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.

In one aspect, the present invention provides 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, and wherein the tag comprises a cell-specific identifier sequence and a unique molecular identifier (UMI) sequence; pooling the tagged cDNA samples form the plurality of single cells; optionally amplifying the pooled cDNA samples to generate a cDNA library comprising double-stranded cDNA; and performing a tagmentation reaction on the pooled tagged cDNA 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.

In certain examples, the method further comprises amplifying the tagged cDNA fragments to generate amplified tagged cDNA fragments. In some examples, amplifying comprises adding additional sequence to the 5' end of the amplification products.

In some examples, the additional sequence comprises primer binding sequence for amplification on a solid support. In some examples, the additional sequence comprises additional index sequences.

In certain examples, the method further comprises amplifying the amplified tagged cDNA fragments on a solid support.

In certain examples, the method further comprises sequencing the amplification products on the solid support.

What is claimed is:

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 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, and wherein the tag comprises a cell-specific identifier sequence and a unique molecular identifier (UMI) sequence;

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

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

performing a tagmentation reaction on the pooled tagged 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.

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

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 on a solid support.

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

7. 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.

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.

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. The method of claim 12, wherein the first strand synthesis primer comprises 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.