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 cell organelles, the method comprising the steps of: 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, wherein the first strand synthesis primer is randomer,

an oligo dT primer, or a mixture of oligo dT primer and randomer primer, 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 RNA from a single cell organelle, and wherein the tag comprises a single cell organelle-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 cell organelles; 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 cell organelles.

[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 and a first transposase adapter sequence, and a UMI sequence, 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-transposase adapter sequence, wherein the second transposase adapter sequence is different from the first transposase adapter sequence, to generate the cDNA library, wherein the cDNA library comprises a plurality of tagged cDNA fragments having the

first transposase adapter sequence on a first strand and the second transposase 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, wherein the first strand synthesis primer is a randomer, an oligo dT primer, or 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 RNA from a single cell organelle, and wherein the tag comprises a single cell organelle-specific identifier sequence and a first transposase 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 transposase adapter sequence, wherein the second transposase adapter sequence is different from the first transposase adapter sequence, to generate the cDNA library, wherein the cDNA library comprises a plurality of tagged cDNA fragments having the first transposase adapter sequence on a first strand and the second transposase adapter sequence on a second strand.

[008] In one example, presented herein are methods and compositions of nucleic acid sequence analysis from a single cell. In some 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 examples, the organelle can be a nuclei

from a single cell. In some examples, the single organelle is obtained from single cell. Other exemplary organelles include but are not limited to mitochondria and ribosome.

CLAIMS

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

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, wherein the first strand synthesis primer is randomer, an oligo dT primer, or a mixture of oligo dT primer and randomer primer, 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 RNA from a single cell organelle, and wherein the tag comprises a single cell organelle-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 cell organelles;

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 cell organelles.

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

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

6. 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, wherein the first strand synthesis primer is a randomer, an oligo dT primer, or 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 RNA from a single cell organelle, and wherein the tag comprises a single cell organelle-specific identifier sequence and a first transposase 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 transposase adapter sequence, wherein the second transposase adapter sequence is different from the first transposase adapter sequence, to generate the cDNA library, wherein the cDNA library comprises a plurality of tagged cDNA fragments having the first transposase adapter sequence on a first strand and the second transposase adapter sequence on a second strand.

7. The method of claim 6, wherein the tag further comprises a UMI sequence.

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

9. The method of any one of claims 1-8, wherein the first strand synthesis primer comprises a first amplification primer binding site and the TSO primer comprises a second amplification primer binding site.

10. The method of any one of claims 4 or 5, wherein the first strand synthesis primer is extended beyond the mRNA and further copies the TSO primer strand to prepare a complementary TSO strand.

11. The method of any one of claims 1-3 or 6-9, 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.

12. The method of claim 10 or claim 11, 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 RNA or mRNA template to encompass the complementary TSO strand and prepare the pooled, tagged, double-stranded cDNA.

13. The method of claim 12, 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.

14. The method of claim 13, wherein the first amplification primer, the second amplification primer, or both the first and the second amplification primer are immobilized on a solid support.

15. The method of claim 14, wherein the solid support is a bead, flow cell, or microwell.

16. The method of claim 14 or 15, further comprising sequencing the amplification products on the solid support.

17. The method of any one of claims 1-16, wherein the tagmentation reaction comprises contacting the double-stranded cDNA with a transposase mixture comprising a Tn5 transposase.

18. The method of claim 17, wherein the second transposase adapter sequence is identical in all the transposons.

19. The method of any one of claims 1 to 18, wherein the first strand synthesis primer comprises a primer comprising a double-stranded portion.

20. The method of claim 19, 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.