

WHAT IS CLAIMED IS:

1. A method of analyzing at least two or more analytes of a single cell, said method comprising:

(a) providing a plurality of contiguity preserving elements (CE), wherein each CE comprises a single cell;

(b) lysing said single cells within said CE, wherein the analytes within said single cell are released within said CE;

(c) providing a first reporter moiety to a first analyte within said single cell of each CE;

(d) providing a second reporter moiety to a second analyte within said single cell of each CE;

(e) modifying said analytes such that at least some of said first and second analytes of said CE comprise said first and second reporter moieties, respectively;

(f) combining the CE comprising said analytes comprising said reporter moieties;

(g) compartmentalizing the CE comprising said first and second analytes comprising said first and second reporter moieties, respectively into a plurality of compartments;

(h) providing a third reporter moiety to said first analyte comprising said first reporter moiety of each CE;

(i) providing a fourth reporter moiety to said second analyte comprising said second reporter moiety of each CE;

(j) further modifying said analytes such that at least some first analytes comprise said first and third reporter moieties and at least some second analytes comprise said second and fourth reporter moieties;

(j) analyzing said analytes comprising said reporter moieties of each compartment, wherein such analysis detects the analytes of a single cell.

2. The method of claim 1, wherein said first and second reporter moieties identify the source of said analytes.

3. The method of claim 1, wherein the combination of said reporter moieties identifies the source of said analytes.

4. The method of any one of claims 1-3, wherein detection of said analytes are done simultaneously.

5. The method of any one of claims 1-4, wherein said first analyte is genomic DNA and the second analyte is cDNA or RNA.

6. The method of claim 5, wherein modifying at least some of the genomic DNA, cDNA or RNA to comprise first and second reporter moieties comprises contacting the genomic DNA, cDNA or RNA with a plurality of transposomes, each transposome comprising a transposase and a transposon sequence comprising the first reporter moiety or a second reporter moiety under conditions such that at least some of the transposon sequences are inserted into the genomic DNA, cDNA or RNA.

7. The method of claim 6, wherein step (g) further comprises removing the transposase from the genomic DNA, cDNA or RNA.

8. The method of claim 7, wherein the transposase is removed subsequent to modifying said analytes at step (e).

9. The method of claim 8, wherein removing the transposase comprises a method selected from the group consisting of adding a detergent, changing temperature, changing pH, adding a protease, adding a chaperone, changing salt concentration, and adding a strand-displacing polymerase.

10. The method of claim 6, wherein step (e) comprises contacting the target nucleic acid with a plurality of transposomes, each transposon comprising a first transposon sequence comprising a first reporter moiety, a second transposon sequence noncontiguous with said first transposon sequence, and a transposase associated with the first transposon sequence and the second transposon sequence.

11. The method of any one of claims 6-10, wherein the first transposon sequences comprises a first primer site and the second transposon sequences comprises a second primer site.

12. The method of claim 13, wherein the first primer site further comprises a first barcode and the second primer site further comprises a second barcode.

13. The method of any one of claims 1-12, wherein the first, second, third, or fourth reporter moiety comprises a barcode.

14. The method of claim 1, wherein one analyte is protein, and optionally wherein the protein is labeled with a nucleic acid reporter moiety, and further optionally wherein the nucleic acid reporter moiety comprises a combinatorially derived set of barcodes.

15. The method of claim 14, wherein said protein is from a single cell.

16. The method of any of claims 1-15, wherein steps (c)-(j) are repeated at least one more time.

17. The method of claim 16, wherein the additional reporter moieties in each of the additional steps are different from said first, second, third, and fourth reporter moieties.

18. A method of analyzing analytes, said method comprising:

(a) providing a contiguity preserving elements (CE), wherein each CE comprises at least one analyte;

(b) compartmentalizing said CE comprising said at least one analyte into a plurality of first compartments;

(c) providing a first set of reporter moieties to the analyte of each CE in said first compartment, wherein said first set of reporter moieties identifies the CE;

(d) modifying said analyte such that at least some analytes of said CE comprise said first reporter moiety;

(e) combining the CE comprising said analyte comprising said first reporter moiety;

(f) compartmentalizing the CE comprising said analyte comprising said first reporter moiety into a plurality of second compartments;

(g) providing a second set of reporter moieties to said analyte comprising said first reporter moiety of each CE;

(h) further modifying said analyte such that at least some analytes comprise said second reporter moiety;

(i) analyzing said analyte comprising said first and second reporter moieties of each second compartment;

wherein said analytes are components of a single cell, wherein each of said first and/or second reporter moieties is unique to said first cell, and wherein said first and/or second reporter moieties identifies the single cell.

19. The method of claim 18, wherein said analyte comprises a nucleic acid.
20. The method of claim 19, wherein said nucleic acid comprises DNA.
21. The method of claim 20, wherein said DNA comprises genomic DNA.
22. The method of claim 19, wherein said nucleic acid comprises RNA or cDNA.
23. The method of claim 18, wherein said analyte is selected from the group consisting of organelle, lipid, carbohydrate, and cellular metabolite.
24. The method of claim 19, wherein step (d) comprises contacting the nucleic acid with a plurality of transposomes, each comprising a transposase and a transposon sequence comprising the first reporter moiety under conditions such that at least some of the transposon sequences insert into the target nucleic acid.
25. The method of claim 19, wherein step (d) comprises contacting the target nucleic acid with a plurality of transposomes, each transposon comprising a first transposon

sequence comprising a first reporter moiety, a second transposon sequence noncontiguous with said first transposon sequence, and a transposase associated with the first transposon sequence and the second transposon sequence.

26. The method of claim 19, wherein step (f) comprises removing the transposase from the compartmentalized first indexed template nucleic acids.

27. The method of claim 26, wherein the transposase is removed subsequent to step (d).

28. The method of claim 26, wherein the transposase is removed prior to step (i).

29. The method of claim 26, wherein removing the transposase comprises a method selected from the group consisting of adding a detergent, changing temperature, changing pH, adding a protease, adding a chaperone, changing salt concentration, and adding a strand-displacing polymerase.

30. The method of any one of claims 24-29, wherein the first transposon sequences comprises a first primer site and the second transposon sequences comprises a second primer site.

31. The method of claim 30, wherein the first primer site further comprises a first barcode and the second primer site further comprises a second barcode.

32. The method of claim 18, wherein said first reporter moiety comprises a primer wherein said analyte is a nucleic acid, and wherein step (d) comprises amplifying said nucleic acid with at least one primer, wherein said primer is the first reporter moiety.

33. The method of claim 18, wherein said first reporter moiety comprises a primer wherein said analyte is a nucleic acid, and wherein step (d) comprises ligating said nucleic acid with at least one primer, wherein said primer is the first reporter moiety.

34. The method of any one of claims 18-33, wherein said first reporter moiety provided to the analyte of each first compartment are different.

35. The method of any one of claims 18-34, further comprising providing a plasmid to said CE in step (c), wherein said plasmid is modified to comprise said first and/or second reporter moieties in steps (d)-(h), wherein said plasmid comprising said first or said reporter moieties identifies said CE.

36. The method of claim 18, wherein said analyte is protein.

37. The method of claim 36, wherein said protein is from a single cell.

38. The method of claim 18-36, wherein steps (c)-(h) are repeated at least one more time.

39. The method of claim 38, wherein the additional sets of reporter moieties in each of the addition steps are different than said first and second sets of reporter moieties.

40. The method of claim 18, wherein said analyte is mRNA or cDNA.

41. The method of claim 40, wherein said mRNA or cDNA is from a single cell.

42. The method of any one of claims 40-41, wherein said CE comprises solid support capable of immobilizing said mRNA or cDNA.

43. The method of claim 42, wherein said solid support comprises oligo (dT) probes immobilized on said solid support.

44. The method of any one of claims 40-43, wherein said first set of reporter moieties comprise first barcode sequence.

45. The method of any one of claims 40-44, wherein said second set of reporter moieties comprise second barcode sequence.

46. The method of any one of claims 40-43, wherein step (d) comprises contacting the nucleic acid with a plurality of transposomes, each comprising a transposase and a transposon sequence comprising the first reporter moiety under conditions such that at least some of the transposon sequences insert into the target nucleic acid.

47. The method of any one of claims 40-43, wherein step (d) comprises contacting the target nucleic acid with a plurality of transposomes, each transposon comprising a first transposon sequence comprising a first reporter moiety, a second transposon sequence noncontiguous with said first transposon sequence, and a transposase associated with the first transposon sequence and the second transposon sequence.

48. The method of any one of claims 46-47, wherein step (f) comprises removing the transposase from the compartmentalized first indexed template nucleic acids.

49. The method of claim 48, wherein the transposase is removed subsequent to step (d).

50. The method of claim 48, wherein the transposase is removed prior to step (i).

51. The method of claim 48, wherein removing the transposase comprises a method selected from the group consisting of adding a detergent, changing temperature, changing pH, adding a protease, adding a chaperone, changing salt concentration, and adding a strand-displacing polymerase.

52. The method of any one of claims 40-51, wherein said first and second reporter moieties identifies the source of said analytes.

53. The method of any one of claims 40-51, wherein the combination of said reporter moieties identifies the source of said analytes.

54. The method of claim 40-53, wherein steps (d)-(i) are repeated at least one more time.

55. The method of claim 54, wherein the additional sets of reporter moieties in each of the addition steps are different than said first and second sets of reporter moieties.

56. The method of any of claims 1-55, wherein said single cell is associated with a disease.

57. The method of claim 56, wherein said single cell is associated with cancer.

58. The method of claim 56, wherein said single cell is associated with genetic disease.

59. A method of obtaining sequence information from a target nucleic acid in a single cell, said method comprising:

- a) introducing a cell suspension to a first droplet actuator;
- b) using electrode mediated droplet operations to dispense an array of droplets comprising cell suspension, each droplet comprises a single cell;
- c) introducing a cell lysis buffer to a second droplet actuator;
- c) using electrode mediated droplet operations to dispense an array of droplets comprising cell lysis buffer;
- d) using electrode mediated droplet operations to combine array of cell lysis buffer droplets and the array of cell suspension droplets to yield an array of cell lysate droplets;
- e) introducing a reagent solution comprising first reporter moiety and enzymes to a third droplet actuator, wherein at least one enzyme is capable of introducing said first reporter moiety to said target nucleic acid, and wherein said first reporter moiety identifies the single cell;
- f) using electrode mediated droplet operations to dispense an array of droplets comprising reagent solution;
- g) using electrode mediated droplet operations to combine an array of cell lysate droplet with reagent droplets to yield an array of first reporter moiety droplets, wherein the reporter moieties are introduced to the nucleic acids of the cell lysate droplets;

h) using electrode mediated droplet operations to dispense an array of droplets comprising wash solution;

i) using electrode mediated droplet operations to combine array of first reporter moiety droplets with wash solution droplets, wherein the enzyme is removed after the reaction from the nucleic acid to yield an array of droplets comprising target nucleic acid, wherein said target nucleic acid comprises first reporter moiety;

(j) repeating steps (e)-(i) at least once, wherein subsequent reagent solutions comprise reporter moieties different from said first reporter moiety to yield an array of droplets comprising target nucleic acid comprising multiple reporter moieties;

(k) obtaining sequence information from said target nucleic acid comprising one or more reporter moieties and associating said sequence information to said single cell.

60. The method of claim 59 further comprising:

(l) introducing a protein labelling reagent solution comprising labels specific for proteins to a droplet actuator;

(m) using electrode mediated droplet operations to dispense an array of droplets comprising protein labelling reagent solution;

(n) using electrode mediated droplet operations to combine an array of protein labelling reagent solution to an array of droplets comprising target nucleic acid comprising multiple reporter moieties, wherein at least a portion of the proteins from said single cell comprises a label unique to said single cell, and identifying at least a portion of the proteins comprising said labels.

61. The method of claim 60, wherein said method of obtaining sequence information from a target nucleic acid is performed simultaneously with identifying at least a portion of the proteins comprising said labels.

62. The method of any of claims 59-61, wherein said target nucleic acid is RNA.

63. The method of any of claims 59-61, wherein said target nucleic acid is DNA.

64. The method of claim 63, wherein said target nucleic acid is genomic DNA.
65. The method of any of claims 59-61, wherein said target nucleic acid is both RNA and DNA.
66. The method of any of claims 59-65, wherein said first reporter moiety is introduced to said target nucleic acid by ligation.
67. The method of any of claims 59-66, further comprising:
- (i) introducing a reagent solution comprising reporter moieties specific for cellular metabolites to a droplet actuator;
 - (ii) using electrode mediated droplet operations to dispense an array of reagent droplets comprising reporter moieties specific for cellular metabolites;
 - (iii) using electrode mediated droplet operations to combine said array of reagent droplets to an array of droplets comprising target nucleic acid comprising multiple reporter moieties, wherein at least a portion of the cellular metabolites from said single cell comprises a reporter moiety unique to said single cell, and identifying at least a portion of the cellular metabolites.
68. The method of any one of claims 1-13, wherein said first, second, third, or fourth reporter moieties comprise primer binding site.
69. The method of any one of claims 1-17, wherein said first, second, or both analytes are nucleic acids.
70. The method of claim 69, wherein the nucleic acids comprising reporter moieties are amplified prior to analysis.
71. The method of any one of claims 69-70, wherein the analysis of the nucleic acid is by sequencing.