(ii) comprising a first bead and a second bead, wherein said first bead comprises a first primer and said second bead comprises a second primer,~~ (ii) at least two nucleic acid molecules of a plurality of nucleic acid molecules comprising a first nucleic acid molecule and a second nucleic acid molecule, ~~wherein said first nucleic acid molecule and said second nucleic acid molecule have different nucleic acid sequences,~~ and (iii) one or more reagents;
- (b) in said partition, (i) using said first nucleic acid molecule, said first primer, and said one or more reagents to generate one or more first amplification products of said first nucleic acid molecule, wherein at least a subset of said one or more first amplification products ~~are~~is attached to ~~a bead of said at least two beads;~~said first bead, and (ii) using said second nucleic acid molecule, said second primer, and said one or more reagents to generate one or more second amplification products of said second nucleic acid molecule, wherein at least a subset of said one or more second amplification products is attached to said second bead;
- (c) recovering said first bead and said second bead from said partition; and
- (d) assaying ~~an~~(i) a first amplification product of said at least said subset of said one or more first amplification products ~~or derivatives thereof~~attached to said first bead, to identify a sequence of said first nucleic acid molecule and (ii) a second amplification product of said at least said subset of said one or more second amplification products attached to said second bead, to identify a sequence of said second nucleic acid molecule.

2. The method of claim 1, wherein (a) comprises bringing (i) a first solution comprising ~~a~~said plurality of nucleic acid molecules ~~comprising said nucleic acid molecule~~ and (ii) a second solution comprising said plurality of ~~beads comprising said at least two~~ beads in contact with a

fluid that is immiscible with said first solution and said second solution, to generate said plurality of partitions.

3. The method of claim 1 or 2, wherein said first bead has attached thereto a plurality of primers comprising said first primer ~~molecules~~ for performing one or more amplification reactions using said first nucleic acid molecule, and (b) comprises using primer molecules of said plurality of primers comprising said first primer ~~molecules~~ to conduct said one or more amplification reactions to generate said ~~amplification product~~ at least said subset of said one or more first amplification products attached to said first bead.

~~4. The method of claim 3, wherein said bead has attached thereto a plurality of additional primer molecules for performing one or more additional amplification reactions using said nucleic acid molecule, which plurality of additional primer molecules are different than said plurality of primer molecules.~~

~~5. The method of claim 4, wherein (b) further comprises using additional primer molecules of said plurality of additional primer molecules to conduct said one or more additional amplification reactions to generate additional amplification products of said one or more amplification products, wherein at least a subset of said additional amplification products are attached to said bead, and wherein (d) further comprises assaying said additional amplification products attached to said bead, or derivatives thereof, to identify a sequence of said nucleic acid molecule.~~

~~6. The method of claim 5, wherein said nucleic acid molecule is a double-stranded nucleic acid molecule, and wherein amplification products corresponding to a first strand of said nucleic acid molecule are generated using said plurality of primer molecules, and amplification products corresponding to a second strand of said nucleic acid molecule are generated using said plurality of additional primer molecules.~~

~~7. The method of any one of claims 3-6, wherein an additional bead of said at least two beads has attached thereto a plurality of additional primer molecules for performing one or more additional amplification reactions using said nucleic acid molecule, which plurality of additional primer molecules are different than said plurality of primer molecules.~~

~~8. The method of claim 7, further comprising:~~

- ~~(e) — using additional primer molecules of said plurality of additional primer molecules to conduct said one or more additional amplification reactions to generate additional amplification products of said one or more amplification products, wherein at least a subset of said additional amplification products are attached to said additional bead of said at least two beads;~~
- ~~(f) — recovering said additional bead from said partition; and~~
- ~~(g) — assaying said additional amplification products attached to said additional bead, or derivatives thereof, to identify a sequence of said nucleic acid molecule.~~

~~9. The method of any one of claims 1-8, wherein each of at least 80% of said plurality of partitions comprises two or more beads of said plurality of beads.~~

~~104.~~ The method of any one of claims 1-~~39~~, wherein each of at least 80% of said plurality of partitions comprises two or more beads of said plurality of beads, optionally wherein each of at least 80% of said plurality of partitions comprises three or more beads of said plurality of beads.

~~11. The method of any one of claims 1-10, wherein said at least two beads are attached to one another.~~

~~12. The method of any one of claims 1-11, further comprising separating partitions of said plurality of partitions that each comprise at least two beads from other partitions of said plurality of partitions that each comprise at most one bead.~~

~~13. The method of claim 12, wherein said separating comprises optically detecting said partitions that each comprise at least two beads or said other partitions that each comprise at most one bead and, based at least in part on said optically detecting, adjusting a direction of flow of a fluid in a fluidic device to provide said partitions that each comprise at least two beads in a first channel of said fluidic device and said other partitions that each comprise at most one bead in a second channel of said fluidic device.~~

~~145.~~ The method of any one of claims ~~1-41-13~~, wherein said one or more reagents comprise nucleic acid molecules comprising priming sequences.

~~156.~~ The method of claim ~~514~~, wherein said nucleic acid molecules comprising said priming sequences further comprise unique molecular identifier sequences.

~~167.~~ The method of claim ~~514~~, wherein said nucleic acid molecules comprising said priming sequences further comprise barcode sequences.

~~178.~~ The method of any one of claims 1-~~716~~, wherein ~~(d) comprises using said one or more amplification products or derivatives thereof as a template~~said one or more reagents comprise one or more polymerizing enzymes.

~~189.~~ The method of any one of claims 1-~~817~~, wherein said plurality of partitions is a plurality of droplets.

~~1910.~~ The method of any one of claims 1-~~918~~, wherein (d) comprises sequencing said first amplification product or derivatives thereof said at least said subset of said one or more first amplification products attached to said first bead and said second amplification product of said at least said subset of said one or more second amplification products attached to said second bead.

~~2011.~~ The method of any one of claims 1-~~1019~~, wherein, in (a), said first bead comprises a plurality of first primers comprising said first primer~~nucleic acid molecule is~~ attached to said first

bead, and said second bead comprises a plurality of second primers comprising said second primer attached to said second bead.

12. The method of claim 11, wherein (b) comprises:

(1) generating a first extension product of said first nucleic acid molecule which comprises a first sequence complementary to said first primer of said plurality of first primers, binding said first extension product to said first primer attached to said first bead, and extending said first primer to generate said first amplification product of said at least said subset of said one or more first amplification products attached to said first bead, and

(2) generating a second extension product of said second nucleic acid molecule which comprises a second sequence complementary to said second primer of said plurality of second primers, binding said second extension product to said second primer attached to said second bead, and extending said second primer to generate said second amplification product of said at least said subset of said one or more second amplification products attached to said second bead.

13. The method of any one of claims 1-12, further comprising generating said plurality of partitions, wherein each of at least 50% of said plurality of partitions comprises at least one bead and at least one nucleic acid template, optionally wherein each of at least 80% of said plurality of partitions comprise at least one bead and at least one nucleic acid template.

14. The method of any one of claims 1-13, wherein, subsequent to (c), said first bead is monoclonal and said second bead is monoclonal.

15. The method of any one of claims 1-14, wherein said first primer and said second primer have different sequences.

16. The method of any one of claims 1-15, wherein said first nucleic acid molecule comprises a first adaptor and said second nucleic acid molecule comprises a second adaptor, wherein said

first adaptor and said second adaptor have different sequences, and wherein said first primer has sequence complementarity with said first adaptor and said second primer has sequence complementarity with said second adaptor.

17. The method of claim 16, wherein said first primer does not have sequence complementarity with said second adaptor and said second primer does not have sequence complementarity with said first adaptor.

18. The method of claim 16 or 17, wherein, in (a), said first primer does not hybridize with said second adaptor and said second primer does not hybridize with said first adaptor.

19. The method of any one of claims 16-18, wherein (b) comprises hybridizing said first primer to said first adaptor and said second primer to said second adaptor.

20. The method of any one of claims 1-19, wherein said plurality of nucleic acid molecules comprises isolated nucleic acid molecules.