CLAIMS

1. A method of exposing cells from a population to at least two different reagents, wherein each cell is exposed to the reagents individually, or in groups of two of more, the method comprising:

(a) distributing cells from the population to a plurality of capture sites in a microfluidic device so that a plurality of capture sites each comprises one or more cells;

(b) providing one or more first reagent(s) to each capture site;

(c) providing one or more second reagent(s) to each capture site, wherein the second reagent(s) is/are different from the first reagent(s) and is/are provided separately from the first reagent(s), and the one or more first reagent(s) and the one or more second reagent(s) are separately provided to each capture site after distributing the cells to the capture sites;

(d) conducting a reaction, whereby the reaction products encode an item of capture site information;

(e) recovering the reaction products; and

(f) analyzing the reaction products, wherein such analysis permits the identification of particular reaction products as having been derived from a single cell or group of cells at a particular capture site.

2. A method of incorporating nucleic acid sequences into reaction products from a cell population, wherein the nucleic acid sequences are incorporated into the reaction products of each cell individually, or in groups of up to 1000, the method comprising:

(a) distributing cells from the population to a plurality of capture sites in a microfluidic device so that a plurality of capture sites each comprises one or more cells;

(b) providing one or more first reagent(s) to each capture site;

(c) providing one or more second reagent(s) to each capture site, wherein the second reagent(s) is/are different from the first reagent(s) and is/are provided separately from the first reagent(s), and the one or more first reagent(s) and the one or more second reagent(s) are separately provided to each capture site after distributing the cells to the capture sites;

(d) conducting a reaction in which nucleic acid sequences are incorporated into the reaction products of each cell or group of cells, individually;

(e) recovering the reaction products; and

(f) analyzing the reaction products, wherein such analysis permits the identification of particular reaction products as having been derived from a single cell or group of cells at a particular capture site.

3. A method of incorporating nucleic acid sequences into nucleic acids of a cell population, wherein the nucleic acid sequences are incorporated into the nucleic acids of each cell individually or in groups of up to 1000, the method comprising:

(a) distributing cells from the population to a plurality of capture sites in a microfluidic device so that a plurality of capture sites each comprises one or more cells;

(b) providing one or more first reagent(s) to each capture site;

(c) providing one or more second reagent(s) to each capture site, wherein the second reagent(s) is/are different from the first reagent(s) and is/are provided separately from the first reagent(s), and the one or more first reagent(s) and the one or more second reagent(s) are separately provided to each capture site after distributing the cells to the capture sites;

(d) conducting a reaction in which nucleic acid sequences are incorporated into the nucleic acids of each cell or group of cells, individually, to produce reaction products;

(e) recovering the reaction products; and

(f) analyzing the reaction products, wherein such analysis permits the identification of particular reaction products as having been derived from a single cell or group of cells at a particular capture site.

4. The method of claim 1, where the distribution is carried out so that a plurality of capture sites each comprise not more than a single cell.

5. The method of claim 1, wherein the reaction incorporates a nucleotide barcode into the reaction products.

6. The method of claim 5, wherein the barcode encodes an item of capture site information.

7. The method of claim 1, wherein the reaction incorporates a nucleic acid sequence that uniquely identifies the molecule into which it is incorporated.

8. The method of claim 3, wherein the reaction comprises reverse transcription of RNA.

9. The method of claim 8, wherein the first reagent(s) comprise a reverse transcription (RT) primer comprising a poly-dT sequence and a first barcode 5' of the poly-dT sequence.

10. The method of claim 9, wherein the RT primer additionally comprises a first unique molecular index (UMI).

11. The method of claim 10, wherein the first UMI is 5' of the poly-dT sequence.

12. The method of claim 9, wherein the RT primer additionally comprises a first linker.

13. The method of claim 12, wherein the first linker is at the 5' end of the RT primer.

14. The method of claim 9, wherein the RT primer additionally comprises an anchor sequence 3' of the poly-dT sequence.

15. The method of claim 8, wherein the reaction additionally comprises second-strand synthesis to produce cDNA.

16. The method of claim 8, wherein the second reagent(s) comprise a 5' oligonucleotide comprising a poly-riboG sequence.

17. The method of claim 16, wherein the 5' oligonucleotide comprises a second barcode 5' of the poly-riboG sequence.

18. The method of claim 16, wherein the 5' oligonucleotide additionally comprises a second UMI.

19. The method of claim 18, wherein the second UMI is 5' of the poly-riboG sequence.

20. The method of claim 16, wherein the 5' oligonucleotide additionally comprises a second linker.

21. The method of claim 20, wherein the second linker is at the 5' end of the 5' oligonucleotide.

22. The method of claim 21, wherein the method comprises producing cDNA, wherein one strand has the structure: 5'-second linker-nucleotide sequence derived from RNA-first linker-3', with a barcode located in between the linkers.

23. The method of claim 22, wherein the first barcode is located adjacent to the first linker.

24. The method of claim 23, wherein the second barcode is located adjacent to the second linker.

25. The method of claim 23, wherein said one strand of cDNA has the structure: 3'-second linker-poly dC-nucleotide sequence derived from RNA-first barcode-first linker-5'.

26. The method of claim 25, wherein said one strand of cDNA has the structure: 3'-second linker-second barcode-poly dC-nucleotide sequence derived from RNA-first barcode-first linker-5'.

27. The method of claim 25, wherein said one strand of cDNA has a structure selected from the group consisting of:

3'-second linker-poly dC-nucleotide sequence derived from RNA-first UMI-first barcode-first linker-5'; and

3'-second linker-poly dC-nucleotide sequence derived from RNA- first barcode-first UMI-first linker-5'.

28. The method of claim 27, wherein said one strand of cDNA has a structure selected from the group consisting of:

3'-second linker-second barcode-second UMI-poly dC-nucleotide sequence derived from RNA-first UMI-first barcode-first linker-5';

3'-second linker-second barcode- second UMI-poly dC-nucleotide sequence derived from RNA- first barcode- first UMI-first linker-5';

3'-second linker- second UMI-second barcode-poly dC-nucleotide sequence derived from RNA-first UMI-first barcode-first linker-5'; and

3'-second linker- second UMI-second barcode-poly dC-nucleotide sequence derived from RNA- first barcode- first UMI-first linker-5'.

29. The method of claim 3, wherein the reaction comprises amplification of DNA.

30. The method of claim 29, wherein the first and/or second reagent(s) comprise first and/or second amplification primers, respectively, wherein the first and/or second amplification primers comprise a first or second barcode, respectively, that is 5' of a primer sequence.

31. The method of claim 30, wherein the first and/or second amplification primers additionally comprise a first or second UMI, respectively.

32. The method of claim 31, wherein the first or second UMI is 5' of the primer sequence.

33. The method claim 30, wherein the first and/or second amplification primer additionally comprises a first or second linker.

34. The method of claim 33, wherein the first or second linker is at the 5' end of the amplification primer.

35. The method of claim 34, wherein the method comprises producing amplicons, wherein one strand has the structure: 5'-second linker-nucleotide sequence derived from cellular DNA-first linker-3', with a barcode located in between the linkers.

36. The method of claim 35, wherein one strand has the structure: 3'-second linker-nucleotide sequence derived from cellular DNA-first barcode-first linker-5'.

37. The method of claim 25, wherein said one strand has the structure: 3'-second linker-second barcode- nucleotide sequence derived from cellular DNA-first barcode-first linker-5'.

38. The method of claim 25, wherein said one strand has a structure selected from the group consisting of:

3'-second linker-nucleotide sequence derived from cellular DNA-first UMI-first barcode-first linker-5'; and

3'-second linker- nucleotide sequence derived from cellular DNA- first barcode-first UMI-first linker-5'.

39. The method of claim 27, wherein said one strand has a structure selected from the group consisting of:

3'-second linker-second barcode-second UMI- nucleotide sequence derived from cellular DNA-first UMI-first barcode-first linker-5';

3'-second linker-second barcode- second UMI- nucleotide sequence derived from cellular DNA- first barcode- first UMI-first linker-5';

3'-second linker- second UMI-second barcode- nucleotide sequence derived from cellular DNA-first UMI-first barcode-first linker-5'; and

3'-second linker- second UMI-second barcode-poly dC-nucleotide sequence derived from cellular DNA- first barcode- first UMI-first linker-5'.

40. The method of claim 1, wherein the microfluidic device comprises a matrix-type microfluidic device comprising:

capture sites arranged in a matrix of R rows and C columns, wherein R and C are integers greater than 1, and wherein the capture sites can be fluidically isolated from one another after distribution of cells to the capture sites;

a set of R first input lines configured to deliver the first reagent(s) to capture sites in a particular row;

a set of C second input lines configured to deliver second reagent(s) to capture sites in a particular column, wherein said delivery is separate from the delivery first reagent(s),

wherein, after the reaction, reaction products are recovered from the microfluidic device in pools of reaction products from individual rows or columns.

41. The method of claim 40, wherein an RT primer is delivered to capture sites via one set of the input lines, and a 5' oligonucleotide is delivered to the capture sites via the other set of input lines.

42. The method of claim 40, wherein a first amplification primer is delivered to capture sites via one set of the input lines, and a second amplification primer is delivered to the capture sites via the other set of input lines.

43. The method of claim 1, wherein all methods steps are performed in the microfluidic device.

44. The method of claim 20, wherein the reaction products are subjected to preamplification using linker primers that anneal to the first and second linkers, wherein the linker primers are the same or different.

45. The method of claim 44, wherein said preamplification is performed in the microfluidic device.

46. The method of claim 1, wherein the reaction products are subjected to tagmentation.

47. The method of claim 1, wherein the reaction incorporates one or more DNA sequencing primer binding sites into the reaction products.

48. The method of claim 1, wherein the reaction products are subjected to DNA sequencing.

49. The method of claim 48, wherein the sequences obtained from DNA sequencing are identified as having been derived from a particular capture site based on one or two barcodes.

50. The method of claim 40, wherein the pools of reaction products are separately subjected to preamplification using linker primers that anneal to the first and second linkers, wherein the linker primers are the same or different.

51. The method of claim 40, wherein the pools of reaction products are combined into one reaction mixture, which is subjected to preamplification using linker primers that anneal to the first and second linkers, wherein the linker primers are the same or different.

52. The method of claim 40, wherein the microfluidic device is sufficiently transparent on at least one surface to permit visualization of cells and/or, when a visualizable label is employed, signals associated with cells or reaction products.

53. The method of claim 52, additionally comprising imaging the cell-occupied capture sites before conducting the reaction.

54. The method of claim 1, wherein the reaction comprises whole transcriptome amplification (WTA), whole genome amplification (WGA), protein proximity ligation, microRNA (mRNA) preamplification, target-specific amplification of RNA or DNA.

55. The method of claim 1, wherein the microfluidic device comprises at least 750 capture sites.

56. The method of claim 1, where the one or more first reagent(s) and the one or more second reagent(s) are provided to a pair of fluidically isolatable chambers in the capture site that are distinct from one another and distinct from a chamber including the capture feature.