

In some embodiments, the method comprises distributing at most one solid support per emulsion, microwell, or well. In some embodiments, the method comprises distributing one solid support and one cell to each of from 1 to 2,000,000 microwells, emulsions, or wells. In some embodiments, cell distribution is random or non-random. In some embodiments, cell distribution is stochastic. In some embodiments, a cell is distributed by a cell sorter. In some embodiments, a cell is distributed by contacting one or more wells, microwells, or emulsions with a dilute solution of cells diluted so that at most one cell is distributed to the one or more wells, microwells, or emulsions.

[0016] In some embodiments, the target specific regions, target specific regions of the plurality of oligonucleotides, or the target specific region of the two or more polynucleotide molecules, comprise sequences complementary to two or more targets of a target panel. In some embodiments, the two or more targets of the target panel are biomarkers. In some embodiments, the biomarkers are biomarkers for a disease or condition. In some embodiments, the disease or condition is a cancer, an infection, a viral infection, an inflammatory disease, a neurodegenerative disease, a fungal disease, a bacterial infection, or any combination thereof. In some embodiments, the panel comprises from: 2-50,000, 2-40,000, 2-30,000, 2-20,000, 2-10,000, 2-9000, 2-8,000, 2-7,000, 2-6,000, 2-5,000, 2-1,000, 2-800, 2-700, 2-600, 2-500, 2-400, 2-300, 2-200, 2-100, 2-75, 2-50, 2-40, 2-30, 2-20, 2-10, or 2-5 biomarkers.

[0016a] One aspect provided is a composition comprising: a single cell and a single bead, 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.

[0016b] One aspect provided is a composition comprising: a lysate from a single cell and a single bead, 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.

[0016c] One aspect provided is a composition comprising a particle, wherein the particle comprises a plurality of oligonucleotides, wherein each of the plurality of oligonucleotides comprises a cellular label sequence, a molecular label sequence, and a target-binding region, wherein the cellular label sequence of each of the plurality of oligonucleotides is the same,

and at least 100 of the plurality of oligonucleotides comprise different molecular label sequences.

[0016d] One aspect provided is a kit comprising a plurality of particles each comprising a plurality of oligonucleotides, wherein each of the plurality of oligonucleotides comprises a cellular label sequence, a molecular label sequence, and a target-binding region, wherein the cellular label sequence of each of the plurality of oligonucleotides is the same, and at least 100 of the plurality of oligonucleotides comprise different molecular label sequences.

[0016e] One aspect provided is droplet comprising: a. a single bead comprising a plurality of oligonucleotides, wherein each of the plurality of oligonucleotides comprises a molecular label sequence, a cellular label sequence, and an oligo dT sequence, and wherein the cellular label sequence of each of the plurality of oligonucleotides is the same and at least 100 of the plurality of oligonucleotides comprise different molecular label sequences; and b. a single cell.

[0016f] One aspect provided is a droplet comprising: a. a single bead comprising a plurality of oligonucleotides, wherein each of the plurality of oligonucleotides comprises a molecular label sequence, a cellular label sequence, and an oligo dT sequence, and wherein the cellular label sequence of each of the plurality of oligonucleotides is the same and at least 100 of the plurality of oligonucleotides comprises different molecular label sequences; and b. a lysate from a single cell.

[0016g] One aspect provided is a method comprising: a. introducing a single cell and a single bead into a droplet, wherein said single bead comprises a plurality of oligonucleotides, wherein each of the plurality of oligonucleotide comprises a cellular label sequence, a target binding region, and a molecular label sequence, and wherein the cellular label sequence of each of the plurality of oligonucleotides is the same and at least 100 of the plurality of oligonucleotides comprises different molecular label sequences; b. lysing said single cell, thereby releasing nucleic acid targets from said cell; and c. attaching said nucleic acid targets to said plurality of oligonucleotides.

INCORPORATION BY REFERENCE

[0017] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0019] **FIG. 1** depicts an exemplary solid support conjugated with an exemplary oligonucleotide.

CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising:
a single cell and a single bead,
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 composition of claim 1, wherein said plurality of oligonucleotides each comprises a
universal label sequence.
3. A composition comprising:
a lysate from a single cell and a single bead,
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.
4. The composition of claim 3, wherein the nucleic acid contents of said lysate from the
single cell are associated with a plurality of oligonucleotides.
5. The composition of any one of claims 1 to 4, wherein said plurality of oligonucleotides is
capable of labeling individual occurrences of target molecules of said single cell or said
lysate from said single cells.
6. The composition of any one of claims 1 to 5, wherein said plurality of oligonucleotides is
capable of labeling individual occurrences of mRNA molecules of said single cell or said
lysate from said single cells via reverse transcription.

7. The composition of any one of claims 1 to 6, wherein said plurality of oligonucleotides comprises at least 100,000 oligonucleotides.
8. The composition of any one of claims 1 to 7, wherein said single cell is a cell selected from the group consisting of a rare cell, a tumor cell, and an immune cell.
9. The composition of any one of claims 1 to 8, wherein said single cell is from a human or a tissue, and/or said single cell is infected with viral polynucleotides.
10. The composition of any one of claims 1 to 9, wherein said bead is magnetic, or said bead comprises silica gel, controlled pore glass, Dynabead, Wang resin, Merrifield resin, Sephadex/Sepharose bead, cellulose bead, polystyrene bead, or any combination thereof.
11. A composition comprising
a particle,
wherein the particle comprises a plurality of oligonucleotides, wherein each of the plurality of oligonucleotides comprises a cellular label sequence, a molecular label sequence, and a target-binding region, wherein the cellular label sequence of each of the plurality of oligonucleotides is the same, and at least 100 of the plurality of oligonucleotides comprise different molecular label sequences.
12. The composition of claim 11, wherein the particle is a bead, and optionally the bead is selected from the group consisting of streptavidin beads, agarose beads, magnetic beads, antibody conjugated beads, protein A conjugated beads, protein G conjugated beads, protein A/G conjugated beads, protein L conjugated beads, oligodT conjugated beads, silica beads, silica-like beads, anti-biotin microbead, anti-fluorochrome microbead, and any combination thereof.
13. The composition of any one of claims 11 to 12, wherein the plurality of oligonucleotides comprises DNA.
14. The composition of any one of claims 1 to 13, wherein at least 1,000 or at least 10,000 of the plurality of oligonucleotides comprise different molecular label sequences.

15. The composition of any one of claims 11 to 14, wherein the plurality of oligonucleotides comprises at least 10,000 oligonucleotides.

16. The composition of any one of claims 1 to 15, wherein each of the plurality of oligonucleotides further comprises at least one of:

- (a) a sample label; and
- (b) a universal label.

17. The composition of any one of claims 1 to 16, wherein the target-binding region comprises a sequence selected from the group consisting of a gene-specific sequence, an oligo-dT sequence, a random multimer sequence, and any combination thereof.

18. The composition of any one of claims 1 to 17, wherein the molecular label sequences comprise random sequences.

19. The composition of any one of claims 11 to 18, wherein the particle comprises a material selected from the group consisting of polydimethylsiloxane (PDMS), polystyrene, glass, polypropylene, agarose, gelatin, hydrogel, paramagnetic, ceramic, plastic, glass, methylstyrene, acrylic polymer, titanium, latex, sepharose, cellulose, nylon, silicone, and any combination thereof.

20. The composition of any one of claims 11 to 19, wherein

- (a) each of the plurality of oligonucleotides comprises a linker functional group, and
- (b) the particle comprises a solid support functional group;

wherein the support functional group and the linker functional group are associated with each other.

21. The composition of claim 20, wherein the linker functional group and the support functional group are individually selected from the group consisting of C6, biotin, streptavidin, primary amine(s), aldehyde(s), ketone(s), and any combination thereof.

22. The composition of any one of claims 11 to 21, wherein the cellular label sequences and/or the molecular label sequences of the plurality of oligonucleotides comprise at least 8 nucleotides.

23. A kit comprising a plurality of particles each comprising a plurality of oligonucleotides, wherein each of the plurality of oligonucleotides comprises a cellular label sequence, a molecular label sequence, and a target-binding region, wherein the cellular label sequence of each of the plurality of oligonucleotides is the same, and at least 100 of the plurality of oligonucleotides comprise different molecular label sequences.

24. The kit of claim 23, wherein the plurality of particles comprises a plurality of beads, and optionally the plurality of beads comprises at least 384 beads.

25. The kit of any one of claims 23 or 24, further comprising a microwell array, wherein each microwell of the microwell array comprises a particle of the plurality particles.

26. The composition of any one of claims 11 to 22, wherein the particle is a microparticle or a nanoparticle.

27. A droplet comprising:

a. a single bead comprising a plurality of oligonucleotides, wherein each of the plurality of oligonucleotides comprises a molecular label sequence, a cellular label sequence, and an oligo dT sequence, and wherein the cellular label sequence of each of the plurality of oligonucleotides is the same and at least 100 of the plurality of oligonucleotides comprise different molecular label sequences; and

b. a single cell.

28. The droplet of claim 27, wherein at least 1,000 of said plurality of oligonucleotides comprise different molecular label sequences.

29. The droplet of claims 27 or 28, wherein said plurality of oligonucleotides comprises at least 100,000 oligonucleotides.

30. The droplet of any one of claims 27 to 29, wherein said droplet is encapsulated by an immiscible liquid.

31. The droplet of any one of claims 27 to 30, wherein said droplet is in an emulsion, and optionally said emulsion is a water-in-oil emulsion.

32. The droplet of any one of claims 27 to 31, wherein the volume of said droplet is between one picoliter and one microliter, and/or the diameter of said droplet is from 0.001 to 150 microns.

33. The droplet of any one of claims 27 to 32, wherein said droplet is an aqueous droplet within a continuous oil phase, or an oil droplet within a continuous aqueous phase, or a droplet within a larger droplet.

34. The droplet of any one of claims 27 to 33, wherein said single cell is a cell selected from the group consisting of: a rare cell, a tumor cell, and an immune cell.

35. The droplet of any one of claims 27 to 34, wherein said single cell is from a human or from a tissue.

36. The droplet of any one of claims 27 to 35, wherein said bead is magnetic.

37. A droplet comprising:

a. a single bead comprising a plurality of oligonucleotides, wherein each of the plurality of oligonucleotides comprises a molecular label sequence, a cellular label sequence, and an oligo dT sequence, and wherein the cellular label sequence of each of the plurality of oligonucleotides is the same and at least 100 of the plurality of oligonucleotides comprises different molecular label sequences; and

b. a lysate from a single cell.

38. The droplet of claim 37, wherein said lysate comprises nucleic acids targets, and optionally said nucleic acid targets are hybridized to said plurality of oligonucleotides.

39. A method comprising:

a. introducing a single cell and a single bead into a droplet, wherein said single bead comprises a plurality of oligonucleotides, wherein each of the plurality of oligonucleotide comprises a cellular label sequence, a target binding region, and a molecular label sequence,

and wherein the cellular label sequence of each of the plurality of oligonucleotides is the same and at least 100 of the plurality of oligonucleotides comprises different molecular label sequences;

- b. lysing said single cell, thereby releasing nucleic acid targets from said cell; and
- c. attaching said nucleic acid targets to said plurality of oligonucleotides.

40. The method of claim 39, wherein said droplet spatially isolates said single cell.

41. The method of claim 39 or 40, wherein said attaching comprises stochastically attaching said nucleic acid targets to said plurality of oligonucleotides.

42. The method of any one of claims 39 to 41, wherein said attaching comprises hybridizing said nucleic acid targets to said plurality of oligonucleotides.

43. The method of any one of claims 39 to 42, further comprising reverse transcribing said nucleic acid targets, thereby generating labelled target cDNAs.

44. The method of claim 43, wherein said reverse transcribing occurs in said droplet.

45. The method of any one of claim 39 to 44, further comprising amplifying said nucleic acid targets, thereby generating labelled amplicons.

46. The method of claim 45, further comprising determining the number of said labelled amplicons in said cell.

47. The method of claim 46, wherein said determining comprises counting the number of unique molecular label sequences for a given amplicon of said labelled amplicons.

48. The method of any one of claims 39 to 47, wherein said nucleic acid targets are RNA.

49. The droplet of any one of claims 27-36, wherein at least 10,000 of said plurality of oligonucleotides comprise different molecular label sequences.

50. The droplet of any one of claims 27 to 36 and 49, wherein each of the plurality of oligonucleotides further comprises (a) a sample label or (b) a universal label.

51. The droplet of any one of claims 27 to 36 and 49 to 50, wherein the bead is selected from the group consisting of streptavidin beads, agarose beads, magnetic beads, antibody conjugated beads, protein A conjugated beads, protein G conjugated beads, protein A/G conjugated beads, protein L conjugated beads, oligodT conjugated beads, silica beads, silica-like beads, anti-biotin microbead, and anti-fluorochrome microbead.

52. The droplet of any one of claims 27 to 38 and 49 to 51, wherein said bead is semi-solid, and optionally said bead comprises a gel.

53. The droplet of claim 52, wherein said bead comprises a gel.

54. The droplet of claim 53, wherein said bead comprises a hydrogel.

55. The droplet of claim 27 to 38 and 49 to 54, wherein said bead comprises a polymer.

56. The method of any one of claims 39 to 48, wherein said bead is semi-solid, and optionally said bead comprises a gel.

57. The method of claim 56, wherein said bead comprises a hydrogel.

58. The method of any one of claims 39 to 48 and 56 to 57, wherein said bead comprises a polymer.