

CLAIMS

What is claimed is:

1. A method of assaying single particles in a plurality of particles, said method comprising:
5 capturing particles of said plurality in separate reaction volumes to produce a plurality of separate reaction volumes containing only one particle each; assaying a plurality of parameters for each particle, wherein said assaying comprises:
10 performing a plurality of reactions on each particle in each separate reaction volume to produce a plurality of reaction products for each particle, wherein at least one reaction product comprises a nucleotide sequence that is added to a target nucleic acid and that encodes the identity of the reaction volume that was the source of the reaction product; and
15 analyzing the reaction products to obtain said results; and associating results of the assay with each particle to produce a data set comprising a plurality of parameters for each particle in said plurality of particles that is in a separate reaction volume.
2. The method of claim 1, wherein at least one of the parameters is
20 assayed in each separate reaction volume.
3. The method of any preceding claim, wherein the contents of the separate reaction volumes are recovered.
4. The method of any preceding claim, additionally comprising
25 optimizing said capturing such that the expected fraction of separate reaction volumes with only one particle each is at least 35% of the total number of separate reaction volumes.
5. The method of any preceding claim, additionally comprising optimizing said capturing such that the expected fraction of separate reaction volumes with only one particle each is at least 50% of the total number of separate reaction volumes.

6. The method of any preceding claim, additionally comprising optimizing said capturing such that the expected fraction of separate reaction volumes with only one particle each is at least 65% of the total number of separate reaction volumes.
7. The method of any preceding claim, additionally comprising optimizing said capturing such that the expected fraction of separate reaction volumes with only one particle each is at least 85% of the total number of separate reaction volumes.
8. The method of any preceding claim, wherein the separate reaction volumes are present within individual compartments of a microfluidic device.
9. The method of any of claims 1-8, wherein the particles are captured in separate reaction volumes prior to adding one or more reagent(s) for performing said plurality of reactions.
10. The method of any preceding claim, wherein the particles are cells.
11. The method of any preceding claim, wherein the particles are selected from nucleic acids, proteins, carbohydrates, lipids, and combinations thereof.
12. The method of any preceding claim, wherein fewer than 40,000 particles are employed in the method.
13. The method of any preceding claim, wherein fewer than 10,000 particles are employed in the method.
14. The method of any preceding claim, wherein the number of particles distributed into separate reaction volumes is greater than 10, greater than 100, or greater than 1000.
15. The method of any of claims 1-3 or 8-14, wherein said capturing is performed by limiting dilution.
16. The method of claim 15, wherein limiting dilution comprises: preparing a series of dilutions of particle suspension;

distributing particles from each dilution into separate compartments of a microfluidic device;

determining the number of particles in each compartment; and

selecting the dilution that produces the highest number of

5 compartments comprising only a single particle for use in capturing single particles in separate reaction volumes.

17. The method of claim 16, wherein the number of particles in each compartment is determined by brightfield microscopy or fluorescence microscopy.

10 18. The method of claim 16, wherein a stain, dye, or label is employed to detect the number of particles in each separate reaction volume.

19. The method of claim 18, wherein the particles are cells, and the stain, dye, or label is a membrane-permeant stain, dye, or label.

15 20. The method of claim 18, wherein the particles are cells, and a cell membrane-permeant nucleic acid dye is employed to detect the number of cells in each separate reaction volume.

21. The method of claim 18, wherein the particles are cells, and the stain, dye, or label is a cell-surface stain, dye, or label.

20 22. The method of claim 18, wherein the particles are cells, and a labeled antibody specific for a cell-surface marker is employed to detect the number of cells in each separate reaction volume.

23. The method of any preceding claim, wherein said capturing comprises mechanical capture at a plurality of capture sites in a microfluidic device.

25 24. The method of claim 23, wherein each capture site comprises:
a capture feature sized to contain only one particle; and
a drain feature, wherein when the capture feature is not occupied by a particle, the drain feature permits the flow of fluid through the capture site.

25. The method of claim 23, wherein the microfluidic device comprises a focusing feature to focus particle flow to each capture site.

26. The method of any preceding claim, wherein the method comprises affinity-based capture and employs a binding partner that binds a particle component.

5 27. The method of claim 26, wherein the binding partner is affixed to a discrete region of the microfluidic device, wherein each discrete region permits binding of only one particle.

28. The method of claim 26, wherein said affinity-based capture comprises:
10 capture of a support comprising the binding partner at a plurality of capture sites in a microfluidic device to produce immobilized supports at each capture site, wherein each immobilized support displays the binding partner in a manner that permits binding of only one particle to each immobilized support; and
binding of particles to the binding partners on the immobilized
15 supports.

29. The method of claim 28, wherein the support is captured by mechanical capture.

30. The method of claim 28, wherein each capture site comprises:
a capture feature sized to contain only one support; and
20 a drain feature, wherein when the capture feature is not occupied by a support, the drain feature permits the flow of fluid through the capture site.

31. The method of claim 28, wherein the microfluidic device comprises a focusing feature to focus support and/or particle flow to each capture site.

32. The method of any of claims 23-25 and 27-31, wherein each capture
25 site is located within a separate compartment of the microfluidic device.

33. The method of claim 32, wherein said capturing comprises passing a solution comprising the particles through the compartments of the microfluidic device, whereby 35% or more of the compartments comprise only a single particle.

34. The method of any of the preceding claims, wherein the separate reaction volumes are present within individual compartments of a microfluidic device, and the method comprises

5 and
determining the number of particles in each separate reaction volume;
disregarding results from any reaction volumes that contain no particles or contain more than a single particle.

35. The method of any of claims 3-34, wherein said recovering comprises separately recovering reaction products from separate reaction volumes.

10 36. The method of any of claims 3-34, wherein said recovering comprises pooling reaction products from a plurality of separate reaction volumes.

37. The method of any preceding claim, wherein at least one assay is selected from detection of a DNA sequence; detection of an RNA sequence; detection of a molecule selected from the group consisting of a protein, carbohydrate, lipid, and any combination thereof; detection of a small molecule; detection of an activity; detection of an ion concentration; detection of an ion potential; and detection of a red-ox potential.

38. The method of any preceding claim, wherein the reactions comprise one or more reactions selected from amplification of DNA, digestion of DNA with a nuclease, ligation of DNA, ligation of (an) adaptor sequence(s) onto a DNA molecule, transposase-mediated incorporation of a transposon into a DNA molecule, DNA sequencing, reverse transcription of RNA, amplification of RNA, and digestion of RNA with an RNase.

39. The method of any preceding claim, wherein the reactions comprise whole genome amplification and/or whole transcriptome amplification.

25 40. The method of any preceding claim, wherein the at least one reaction product is produced by a reaction comprising nucleic acid amplification using at least two amplification primers, wherein each amplification primer comprises a barcode sequence, and the combination of barcode sequences encodes the identity of the reaction volume that was the source of the reaction product.

41. The method of claim 40, wherein the nucleic acid amplification uses a pair of inner primers and a pair of outer primers, wherein:

the inner primers comprise:

5 a forward, inner primer comprising a first nucleotide tag, a first barcode nucleotide sequence, and a target-specific portion; and
a reverse, inner primer comprising a target-specific portion, a first barcode nucleotide sequence, and a second nucleotide tag; and

the outer primers comprise:

10 a forward, outer primer comprising a second barcode nucleotide sequence and a first nucleotide tag-specific portion; and
a reverse, outer primer comprising a second nucleotide tag-specific portion and a second barcode nucleotide sequence;

wherein the outer primers are in excess of the inner primers; and

15 the nucleic acid amplification produces an amplicon comprising 5'-second barcode nucleotide sequence-first nucleotide tag sequence-first barcode nucleotide sequence-target nucleotide sequence-first barcode nucleotide sequence-second nucleotide tag sequence-second barcode nucleotide sequence-3'.

42. The method of claim 40, wherein the nucleic acid amplification uses a pair of inner primers, a pair of stuffer primers, and a pair of outer primers, wherein:

20 the inner primers comprise:

a forward, inner primer comprising a first nucleotide tag and a target-specific portion; and
a reverse, inner primer comprising a target-specific portion and a second nucleotide tag;

25 the stuffer primers comprise:

a forward, stuffer primer comprising a third nucleotide tag, a first barcode nucleotide sequence, and a first nucleotide tag-specific portion; and
a reverse, stuffer primer comprising a second nucleotide tag-specific portion, a first barcode nucleotide sequence, a fourth nucleotide tag; and

30 the outer primers comprise:

a forward, outer primer comprising a second barcode nucleotide sequence and a third nucleotide tag-specific portion; and

a reverse, outer primer comprising a fourth nucleotide tag-specific portion and a second barcode nucleotide sequence;

wherein the outer primers are in excess of the stuffer primers, which are in excess of the inner primers; and

5 the nucleic acid amplification produces an amplicon comprising 5'-second barcode nucleotide sequence-third nucleotide tag sequence-first barcode nucleotide sequence-first nucleotide tag sequence-target nucleotide sequence-second nucleotide tag sequence-first barcode nucleotide sequence-fourth nucleotide tag sequence-second barcode nucleotide sequence-3'.

10 43. The method of claim 41, wherein the separate reaction volumes are separate compartments of a microfluidic device, the separate compartments being arranged as an array defined by rows and columns, wherein the combination of the first and second barcodes in each amplicon identifies the row and column of the compartment that was the source of the amplicon.

15 44. The method of claim 43, wherein said recovering comprises pooling amplicons from compartments in a row or in a column.

20 45. The method of claim 41, wherein the outer primers additionally comprise first and second primer binding sites that are capable of being bound by DNA sequencing primers, and wherein the nucleic acid amplification produces an amplicon comprising 5'-first primer binding site-second barcode nucleotide sequence-first nucleotide tag sequence-first barcode nucleotide sequence-target nucleotide sequence-first barcode nucleotide sequence-second nucleotide tag sequence-second barcode nucleotide sequence-second primer binding site-3'.

25 46. The method of claim 45, wherein said analyzing comprises sequencing the amplification products.

47. The method of any preceding claim, wherein the plurality of reactions is performed on intact particles.

48. The method of any preceding claim, wherein the plurality of reactions is performed on disrupted particles.

49. The method of claim 48, wherein the plurality of reactions is performed on lysed cells.

50. The method of any preceding claim, wherein the particles are treated with an agent that elicits biological response prior to performing the plurality of reactions.

5 51. The method of any preceding claim, wherein the method comprises determining the presence or amount of one or more target nucleic acids in or associated with each particle.

10 52. The method of any preceding claim, wherein the method comprises determining the copy numbers of one or more DNA molecule(s) in or associated with each particle.

53. The method of any preceding claim, wherein the method comprises determining the genotype(s) at one or more loci in or associated with each particle.

54. The method of any preceding claim, wherein the method comprises determining a haplotype for a plurality of loci in or associated with each particle.

15 55. The method of any preceding claim, wherein the method comprises determining the expression level(s) of one or more RNA molecule(s) in or associated with each particle.

20 56. The method of any preceding claim, wherein the method comprises determining the nucleotide sequence(s) of one or more RNA molecule(s) in or associated with each particle.

57. The method of any preceding claim, wherein the method comprises determining the expression level(s) of one or more proteins in or associated with each particle.

25 58. The method of any preceding claim, wherein the results of the analysis indicate the presence of:

two or more copy number variations that are correlated with a phenotype;

two or more mutations that are correlated with a phenotype; or
at least one copy number variation and at least one mutation that are,
together, correlated with a phenotype.

5 59. The method of claim 58, wherein the phenotype is selected from the group consisting of risk, presence, severity, prognosis, and responsiveness to a specific therapy of a disease.

60. The method of claim 59, wherein the phenotype comprises resistance to a drug.

10 61. The method of any preceding claim, where the results of analysis indicate the occurrence of genomic recombination.

62. The method of any preceding claim, where the results of analysis indicate co-expression of particular splice variants.

63. The method of any preceding claim, where the results of analysis indicate co-expression of particular light and heavy chains in B cells.

15 64. The method of any preceding claim, where the results of analysis indicate the presence of a particular pathogen in a particular host cell.

65. A method of isolating a single particle, said method comprising:
capture of a support comprising a binding partner at a capture site on
a substrate to produce an immobilized support at the capture site, wherein the binding
20 partner binds a particle component, and the immobilized support displays the binding
partner in a manner that permits binding of only one particle to each immobilized support;
and
binding of a particle to the binding partner on the immobilized
support.

25 66. The method of claim 65, wherein the capture comprises mechanical capture of a support.

67. The method of claim 65, wherein the capture site comprises:

a capture feature sized to contain only one support; and
a drain feature, wherein when the capture feature is not occupied by a support, the drain feature permits the flow of fluid through the capture site.

5 68. The method of claim 65, wherein the substrate comprises a focusing feature to focus flow to each capture site.

69. The method of any of claims 65-68, wherein the substrate comprises a plurality of capture sites.

70. The method of claim 69, wherein each capture site is located within a separate compartment of a microfluidic device.

10 71. The method of claim 70, wherein the method comprises passing a solution comprising the particles through the compartments of the microfluidic device, whereby 35% or more of the compartments comprise only a single particle.

CLAIMS

What is claimed is:

1. A method of assaying single particles in a plurality of particles, said method comprising:
 - capturing particles of said plurality in separate reaction volumes to produce a plurality of separate reaction volumes containing only one particle each such that the expected fraction of separate reaction volumes with only one particle each is at least 35% of the total number of separate reaction volumes;
 - assaying a plurality of parameters for each particle(s) in each separate reaction volume, and wherein said assaying comprises:
 - performing a plurality of reactions on each particle in each separate reaction volume to produce a plurality of reaction products for each particle(s) in each separate reaction volume,
 - wherein at least one reaction product is an amplicon produced by a reaction comprising nucleic acid amplification using at least two amplification primers, wherein each amplification primer comprises a barcode sequence;
 - wherein the separate reaction volumes are separate compartments of a microfluidic device, the separate compartments being arranged as an array defined by rows and columns, wherein the combination of the first and second barcodes in each amplicon identifies the row and column of the compartment that was the source of the amplicon;
 - recovering the contents of the separate reaction volumes by pooling reaction products from compartments in a row or in a column; and
 - analyzing the reaction products to obtain results;
 - disregarding results from any reaction volumes that contain no particles or contain more than a single particle; and
 - associating remaining results of the assay with each separate reaction volume containing only one particle to produce a data set comprising a plurality of parameters for each particle in said plurality of particles that is in a separate reaction volume.

2. The method of claim 1, additionally comprising optimizing said capturing such that the expected fraction of separate reaction volumes with only one particle each is at least 50% of the total number of separate reaction volumes.

3. The method of claim 1, additionally comprising optimizing said capturing such that the expected fraction of separate reaction volumes with only one particle each is at least 65% of the total number of separate reaction volumes.

4. The method of claim 1, additionally comprising optimizing said capturing such that the expected fraction of separate reaction volumes with only one particle each is at least 85% of the total number of separate reaction volumes.

5. The method of any of claims 1-4, wherein the particles are captured in separate reaction volumes prior to adding one or more reagent(s) for performing said plurality of reactions.

6. The method of any of claims 1-5, wherein the particles are cells.

7. The method of any of claims 1-6, wherein fewer than 40,000 particles are employed in the method.

8. The method of claim 7, wherein fewer than 10,000 particles are employed in the method.

9. The method of any of claims 1-8, wherein the number of particles distributed into separate reaction volumes is greater than 10, greater than 100, or greater than 1000.

10. The method of any of claims 1-9, wherein said capturing is performed by limiting dilution.

11. The method of claim 10, wherein limiting dilution comprises:
preparing a series of dilutions of particle suspension;
distributing particles from each dilution into separate compartments of a microfluidic device;
determining the number of particles in each compartment; and

selecting the dilution that produces the highest number of compartments comprising only a single particle for use in capturing single particles in separate reaction volumes.

12. The method of claim 11, wherein the number of particles in each compartment is determined by brightfield microscopy or fluorescence microscopy.

13. The method of claim 11, wherein a stain, dye, or label is employed to detect the number of particles in each separate reaction volume.

14. The method of claim 13, wherein the particles are cells, and the stain, dye, or label is a membrane-permeant stain, dye, or label.

15. The method of claim 13, wherein the particles are cells, and a cell membrane-permeant nucleic acid dye is employed to detect the number of cells in each separate reaction volume.

16. The method of claim 13, wherein the particles are cells, and the stain, dye, or label is a cell-surface stain, dye, or label.

17. The method of claim 13, wherein the particles are cells, and a labeled antibody specific for a cell-surface marker is employed to detect the number of cells in each separate reaction volume.

18. The method of any of claims 1-17, wherein said capturing comprises mechanical capture at a plurality of capture sites in a microfluidic device.

19. The method of claim 18, wherein each capture site comprises:
a capture feature sized to contain only one particle; and
a drain feature, wherein when the capture feature is not occupied by a particle, the drain feature permits the flow of fluid through the capture site.

20. The method of claim 18, wherein the microfluidic device comprises a focusing feature to focus particle flow to each capture site.

21. The method of any of claims 1-20, wherein the method comprises affinity-based capture and employs a binding partner that binds a particle component.

22. The method of claim 21, wherein the binding partner is affixed to a discrete region of the microfluidic device, wherein each discrete region permits binding of only one particle.

23. The method of claim 21, wherein said affinity-based capture comprises:

capture of a support comprising the binding partner at a plurality of capture sites in a microfluidic device to produce immobilized supports at each capture site, wherein each immobilized support displays the binding partner in a manner that permits binding of only one particle to each immobilized support; and

binding of particles to the binding partners on the immobilized supports.

24. The method of claim 23, wherein the support is captured by mechanical capture.

25. The method of claim 23, wherein each capture site comprises:
a capture feature sized to contain only one support; and
a drain feature, wherein when the capture feature is not occupied by a support, the drain feature permits the flow of fluid through the capture site.

26. The method of claim 23, wherein the microfluidic device comprises a focusing feature to focus support and/or particle flow to each capture site.

27. The method of any of claims 18-20 and 22-26, wherein each capture site is located within a separate compartment of the microfluidic device.

28. The method of any of claims 1-27, wherein at least one assay is selected from detection of a DNA sequence; detection of an RNA sequence; detection of a molecule selected from the group consisting of a protein, carbohydrate, lipid, and any combination thereof; detection of a small molecule; detection of an activity; detection of an ion concentration; detection of an ion potential; and detection of a red-ox potential.

29. The method of any of claims 1-28, wherein the reactions comprise whole genome amplification and/or whole transcriptome amplification.

30. The method of any of claims 1-29, wherein the nucleic acid amplification uses a pair of inner primers and a pair of outer primers, wherein:

the inner primers comprise:

a forward, inner primer comprising a first nucleotide tag, a first barcode nucleotide sequence, and a target-specific portion; and

a reverse, inner primer comprising a target-specific portion, a first barcode nucleotide sequence, and a second nucleotide tag; and

the outer primers comprise:

a forward, outer primer comprising a second barcode nucleotide sequence and a first nucleotide tag-specific portion; and

a reverse, outer primer comprising a second nucleotide tag-specific portion and a second barcode nucleotide sequence;

wherein the outer primers are in excess of the inner primers; and

the nucleic acid amplification produces an amplicon comprising 5'-second barcode nucleotide sequence-first nucleotide tag sequence-first barcode nucleotide sequence-target nucleotide sequence-first barcode nucleotide sequence-second nucleotide tag sequence-second barcode nucleotide sequence-3'.

31. The method of any of claims 1-29, wherein the nucleic acid amplification uses a pair of inner primers, a pair of stuffer primers, and a pair of outer primers, wherein:

the inner primers comprise:

a forward, inner primer comprising a first nucleotide tag and a target-specific portion; and

a reverse, inner primer comprising a target-specific portion and a second nucleotide tag;

the stuffer primers comprise:

a forward, stuffer primer comprising a third nucleotide tag, a first barcode nucleotide sequence, and a first nucleotide tag-specific portion; and

a reverse, stuffer primer comprising a second nucleotide tag-specific portion, a first barcode nucleotide sequence, a fourth nucleotide tag; and

the outer primers comprise:

a forward, outer primer comprising a second barcode nucleotide sequence and a third nucleotide tag-specific portion; and

a reverse, outer primer comprising a fourth nucleotide tag-specific portion and a second barcode nucleotide sequence;

wherein the outer primers are in excess of the stuffer primers, which are in excess of the inner primers; and

the nucleic acid amplification produces an amplicon comprising 5'-second barcode nucleotide sequence-third nucleotide tag sequence-first barcode nucleotide sequence-first nucleotide tag sequence-target nucleotide sequence-second nucleotide tag sequence-first barcode nucleotide sequence-fourth nucleotide tag sequence-second barcode nucleotide sequence-3'.

32. The method of claim 30, wherein the outer primers additionally comprise first and second primer binding sites that are capable of being bound by DNA sequencing primers, and wherein the nucleic acid amplification produces an amplicon comprising 5'-first primer binding site-second barcode nucleotide sequence-first nucleotide tag sequence-first barcode nucleotide sequence-target nucleotide sequence-first barcode nucleotide sequence-second nucleotide tag sequence-second barcode nucleotide sequence-second primer binding site-3'.

33. The method of claim 32, wherein said analyzing comprises sequencing the amplification products.

34. The method of any of claims 1-33, wherein the plurality of reactions is performed on disrupted particles.

35. The method of claim 34, wherein the plurality of reactions is performed on lysed cells.

36. The method of any of claims 1-35, wherein the particles are treated with an agent that elicits biological response prior to performing the plurality of reactions.

37. The method of any of claims 1-36, wherein the method comprises determining the presence or amount of one or more target nucleic acids in or associated with each particle.

38. The method of any of claims 1-37, wherein the method comprises determining the copy numbers of one or more DNA molecule(s) in or associated with each particle.

39. The method of any of claims 1-38, wherein the method comprises determining the genotype(s) at one or more loci in or associated with each particle.

40. The method of any of claims 1-39, wherein the method comprises determining a haplotype for a plurality of loci in or associated with each particle.

41. The method of any of claims 1-40, wherein the method comprises determining the expression level(s) of one or more RNA molecule(s) in or associated with each particle.

42. The method of any of claims 1-41, wherein the method comprises determining the nucleotide sequence(s) of one or more RNA molecule(s) in or associated with each particle.

43. The method of any of claims 1-42, wherein the method comprises determining the expression level(s) of one or more proteins in or associated with each particle.

44. The method of any of claims 1-43, wherein the results of the analysis indicate the presence of:

two or more copy number variations that are correlated with a phenotype;

two or more mutations that are correlated with a phenotype; or

at least one copy number variation and at least one mutation that are, together, correlated with a phenotype.

45. The method of claim 44, wherein the phenotype is selected from the group consisting of risk, presence, severity, prognosis, and responsiveness to a specific therapy of a disease.

46. The method of claim 45, wherein the phenotype comprises resistance to a drug.

47. The method of any of claims 1-46, where the results of analysis indicate the occurrence of genomic recombination.

48. The method of any of claims 1-47, where the results of analysis indicate co-expression of particular splice variants.

49. The method of any of claims 1-48, where the results of analysis indicate co-expression of particular light and heavy chains in B cells.

50. The method of any of claims 1-49, where the results of analysis indicate the presence of a particular pathogen in a particular host cell.