in population averages. Similarly, distinct cell types in a complex tissue (e.g. the brain) can only be studied if cells are analyzed individually.

[005] There are often no suitable cell-surface markers to use in isolating single cells for study, and even when there are, a small number of single cells are not sufficient to capture the range of natural variation in gene expression. What is needed is a method of preparing cDNA libraries which can be used to analyze gene expression in a plurality of single cells.

SEQUENCE LISTING

[006] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled IP-1388-US_SequenceListing.txt, created September 15, 2015, which is 4 Kb in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

SUMMARY

[007] Presented herein are methods and compositions for nucleic acid analysis from single cells and/or nucleic acid from single cell nuclei and organelles. Some methods and compositions can be used for multiplexed single cell gene expression analysis. Some methods and compositions include the use of droplets and/or beads bearing unique barcodes such as unique molecular barcodes (UMI).

[007a] In one aspect, presented herein is a method of preparing a cDNA library from a plurality of single cells, the method comprising the steps of: releasing mRNA from each single cell to provide a plurality of individual mRNA samples, wherein the mRNA in each individual mRNA sample is from a single cell; synthesizing a first strand of cDNA from the mRNA in each individual mRNA sample with a mixture comprising (i) a first strand synthesis primer comprising a tag and incorporating the tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA

sample is complementary to mRNA from a single cell, and wherein the tag comprises a cell-specific identifier sequence and a unique molecular identifier (UMI) sequence and (ii) a template switching oligonucleotide (TSO) primer; pooling the tagged cDNA samples from the plurality of single cells; amplifying the pooled, tagged cDNA to generate a pooled, tagged, double-stranded cDNA; and performing a tagmentation reaction on the pooled, tagged, double-stranded cDNA samples to simultaneously cleave each cDNA and incorporate an adapter into each strand of the cDNA, thereby generating the cDNA library from the plurality of single cells.

[007b] In another aspect, presented herein is a method of preparing a cDNA library from a plurality of single cells comprising: releasing mRNA from each single cell to provide a plurality of individual mRNA samples, wherein the mRNA in each individual mRNA sample is from a single cell; synthesizing a first strand of cDNA from the mRNA in each individual mRNA sample with a mixture comprising (i) a first strand synthesis primer comprising a tag, wherein the first strand synthesis primer is a mixture of oligo dT primer and randomer primer, thereby incorporating the tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to mRNA from a single cell, and wherein the tag comprises a cell-specific identifier sequence, a first-read sequencing adapter sequence, and a different second portion, and (ii) a TSO primer; pooling the tagged cDNA samples; amplifying the pooled, tagged cDNA to generate pooled, tagged, double-stranded cDNA; and performing a tagmentation reaction on the pooled, tagged, double-stranded cDNA by contacting the pooled, tagged cDNA with a plurality of transposome complexes each comprising a transposase complexed with a transposon comprising a second-read sequencing adapter sequence, wherein the second-read sequencing adapter sequence is different from the first-read sequencing adapter sequence, to generate the cDNA library, wherein the cDNA library comprises a plurality of tagged cDNA fragments having the first-read sequencing adapter sequence on a first strand and the second-read sequencing adapter sequence on a second strand.

[007c] In another aspect, presented herein is a method of preparing a cDNA library from a plurality of single cell organelles comprising: spatially separating single cell organelles; releasing mRNA, microRNA, small interfering RNA, ribosomal RNA, and/or mitochondrial RNA from each single cell organelle to provide a plurality of individual RNA samples, wherein the RNA in each individual RNA sample is from a single cell organelle; synthesizing a first strand of cDNA from the RNA in each individual RNA sample with a mixture comprising (i) a first strand synthesis primer comprising a tag, thereby incorporating the tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to RNA from a single cell organelle, and wherein the tag comprises a cell organelle-specific identifier sequence and a first-read sequencing adapter sequence, and (ii) a TSO primer; pooling the tagged cDNA samples from the plurality of single cell organelles; amplifying the pooled, tagged cDNA to generate pooled, tagged, double-stranded cDNA; and performing a tagmentation reaction on the pooled, tagged double-stranded cDNA samples by contacting the pooled, tagged cDNA with a plurality of transposome complexes each comprising a transposase complexed with a transposon comprising a second-read sequencing adapter sequence, wherein the second-read sequencing adapter sequence is different from the first-read sequencing adapter sequence, to generate the cDNA library, wherein the cDNA library comprises a plurality of tagged cDNA fragments having the first-read sequencing adapter sequence on a first strand and the second-read sequencing adapter sequence on a second strand.

[008] In one ~~embodiment~~example, presented herein are methods and compositions of nucleic acid sequence analysis from a single cell. In some ~~embodiment~~examples, the methods and compositions of nucleic acid sequence analysis can be used to prepare sequencing library from nucleic acid from a single organelle. In some ~~embodiment~~examples, the organelle can be a nuclei from a single cell. In some ~~embodiment~~examples, the single organelle is obtained from single cell. Other exemplary organelles include but are not limited to mitochondria and ribosome.

[009] In one ~~embodiment~~example, the methods include releasing nuclei from cells to provide plurality of nuclei, wherein each nucleus is from a single cell. The nuclei are spatially separated from each other such that one nucleus is present at a spatial compartment. A first strand of cDNA is synthesized from the mRNA in each individual mRNA sample with a first strand synthesis primer. In some ~~embodiment~~examples, the first strand synthesis primer is an oligo dT primer further comprising a first amplification primer binding site. In some ~~embodiment~~examples, the first strand synthesis primer is a randomer. In some ~~embodiment~~examples, the first strand synthesis primer is a randomer further comprising a first amplification primer binding site. In some ~~embodiment~~examples, the first strand synthesis primer is a mixture of oligo dT primer and randomer each further comprising a first amplification primer binding site. In some ~~embodiment~~examples, the method further includes incorporating a template switching oligonucleotide primer (TSO primer) along with the mixture of oligo dT primer and randomer, each of oligo dT primer and randomer further comprising a first amplification primer binding site. In some ~~embodiment~~examples, the TSO primer further comprises a second amplification primer binding site. In some ~~embodiment~~examples, the first strand synthesis primer is extended beyond the mRNA template and further copies the TSO primer strand. In some ~~embodiment~~examples, the second strand of cDNA is synthesized using the TSO primer. In some ~~embodiment~~examples, the second strand of cDNA is synthesized using the second amplification primer complimentary to the first strand of cDNA that is extended beyond the mRNA template to encompass the complimentary TSO strand. In some ~~embodiment~~examples, the double stranded cDNA is amplified with first and second amplification primers. In some ~~embodiment~~examples, the first, second or both first and second amplification primers are immobilized on a solid support. Exemplary solid supports include beads, flow cells, microwells.

[0010] In some ~~embodiment~~examples, the double stranded cDNA is subjected to tagmentation reaction such that barcodes can be introduced into the double stranded cDNA. Exemplary methods of tagmentation are disclosed in U.S. patent 9,115,396; 9,080,211; 9,040,256; U.S. patent application publication 2014/0194324. Each of which is

incorporated herein by reference in its entirety. In some ~~embodiment~~examples, the barcodes can be used to determine the contiguity information of the sequence. In some ~~embodiment~~examples, the barcodes can be used as a source identifier.

[0011] In some ~~embodiment~~examples, the method includes incorporating a tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to mRNA from a single cell. In one ~~embodiment~~example, the tag comprises a cell-specific identifier sequence and a unique molecular identifier (UMI) sequence. In some ~~embodiment~~examples, the first strand synthesis primer comprises a tag. In some ~~embodiment~~examples, the TSO primer comprises a tag. In some ~~embodiment~~examples, tagged cDNA from the nuclei of single cells can be pooled and optionally amplified.

[0012] In some ~~embodiment~~examples, the methods include preparing sequencing library from microRNA (miRNA), small interfering RNA (siRNA), ribosomal RNA, or mitochondrial DNA from a single cell. The method includes releasing the cell organelles from a single cell to provide plurality of organelles. The organelles are spatially separated from each other such that one organelle is present at a spatial compartment. A first strand of DNA is synthesized from the microRNA (miRNA), small interfering RNA (siRNA), ribosomal RNA, or mitochondrial DNA with a first strand synthesis primer. In some ~~embodiment~~examples, the first strand synthesis primer is a randomer. In some ~~embodiment~~examples, the first strand synthesis primer is a randomer further comprising a primer binding site. In some ~~embodiment~~examples, the first strand synthesis primer is a mixture of a first strand specific synthesis primer and a randomer. In some ~~embodiment~~examples, the first strand synthesis primer is a mixture of a first strand specific synthesis primer and a randomer, each further comprising a first amplification primer binding site.

[0013] In some ~~embodiment~~examples, the primer that binds to the first primer binding site is an amplification primer. In some ~~embodiment~~examples, the method further includes incorporating a template switching oligonucleotide primer (TSO primer) along with the mixture of a first strand specific synthesis primer and a randomer, each of first strand specific

synthesis primer and a randomer further comprising a first primer binding site. In some ~~embodiment~~examples, the TSO primer further comprises a second primer binding site. In some ~~embodiment~~examples, the primer that binds to the second primer binding site is an amplification primer. In some ~~embodiment~~examples, the first strand synthesis primer is extended beyond the microRNA (miRNA), small interfering RNA (siRNA), ribosomal RNA, or mitochondrial DNA template and further copies the TSO primer strand. In some ~~embodiment~~examples, the second strand of DNA is synthesized using the TSO primer. In some ~~embodiment~~examples, the second strand of DNA is synthesized using the second primer complimentary to the first strand of DNA that is extended beyond the template RNA or DNA to encompass the complimentary TSO strand. In some ~~embodiment~~examples, the double stranded DNA is amplified with first and second primers.

[0014] In one ~~embodiment~~example, the organelles such as nuclei, mitochondria, ribosomes are spatially separated by fluorescence activated cell sorting (FACS) and each organelle is sorted into a spatial compartment, e.g., single microwell on a Fluidigm C1 chip. In some ~~embodiment~~examples, each organelle is spatially separated into a spatial compartment by being immobilized on a solid surface. For example, through an antibody, wherein the antibody specifically binds to the organelle and the antibody is immobilized on a solid surface. In some ~~embodiment~~examples, the solid surface is a flow cell or a bead.

[0015] In some ~~embodiment~~examples, the randomers comprise one or more quasi-random primers that are selected from the group consisting of an AT-rich set of random amplification primers; a set of random amplification primers comprising AT-rich 5' termini; a set of variable-length random amplification primers, wherein each primer comprises a random 3' portion and a degenerate 5' terminus, the degenerate 5' terminus of which can be proportional in length to the A/T content of the random 3' portion of the primer; a set of Tm-normalized random primers, wherein each primer of the set comprises one or more base analogues that can normalize the Tm of each primer to the Tm of other primers in the set of primers; a set of random primers, wherein each primer comprises a random 3' portion and a constant 5' priming portion; a set of random

amplification primers, wherein each primer comprises a random 3' portion and a constant 5' priming portion, and wherein the random 3' portion comprises RNA; a set of random amplification primers, wherein each primer comprises a random 3' portion and a constant 5' priming portion, and wherein the random 3' portion comprises at least one non-natural base selected from the group consisting of nucleic acids including 2'-deoxy-2-thiothymidine (2-thio-dT), 2-aminopurine-2'-deoxyriboside (2-amino-dA), N4-ethyl-2'-deoxycytidine (N4-Et-dC), N4-methyl deoxycytidine (N4-Me-dC), 2'-deoxyinosine, 7-deazaguanine (7-deaza-G), 7-iodo-7-deazaguanine (I-deazaG), 7-methyl-7-deazaguanine, (MecG), 7-ethyl-7-deazaguanine (EtcG) and any combination of the foregoing sets of primers. The quasi-random primers set forth above are described in further detail herein. In some ~~embodiment~~examples described herein, the quasi-random primers are provided in pairs or sets.

[0016] One ~~embodiment~~example presented herein is a method of preparing a cDNA library from a plurality of single cells, the method comprising the steps of: releasing mRNA from each single cell to provide a plurality of individual mRNA samples, wherein the mRNA in each individual mRNA sample is from a single cell; synthesizing a first strand of cDNA from the mRNA in each individual mRNA sample with a first strand synthesis primer and incorporating a tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to mRNA from a single cell. In one ~~embodiment~~example, the tag comprises a cell-specific identifier sequence and a unique molecular identifier (UMI) sequence. In some ~~embodiment~~examples, the tag comprises a cell-specific identifier sequence without the UMI. The method further comprises pooling the tagged cDNA samples; optionally amplifying the pooled cDNA samples to generate a cDNA library comprising double-stranded cDNA; and performing a tagmentation reaction to simultaneously cleave each cDNA and incorporate an adapter into each strand of the cDNA, thereby generating a plurality of tagged cDNA fragments. In some ~~embodiment~~examples, sufficient number of single cells is present, the amplification of cDNA can be avoided. The second strand of cDNA is

synthesized using a template switching oligonucleotide primer (TSO primer), followed by symmetric Nextera.

[0017] In certain ~~embodiment~~examples, the method further comprises amplifying the tagged cDNA fragments to generate amplified tagged cDNA fragments. In some ~~aspects~~example, amplifying comprises adding additional sequence to the 5' end of the amplification products.

[0018] In some ~~aspects~~example, the additional sequence comprises primer binding sequence for amplification on a solid support. In some ~~aspects~~example, the additional sequence comprises additional index sequences.

[0019] In certain ~~embodiment~~examples, the method further comprises amplifying the amplified tagged cDNA fragments on a solid support.

[0020] In certain ~~embodiment~~examples, the method further comprises sequencing the amplification products on the solid support.

[0021] In some ~~aspects~~example, the tagmentation reaction comprises contacting the double-stranded cDNA with a transposase mixture comprising adapter sequences that are not found in the first strand synthesis primer.

[0022] In some ~~aspects~~example, the transposase mixture consists essentially of transposomes having one type of adapter sequence.

[0023] In certain ~~embodiment~~examples, the method further comprises sequencing the tagged cDNA fragments.

[0024] In some ~~aspects~~example, sequencing comprises 3' tag counting.

[0025] In some ~~aspects~~example, sequencing comprises whole transcriptome analysis.

[0026] In some ~~aspects~~example, first strand synthesis is performed using a mixture of random primers, the random primers further comprising a tag.

[0027] In some ~~aspects~~example, the first strand synthesis primer comprises a double-stranded portion. In some ~~embodiment~~examples, the first strand synthesis primer comprising a double-stranded portion further comprises a single stranded loop at one end. In some ~~aspects~~example, the first strand synthesis primer comprises a region capable of forming a hairpin.

[0028] In some ~~aspects~~example, the first strand synthesis primer reduces concatenation byproducts compared to a single-stranded first strand synthesis primer.

[0029] In some ~~aspects~~example, the first strand synthesis primer comprises a region of RNA.

[0030] In some ~~aspects~~example, the first strand synthesis primer is hybridized to a complementary oligonucleotide, thereby forming a double stranded portion.

[0031] Also presented herein is a plurality of beads, wherein each bead comprises a plurality of oligonucleotides, each oligonucleotide comprising: (a) a linker; (b) an amplification primer binding site; (c) optionally a Unique Molecular Identifier which differs for each oligonucleotide; (d) a bead-specific sequence that is the same on each oligonucleotide on the bead but is different on other beads; and (e) a capture sequence for capturing mRNAs and priming reverse transcription.

[0032] In some ~~aspects~~example, the capture sequence comprises oligo-dT.

[0033] In some ~~aspects~~example, each bead is in a separate droplet segregated from other beads.

[0034] The details of one or more ~~embodiment~~examples are set forth in the accompanying drawings and the description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

CLAIMS

1. A method of preparing a cDNA library from a plurality of single cells, the method comprising the steps of:

releasing mRNA from each single cell to provide a plurality of individual mRNA samples, wherein the mRNA in each individual mRNA sample is from a single cell;

synthesizing a first strand of cDNA from the mRNA in each individual mRNA sample with a mixture comprising (i) a first strand synthesis primer comprising a tag and incorporating ~~a~~ the tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to mRNA from a single cell, and wherein the tag comprises a cell-specific identifier sequence and ~~optionally~~ a unique molecular identifier (UMI) sequence and (ii) a template switching oligonucleotide (TSO) primer;

pooling the tagged cDNA samples from the plurality of single cells;

~~optionally~~ amplifying the pooled, tagged cDNA ~~samples~~ to generate a ~~cDNA library comprising~~pooled, tagged, double-stranded cDNA; and

performing a tagmentation reaction on the pooled, tagged, double-stranded cDNA samples to simultaneously cleave each cDNA and incorporate an adapter into each strand of the cDNA, thereby generating the cDNA library from the plurality of single cells~~a plurality of tagged cDNA fragments~~.

~~2. The method of claim 1, further comprising amplifying the tagged cDNA fragments to generate amplified tagged cDNA fragments.~~

~~3. The method of claim 2, wherein amplifying comprises adding additional sequence to the 5' end of the amplification products.~~

~~4. The method of claim 3, wherein the additional sequence comprises primer binding sequence for amplification on a solid support.~~

~~5. The method of claim 4, further comprising amplifying the amplified tagged cDNA fragments on a solid support.~~

~~6. The method of claim 5, further comprising sequencing the amplification products on the solid support.~~

~~7~~2. The method of claim 1, wherein the tagmentation reaction comprises contacting the double-stranded cDNA with a transposase mixture comprising adapter sequences that are not found in the first strand synthesis primer.

3. The method of claim 1 or claim 2, wherein the first strand synthesis primer further comprises an amplification primer binding site.

4. A method of preparing a cDNA library from a plurality of single cells comprising:

releasing mRNA from each single cell to provide a plurality of individual mRNA samples, wherein the mRNA in each individual mRNA sample is from a single cell;

synthesizing a first strand of cDNA from the mRNA in each individual mRNA sample with a mixture comprising (i) a first strand synthesis primer comprising a tag, wherein the first strand synthesis primer is a mixture of oligo dT primer and randomer primer, thereby incorporating the tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to mRNA from a single cell, and wherein the tag comprises a cell-specific identifier sequence, a first-read sequencing adapter sequence, and a different second portion, and (ii) a TSO primer;

pooling the tagged cDNA samples;

amplifying the pooled, tagged cDNA to generate pooled, tagged, double-stranded cDNA; and

performing a tagmentation reaction on the pooled, tagged, double-stranded cDNA by contacting the pooled, tagged cDNA with a plurality of transposome complexes each comprising a transposase complexed with a transposon comprising a

second-read sequencing adapter sequence, wherein the second-read sequencing adapter sequence is different from the first-read sequencing adapter sequence, to generate the cDNA library, wherein the cDNA library comprises a plurality of tagged cDNA fragments having the first-read sequencing adapter sequence on a first strand and the second-read sequencing adapter sequence on a second strand.

5. The method of claim 4, wherein the different section portion comprises a UMI sequence.

6. The method of claim 4 or 5, wherein each oligo dT primer and randomer primer further comprises an amplification primer binding site.

7. A method of preparing a cDNA library from a plurality of single cell organelles comprising:

spatially separating single cell organelles;

releasing mRNA, microRNA, small interfering RNA, ribosomal RNA, and/or mitochondrial RNA from each single cell organelle to provide a plurality of individual RNA samples, wherein the RNA in each individual RNA sample is from a single cell organelle;

synthesizing a first strand of cDNA from the RNA in each individual RNA sample with a mixture comprising (i) a first strand synthesis primer comprising a tag, thereby incorporating the tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to RNA from a single cell organelle, and wherein the tag comprises a cell organelle-specific identifier sequence and a first-read sequencing adapter sequence, and (ii) a TSO primer;

pooling the tagged cDNA samples from the plurality of single cell organelles;

amplifying the pooled, tagged cDNA to generate pooled, tagged, double-stranded cDNA; and

performing a tagmentation reaction on the pooled, tagged double-stranded cDNA samples by contacting the pooled, tagged cDNA with a plurality of transposome complexes each comprising a transposase complexed with a transposon comprising a

second-read sequencing adapter sequence, wherein the second-read sequencing adapter sequence is different from the first-read sequencing adapter sequence, to generate the cDNA library, wherein the cDNA library comprises a plurality of tagged cDNA fragments having the first-read sequencing adapter sequence on a first strand and the second-read sequencing adapter sequence on a second strand.

8. The method of claim 7, wherein the tag further comprises a unique molecular identifier (UMI) sequence.

9. The method of claim 7 or 8, wherein first strand synthesis primer further comprises an amplification primer binding site.

10. The method of any one of claims 1-9, wherein the first strand synthesis primer comprises a first amplification primer binding site and the TSO primer comprises a second amplification primer binding site.

11. The method of any one of claims 1-6 or 10, wherein the first strand synthesis primer is extended beyond the mRNA and further copies the TSO primer strand to prepare a complementary TSO strand.

12. The method of any one of claims 7-10, wherein the first strand synthesis primer is extended beyond the microRNA, small interfering RNA, ribosomal RNA, and/or mitochondrial RNA and further copies the TSO primer strand to prepare a complementary TSO strand.

13. The method of claim 11 or claim 12, wherein the second strand of cDNA is synthesized using a second amplification primer complementary to the first strand of cDNA that is extended beyond the mRNA template to encompass the complementary TSO strand and prepare the pooled, tagged, double-stranded cDNA.

14. The method of claim 13, wherein the pooled, tagged, double-stranded cDNA is amplified with a first amplification primer that binds to the first amplification primer binding site and a second amplification primer that binds to the second amplification primer binding site.

15. The method of claim 14, wherein the first amplification primer, the second amplification primer, or both the first and the second amplification primer are immobilized on a solid support.

16. The method of claim 15, wherein the solid support is a bead, flow cell, or microwell.

17. The method of claim 15 or 16, further comprising sequencing the amplification products on the solid support.

18. The method of any one of claims 1-17, wherein the tagmentation reaction comprises contacting the double-stranded cDNA with a transposase mixture comprising a Tn5 transposase.

19. The method of claim 18, wherein the second-read sequencing adapter sequence is identical in all the transposons.

~~8. The method of claim 7, wherein the transposase mixture consists essentially of transposomes having one type of adapter sequence.~~

~~9. The method of claim 1, further comprising sequencing the tagged cDNA fragments.~~

~~10. The method of claim 9, wherein sequencing comprises 3' tag counting.~~

~~11. The method of claim 9, wherein sequencing comprises whole transcriptome analysis.~~

~~12. The method of claim 1, first strand synthesis is performed using a mixture of random primers, the random primers further comprising a tag.~~

~~13~~20. The method of any one of claims 1 to 1~~92~~, wherein the first strand synthesis primer comprises a primer comprises a double-stranded portion.

21. The method of claim 20, wherein:

(a) the first strand synthesis primer reduces concatenation byproducts compared to a single-stranded first strand synthesis primer;

(b) the first strand synthesis primer comprises a primer comprising a region capable of forming a hairpin;

(c) the first strand synthesis primer comprises a primer comprising a region of RNA; and/or

(d) the first strand synthesis primer comprises a primer hybridized to a complementary oligonucleotide, thereby forming a double stranded portion.

~~14. The method of claim 13, wherein the first strand synthesis primer reduces concatenation byproducts compared to a single-stranded first strand synthesis primer.~~

~~15. The method of claim 13, wherein the first strand synthesis primer comprises a region capable of forming a hairpin.~~

~~16. The method of claim 13, wherein the first strand synthesis primer comprises a region of RNA.~~

~~17. The method of claim 13, wherein the first strand synthesis primer is hybridized to a complementary oligonucleotide, thereby forming a double stranded portion.~~

~~18. A plurality of beads, wherein each bead comprises a plurality of oligonucleotides, each oligonucleotide comprising:~~

~~(a) a linker;~~

~~(b) an amplification primer binding site;~~

~~(c) optionally a Unique Molecular Identifier which differs for each oligonucleotide;~~

~~(d) a bead-specific sequence that is the same on each oligonucleotide on the bead but is different on other beads; and~~

~~(e) a capture sequence for capturing mRNAs and priming reverse transcription.~~

~~19. The plurality of beads of claim 18, wherein the capture sequence comprises oligo-dT.~~

~~20. The plurality of beads of claim 18, wherein each bead is in a separate droplet segregated from other beads.~~