

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).

[008] In one aspect, the present disclosure refers to 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 first strand synthesis primer mixture, wherein the first strand synthesis primer mixture comprises a mixture of an oligo dT primer and a randomer primer, wherein the oligo dT primer and the randomer primer each comprise a tag to incorporate 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 each tag of each tagged cDNA sample has a same first portion comprising a cell-specific identifier sequence, a same first-read sequencing adapter, and a different second portion; pooling the tagged cDNA samples from a plurality of single cells; amplifying the pooled, tagged cDNA samples to generate pooled, tagged, double-stranded cDNA; and performing a tagmentation reaction on the pooled, tagged, double-stranded cDNA by contacting the pooled, tagged, double-stranded cDNA with a plurality of transposome complexes each comprising a transposase complexed with a transposon comprising a transposon end sequence and a second-read sequencing adapter sequence, wherein the second-read sequencing adapter sequence is different from the first-read sequencing adapter sequence and is identical in all the transposons, 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. In yet another aspect, the present disclosure refers to a method of preparing a cDNA sequencing library from a plurality of single cell organelles comprising: spatially separating single cell organelles, optionally wherein the organelles are selected from nuclei, mitochondria, and ribosomes; 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 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-specific identifier sequence and optionally a unique molecular identifier (UMI) sequence, optionally wherein the first strand synthesis primer is a mixture of oligo dT primer and randomer primers; pooling the tagged cDNA samples from the plurality of single cell organelles; amplifying the pooled cDNA samples 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 to simultaneously cleave each cDNA and incorporate an adapter into each strand of the cDNA, thereby generating a cDNA library from the plurality of single cell organelles. Also described 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.

[009] In one 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 examples, the first strand synthesis primer is an oligo dT primer further comprising a first amplification primer binding site. In some examples, the first strand synthesis primer is a randomer. In some examples, the first strand synthesis primer is a randomer further comprising a first amplification primer binding site. In some 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 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 examples, the TSO primer further comprises a second amplification primer binding site. In some examples, the first strand synthesis primer is extended beyond the mRNA template and further copies the TSO primer strand. In some examples, the second strand of cDNA is synthesized using the TSO primer. In some examples, the second strand of cDNA is synthesized using the second

CLAIMS

1. 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 first strand synthesis primer mixture, wherein the first strand synthesis primer mixture comprises a mixture of an oligo dT primer and a randomer primer, wherein the oligo dT primer and the randomer primer each comprise a tag to incorporate 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 each tag of each tagged cDNA sample has a same first portion comprising a cell-specific identifier sequence, a same first-read sequencing adapter sequence, and a different second portion;

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

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

performing a tagmentation reaction on the pooled, tagged, double-stranded cDNA by contacting the pooled, tagged, double-stranded cDNA with a plurality of transposome complexes each comprising a transposase complexed with a transposon comprising a transposon end sequence and a second-read sequencing adapter sequence, wherein the second-read sequencing adapter sequence is different from the first-read sequencing adapter sequence and is identical in all the transposons, 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.

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

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

4. The method of claim 3, wherein the additional sequence comprises a primer binding sequence for hybridization to a complementary primer binding sequence on a solid support and amplification of the tagged cDNA fragments on the solid support.

5. The method of claim 4, further comprising hybridizing the amplified, tagged cDNA fragments to the complementary primer binding sequence on the solid support, and amplifying the amplified tagged cDNA fragments on the solid support.

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

7. The method of claim 1, further comprising sequencing the tagged cDNA fragments of the cDNA library.

8. The method of claim 7, wherein sequencing comprises 3' tag counting.

9. The method of claim 7, wherein sequencing comprises whole transcriptome analysis.

10. The method of claim 1, wherein the first strand synthesis primer mixture comprises a primer comprising a double-stranded portion.

11. The method of claim 10, wherein the first strand synthesis primer mixture reduces concatenation byproducts compared to a single-stranded first strand synthesis primer.

12. The method of claim 10, wherein the first strand synthesis primer mixture comprises a primer comprising a region capable of forming a hairpin or the first strand synthesis primer mixture comprises a primer comprising a region of RNA.

13. The method of claim 10, wherein the first strand synthesis primer mixture comprises a first strand synthesis primer hybridized to a complementary oligonucleotide, thereby forming a double stranded portion.

14. The method of claim 1, wherein the tag comprises a unique molecular identifier (UMI) sequence.

15. The method of claim 1, wherein the first synthesis primer mixture is attached to a bead and the synthesizing comprises synthesizing tagged cDNA samples on the bead, optionally wherein pooling the tagged cDNA samples comprises pooling the beads with the attached tagged cDNA samples.

16. The method of claim 15, comprising encapsulating each single cell in a droplet with the bead before releasing the mRNA from each single cell, optionally wherein the releasing and synthesizing of each tagged cDNA sample is performed in the droplet.

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

spatially separating single cell organelles, optionally wherein the organelles are selected from nuclei, mitochondria, and ribosomes;

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 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-specific identifier sequence and optionally a unique molecular identifier (UMI) sequence, optionally wherein the first strand synthesis primer is a mixture of oligo dT primer and randomer primers;

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

amplifying the pooled cDNA samples 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 to simultaneously cleave each cDNA and incorporate an adapter into each strand of the cDNA, thereby generating a cDNA library from the plurality of single cell organelles.

18. The method of claim 17, wherein the first strand synthesis primer is a mixture of oligo dT primer and randomer primers.

19. The method of claim 17 or claim 18, wherein the tag further a first-read sequencing adapter sequence.

20. The method of claim 19, comprising:
amplifying the pooled cDNA samples to generate pooled, tagged, double-stranded cDNA;
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; and
optionally amplifying the tagged cDNA fragments of the cDNA library to generate amplified, tagged cDNA fragments.