Claims:

1. A capture object comprising a plurality of capture oligonucleotides, wherein each capture oligonucleotide of said plurality comprises:

a priming sequence;

a capture sequence; and

a barcode sequence comprising three or more cassetable oligonucleotide sequences, each cassetable oligonucleotide sequence being non-identical to the other cassetable oligonucleotide sequences of said barcode sequence, and,

wherein each capture oligonucleotide of said plurality comprises the same barcode sequence.

2. The capture object of claim 1, wherein each capture oligonucleotide of said plurality comprises a 5'-most nucleotide and a 3'-most nucleotide,

wherein said priming sequence is adjacent to or comprises said 5'-most nucleotide,

wherein said capture sequence is adjacent to or comprises said 3'-most nucleotide, and

wherein said barcode sequence is located 3' to said priming sequence and 5' to said capture sequence.

3. The capture object of any one of claims 1-2, wherein each of said three or more cassetable oligonucleotide sequences comprises 8 to 12 nucleotides.

4. The capture object of any one of claims 1-3, wherein said three or more cassetable oligonucleotide sequences of said barcode sequence are linked in tandem without any intervening oligonucleotide sequences.

5. The capture object of any one of claims 1-4, wherein each of said three or more cassetable oligonucleotide sequences of said barcode sequence has a sequence of any one of SEQ ID NOs: 1-40.

6. The capture object of any one of claims 1-5, wherein said barcode sequence comprises four cassetable oligonucleotide sequences.

7. The capture object of any one of claims 1-6, wherein each capture oligonucleotide of said plurality further comprises a unique molecule identifier (UMI) sequence.

8. The capture object of claim 7, wherein said UMI is located 3' to said priming sequence and 5' to said capture sequence.

9 The capture object of any one of claims 1-8, wherein each capture oligonucleotide further comprises a restriction site comprising a recognition sequence of at least 8 base pairs.

10. The capture object of any one of claims 1-8, wherein said capture sequence comprises a poly-dT sequence, a random hexamer sequence, a gene specific sequence, or a mosaic end sequence.

11. A plurality of capture objects, wherein each capture object of said plurality is a capture object of any one of claims 1-10, wherein said barcode sequence of said capture oligonucleotides of each capture object of said plurality is different from the barcode sequence of the capture oligonucleotides of every other capture object of said plurality.

12. A hybridization probe comprising:

an oligonucleotide sequence comprising a sequence of any one of SEQ ID NOs: 41 to 80 and a fluorescent label.

13. A reagent comprising a plurality of hybridization probes, wherein each hybridization probe of said plurality is a hybridization probe according to claim 12, and wherein each hybridization probe of said plurality (i) comprises an oligonucleotide sequence which is non-identical to the oligonucleotide sequence of every other hybridization probe of the plurality and (ii) comprises a fluorescent label which is spectrally distinguishable from the fluorescent label of every other hybridization probe of said plurality.

14. The reagent of claim 13, wherein:

a first hybridization probe of said plurality comprises a sequence selected from a first subset of SEQ ID NOs: 41-80, and a first fluorescent label;

a second hybridization probe of said plurality comprises a sequence selected from a second subset of SEQ ID NOs: 41-80, and a second fluorescent label which is spectrally distinguishable from said first fluorescent label,

a third hybridization probe of said plurality comprises a sequence selected from a third subset of SEQ ID NOs: 41-80, and a third fluorescent label which is spectrally distinguishable from each of said first and second fluorescent labels,

wherein said first, second, and third subsets of SEQ ID NOs: 41-80 are non-overlapping subsets.

15. A method of in-situ identification of one or more capture objects within a microfluidic device, said method comprising:

disposing a single capture object of said one or more capture objects within an isolation region of each of one or more sequestration pens located within an enclosure of said microfluidic device, wherein each capture object comprises a plurality of capture oligonucleotides, and wherein each capture oligonucleotide of said plurality comprises:

a priming sequence;

a capture sequence; and

a barcode sequence, wherein said barcode sequence comprises three or more cassetable oligonucleotide sequences, each cassetable oligonucleotide sequence being non-identical to the other cassetable oligonucleotide sequences of said barcode sequence;

flowing a first reagent solution comprising a first set of hybridization probes into a flow region within said enclosure of said microfluidic device, wherein said flow region is fluidically connected to each of said one or more sequestration pens, and wherein each hybridization probe of said first set comprises:

an oligonucleotide sequence complementary to a cassetable oligonucleotide sequence comprised by any of said barcode sequences of any of said capture oligonucleotides of any of said one or more capture objects, wherein said complementary oligonucleotide sequence of each hybridization probe in said first set is non-identical to every other complementary oligonucleotide sequence of said hybridization probes in said first set; and

a fluorescent label selected from a set of spectrally distinguishable fluorescent labels, wherein said fluorescent label of each hybridization probe in said first set is different from the fluorescent label of every other hybridization probe in said first set of hybridization probes;

hybridizing said hybridization probes of said first set to corresponding cassetable oligonucleotide sequences in any of said barcode sequences of any of said capture oligonucleotides of any of said one or more capture objects;

detecting, for each hybridization probe of said first set of hybridization probes, a corresponding fluorescent signal associated with any of said one or more capture objects; and

generating a record, for each capture object disposed within one of said one or more sequestration pens, comprising (i) a location of said sequestration pen within said enclosure of said microfluidic device, and (ii) an association or non-association of said corresponding fluorescent signal of each hybridization probe of said first set of hybridization probes with said capture object, wherein said record of associations and non-associations constitute a barcode which links said capture object with said sequestration pen.

16. The method of claim 15, further comprising:

flowing an n^{th} reagent solution comprising an n^{th} set of hybridization probes into said flow region of said microfluidic device, wherein each hybridization probe of said n^{th} set comprises:

an oligonucleotide sequence complementary to a cassetable oligonucleotide sequence comprised by any of said barcode sequences of any of said capture oligonucleotides of any of said one or more capture objects, wherein said complementary oligonucleotide sequence of each hybridization probe in said n^{th} set is non-identical to every other complementary oligonucleotide sequence of said hybridization probes in said n^{th} set and any other set of hybridization probes flowed into said flow region of said microfluidic device; and

a fluorescent label selected from a set of spectrally distinguishable fluorescent labels, wherein said fluorescent label of each hybridization probe in said n^{th} set is different from the fluorescent label of every other hybridization probe in said n^{th} set of hybridization probes;

hybridizing said hybridization probes of said n^{th} set to corresponding cassetable oligonucleotide sequences in any of said barcode sequences of any of said capture oligonucleotides of any of said one or more capture objects;

detecting, for each hybridization probe of said n^{th} set of hybridization probes, a corresponding fluorescent signal associated with any of said one or more capture objects; and

supplementing said record, for each capture object disposed within one of said one or more sequestration pens, with an association or non-association of said corresponding fluorescent signal of each hybridization probe of said n^{th} set of hybridization probes with said capture object,

wherein n is a set of positive integers having values of {2,..., m},

wherein m is a positive integer having a value of 2 or greater,

wherein the foregoing steps of flowing said n^{th} reagent, hybridizing said n^{th} set of hybridization probes, detecting said corresponding fluorescent signals, and supplementing said records are repeated for each value of n in said set of positive integers {2, ..., m}, and,

wherein m has a value greater than or equal to 3 and less than or equal to 20.

17. The method of any one of claims 15-16, wherein each barcode sequence of each capture oligonucleotide of each capture object comprises three or four cassetable oligonucleotide sequences.

18. The method of any one of claims 16-17, wherein said first set of hybridization probes and each of said n^{th} sets of hybridization probes comprise three or four hybridization probes.

19. The method of any one of claims 15-18, further comprising disposing one or more biological cells within said one or more sequestration pens of said microfluidic device, wherein each one of said one or more biological cells are disposed in a different one of said one or more sequestration pens.

20. The method of any one of claims 15-19, wherein said enclosure of said microfluidic device further comprises a dielectrophoretic (DEP) configuration, and wherein disposing said one or more capture objects into one or more sequestration pens is performed using dielectrophoretic (DEP) force.

21. The method of any one of claims 15-19, wherein said enclosure of said microfluidic device further comprises a dielectrophoretic (DEP) configuration, and said disposing said one

or more biological cells within said one or more sequestration pens is performed using dielectrophoretic (DEP) forces.

22. A method of correlating genomic data with a biological cell in a microfluidic device, comprising:

disposing a capture object into a sequestration pen of a microfluidic device, wherein said capture object comprises a plurality of capture oligonucleotides, wherein each capture oligonucleotide of said plurality comprises:

a priming sequence;

a capture sequence; and

a barcode sequence, wherein said barcode sequence comprises three or more cassetable oligonucleotide sequences, each cassetable oligonucleotide sequence being non-identical to the other cassetable oligonucleotide sequences of said barcode sequence; and

wherein each capture oligonucleotide of said plurality comprises said same barcode sequence;

identifying said barcode sequence of said plurality of capture oligonucleotides in-situ and recording an association between said identified barcode sequence and said sequestration pen;

disposing said biological cell into said sequestration pen;

lysing said biological cell and allowing nucleic acids released from said lysed biological cell to be captured by said plurality of capture oligonucleotides comprised by said capture object;

transcribing said captured nucleic acids, thereby producing a plurality of barcoded cDNAs, each barcoded cDNA comprising a complementary captured nucleic acid sequence covalently linked to one of said capture oligonucleotides;

sequencing said transcribed nucleic acids and said barcode sequence, thereby obtaining read sequences of said plurality of transcribed nucleic acids associated with read sequences of said barcode sequence;

identifying said barcode sequence based upon said read sequences; and

using said read sequence-identified barcode sequence and said in situ-identified barcode sequence to link said read sequences of said plurality of transcribed nucleic acids with said sequestration pen and thereby correlate said read sequences of said plurality of transcribed nucleic acids with said biological cell placed into said sequestration pen.

23. The method of claim 22, further comprising:

observing a phenotype of said biological cell; and

correlating said read sequences of said plurality of transcribed nucleic acids with said phenotype of said biological cell.

24. The method of any one of claims 22-23, wherein identifying said barcode sequence of said plurality of capture oligonucleotide in-situ comprises performing the method of claim 15.

25. The method of any one of claims 22-24, further comprising:

disposing a plurality of capture objects into a corresponding plurality of sequestration pens of said microfluidic device;

disposing a plurality of biological cells into said corresponding plurality of sequestration pens, and

processing each of said plurality of capture objects and plurality of biological cells according to said additional steps of said method.

26. A kit for producing a nucleic acid library, comprising:

a microfluidic device comprising:

an enclosure, wherein said enclosure comprises a flow region and a plurality of sequestration pens opening off of said flow region; and,

a dielectrophoretic (DEP) configuration; and

a plurality of capture objects, wherein each capture object of said plurality comprises a plurality of capture oligonucleotides, each capture oligonucleotide of said plurality comprising:

a capture sequence; and

a barcode sequence comprising at least three cassetable oligonucleotide sequences, wherein each cassetable oligonucleotide sequence of said barcode sequence is non-identical to the other cassetable oligonucleotide sequences of said barcode sequence, and wherein each capture oligonucleotide of said plurality comprises the same barcode sequence; and wherein said plurality of capture objects is a plurality of capture objects according to claim 11.

27. The kit of claim 26, further comprising:

a plurality of hybridization probes, each hybridization probe comprising:

an oligonucleotide sequence complementary to any one of said cassetable oligonucleotide sequences of said plurality of capture oligonucleotides of any one of said plurality of capture objects; and

a label,

wherein said complementary sequence of each hybridization probe of said plurality is complementary to a different cassetable oligonucleotide sequence; and

wherein said label of each hybridization probe of said plurality is selected from a set of spectrally distinguishable labels.

28. A method of providing a barcoded cDNA library from a biological cell, comprising:

disposing said biological cell within a sequestration pen located within an enclosure of a microfluidic device;

disposing a capture object within said sequestration pen, wherein said capture object comprises a plurality of capture oligonucleotides, each capture oligonucleotide of said plurality comprising:

a priming sequence that binds a primer;

a capture sequence; and

a barcode sequence, wherein said barcode sequence comprises three or more cassetable oligonucleotide sequences, each cassetable oligonucleotide sequence being non-identical to every other cassetable oligonucleotide sequences of said barcode sequence; and wherein said barcode sequence of each capture oligonucleotide of said plurality is the same;

lysing said biological cell and allowing nucleic acids released from said lysed biological cell to be captured by said plurality of capture oligonucleotides comprised by said capture object; and

transcribing said captured nucleic acids, thereby producing a plurality of barcoded cDNAs decorating said capture object, each barcoded cDNA comprising (i) an oligonucleotide sequence complementary to a corresponding one of said captured nucleic acids, covalently linked to (ii) one of said plurality of capture oligonucleotides.

29. The method of claim 28, wherein said capture sequence of one or more of said plurality of capture oligonucleotides comprises a gene-specific primer sequence, wherein said gene-specific primer sequence targets an mRNA sequence encoding a T cell receptor (TCR) or a B-cell receptor (BCR).

30. The method of any one of claims 28-29, further comprising: identifying said barcode sequence of said plurality of capture oligonucleotides of said capture object in situ, while said capture object is located within said sequestration pen, wherein said identifying said barcode is performed using the method of claim 15.

31. The method of any one of claims 28-30, wherein said enclosure of said microfluidic device further comprises a dielectrophoretic (DEP) configuration, and wherein disposing said biological cell and/or disposing said capture object is performed by applying a dielectrophoretic (DEP) force on or proximal to said biological cell and/or said capture object.

32. A method of providing a barcoded sequencing library, comprising:

amplifying a cDNA library of a capture object obtained by a method of claim 28; and

tagmenting said amplified DNA library, thereby producing a sequencing library.

33. A method of providing a barcoded genomic DNA library from a biological micro-object, comprising:

disposing a biological micro-object comprising genomic DNA within a sequestration pen located within an enclosure of a microfluidic device;

contacting said biological micro-object with a lysing reagent capable of disrupting a nuclear envelope of said biological micro-object, thereby releasing genomic DNA of said biological micro-object;

tagmenting said released genomic DNA, thereby producing a plurality of tagmented genomic DNA fragments having a first end defined by a first tagmentation insert sequence and a second end defined by a second tagmentation insert sequence;

disposing a capture object within said sequestration pen, wherein said capture object comprises a plurality of capture oligonucleotides, each capture oligonucleotide of said plurality comprising:

a first priming sequence;

a first tagmentation insert capture sequence; and

a barcode sequence, wherein said barcode sequence comprises three or more cassetable oligonucleotide sequences, each cassetable oligonucleotide sequence being non-identical to every other cassetable oligonucleotide sequence of said barcode sequence, and wherein said barcode sequence of each capture oligonucleotide of said plurality is the same;

contacting ones of said plurality of tagmented genomic DNA fragments with (i) said first tagmentation insert capture sequence of ones of said plurality of capture oligonucleotides of said capture object, (ii) an amplification oligonucleotide comprising a second priming sequence linked to a second tagmentation insert capture sequence, a randomized primer sequence, or a gene-specific primer sequence, and (iii) an enzymatic mixture comprising a strand displacement enzyme and a polymerase;

incubating said contacted plurality of tagmented genomic DNA fragments for a period of time, thereby simultaneously amplifying said ones of said plurality of tagmented genomic DNA fragments and adding said capture oligonucleotide and said amplification oligonucleotide to the ends of said ones of said plurality of tagmented genomic DNA fragments to produce said barcoded genomic DNA library; and

exporting said barcoded genomic DNA library from said microfluidic device.

34. The method of claim 33, further comprising: identifying said barcode sequence of said plurality of capture oligonucleotides of said capture object in situ, while said capture object is located within said sequestration pen, wherein said identifying said barcode sequence is performed using a method of claim 15.

35. The method of any one of claims 33-34, wherein said enclosure of said microfluidic device further comprises a dielectrophoretic (DEP) configuration, and wherein disposing said biological micro-object and/or disposing said capture object is performed by applying a dielectrophoretic (DEP) force on or proximal to said biological cell and/or said capture object.

36. A method of providing a barcoded cDNA library and a barcoded genomic DNA library from a single biological cell, comprising:

disposing said biological cell within a sequestration pen located within an enclosure of a microfluidic device;

disposing a first capture object within said sequestration pen, wherein said first capture object comprises a plurality of capture oligonucleotides, each capture oligonucleotide of said plurality comprising:

a first priming sequence;

a first capture sequence; and

a first barcode sequence, wherein said first barcode sequence comprises three or more cassetable oligonucleotide sequences, each cassetable oligonucleotide sequence being non-identical to every other cassetable oligonucleotide sequence of said first barcode sequence, and wherein said barcode sequence of each capture oligonucleotide of said plurality is the same;

obtaining said barcoded cDNA library by performing the method of claim 28, wherein lysing said biological cell is performed such that a plasma membrane of said biological cell is degraded, releasing cytoplasmic RNA from said biological cell, while leaving a nuclear envelope of said biological cell intact, thereby providing said first capture object decorated with said barcoded cDNA library from said RNA of said biological cell;

exporting said cDNA library-decorated first capture object from said microfluidic device;

disposing a second capture object within said sequestration pen, wherein said second capture object comprises a plurality of capture oligonucleotides, each comprising:

a second priming sequence;

a first tagmentation insert capture sequence; and

a second barcode sequence, wherein said second barcode sequence comprises three or more cassetable oligonucleotide sequences, each cassetable oligonucleotide sequence being non-identical to every other cassetable oligonucleotide sequence of said second barcode sequence, and wherein and said barcode sequence of each capture oligonucleotide of said plurality is the same;

obtaining said barcoded genomic DNA library by performing the method of claim 33, wherein a plurality of tagmented genomic DNA fragments from said biological cell are contacted with said first tagmentation insert capture sequence of ones of said plurality of capture oligonucleotides of said second capture object, thereby providing said barcoded genomic DNA library from said genomic DNA of said biological cell; and

exporting said barcoded genomic DNA library from said microfluidic device.

37. A method of providing a barcoded B cell receptor (BCR) sequencing library, comprising:

generating a barcoded cDNA library from a B lymphocyte, wherein said generating is performed according to the method of claim 28, wherein said barcoded cDNA library decorates

a capture object comprising a plurality of capture oligonucleotides, each capture oligonucleotide of said plurality comprising a Not1 restriction site sequence;

amplifying said barcoded cDNA library;

selecting for barcoded BCR sequences from said barcoded cDNA library, thereby producing a library enriched for barcoded BCR sequences;

circularizing sequences from said library enriched for barcoded BCR sequences, thereby producing a library of circularized barcoded BCR sequences;

relinearizing said library of circularized barcoded BCR sequences to provide a library of rearranged barcoded BCR sequences, each presenting a constant (C) region of said BCR sequence 3' to a respective variable (V) sub-region and/or a respective diversity (D) sub-region; and,

adding a sequencing adaptor and sub-selecting for said V sub-region and/or said D sub-region, thereby producing a barcoded BCR sequencing library.

38. The method of claim 37, wherein said selecting for a BCR sequence region comprises performing a polymerase chain reaction (PCR) selective for BCR sequences, thereby producing said library of barcoded BCR region selective amplified DNA.