

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; 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 comprising a first-read sequencing adapter sequence, 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; 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 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. 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.

16. The method of claim 15, wherein pooling the tagged cDNA samples comprises pooling the beads with the attached tagged cDNA samples.

17. The method of claim 15 or claim 16, comprising encapsulating each single cell in a droplet with the bead before releasing the mRNA from each single cell.

18. The method of claim 17, wherein the releasing and synthesizing of each tagged cDNA sample is performed in the droplet.

19. A method of preparing a cDNA sequencing 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 first strand synthesis primer comprising a tag comprising a first-read sequencing adapter sequence, 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;

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 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.

20. The method of claim 19, wherein the first strand synthesis primer is a mixture of oligo dT primer and randomer primers.

21. The method of claim 19 or claim 20, wherein the tag further comprises a unique molecular identifier (UMI) sequence.

22. The method of any one of claims 19-21, comprising amplifying the tagged cDNA fragments of the cDNA library to generate amplified, tagged cDNA fragments.

23. The method of any one of claims 19-22, wherein the organelles are selected from nuclei, mitochondria, and ribosomes