cleavage sequence is a thrombin cleavage sequence. In some instances, said amino acid sequence comprises a MRSSHHHHHHSSGLVPRGS (SEQ ID NO: 7) amino acid sequence at said N-terminus. In some instances, said engineered reverse transcription enzyme comprises an amino acid sequence according to SEQ ID NO: 6. In some instances, said engineered reverse transcription enzyme comprises an amino acid sequence according to SEQ ID NO: 5. In some instances, said engineered reverse transcription enzyme has improved ability to capture full-length transcripts as compared to a reverse transcriptase enzyme consisting of SEQ ID NO: 3. In some instances, said engineered reverse transcription enzyme has higher resistance to cell lysate as compared to a reverse transcriptase enzyme consisting of SEQ ID NO: 3. In some instances, said engineered reverse transcription enzyme has higher activity in a reaction volume of less than 1 nanoliter as compared to a reverse transcriptase enzyme consisting of SEQ ID NO: 3. In some instances, said engineered reverse transcription enzyme has increased thermal stability and reverse transcription processivity as compared to a reverse transcriptase enzyme consisting of SEQ ID NO: 3. In some instances, said engineered reverse transcription enzyme comprises terminal transferase activity and template switching ability.

[0005a] In one aspect, provided herein is an engineered reverse transcription enzyme, comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 3, wherein said amino acid sequence comprises: (i) a truncation of amino acids to 40 amino acids from the N-terminus relative to SEQ ID NO: 3; and (ii) wherein said amino acid sequence comprises a plurality of mutations selected from the group consisting of an E69 mutation, an Ll39 mutation, a D200 mutation, an E302 mutation, a T306 mutation, a W313 mutation, a T330 mutation, an L435 mutation, a P448 mutation, a D449 mutation, an N454 mutation, a D524 mutation, an L603 mutation, and an E607 mutation relative to SEQ ID NO: 3.

[0006] Disclosed herein, in some embodiments, are methods for nucleic acid sample processing, comprising: (a) providing a template ribonucleic acid (RNA) molecule in a reaction volume, and (b) using an engineered reverse transcription enzyme to reverse transcribe said RNA molecule to a complementary DNA molecule, wherein said engineered reverse transcription enzyme comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 3, wherein said amino acid sequence comprises: (i) a truncation of at least 15 amino acids from the N-terminus relative to SEQ ID NO: 3; and (ii) one or more mutations selected from the group consisting of an E69 mutation, an L139 mutation, a D200 mutation, an E302 mutation, a T306 mutation, a W313 mutation, a T330 mutation, an L435 mutation, a P448 mutation, a D449 mutation, an N454 mutation, a D524 mutation, an L603 mutation, and an E607 mutation relative

to SEQ ID NO: 3. In some instances, said reaction volume is less than 1 nanoliter. In some instances, said reaction volume is less than 500 picoliters. In some instances, said reaction volume is a droplet in an emulsion. In some instances, said reaction volume is a well. In some instances, said reaction volume further comprises a plurality of nucleic acid barcode molecules comprising a barcode sequence. In some instances, said RNA molecule is a messenger RNA (mRNA) molecule, wherein said plurality of nucleic acid barcode molecules further comprise an oligo(dT) sequence, and wherein said engineered reverse transcription enzyme reverse transcribes said mRNA molecule into said complementary DNA molecule using said oligo(dT) sequence, wherein said complementary DNA molecule comprises said barcode sequence. In some instances, said RNA molecule is a messenger RNA (mRNA) molecule, wherein said reaction volume further comprises a nucleic acid molecule comprising an oligo(dT) sequence, wherein said plurality of nucleic acid barcode molecules further comprise a template switching sequence, wherein said engineered reverse transcription enzyme reverse transcribes said mRNA molecule using said nucleic acid molecule comprising said oligo(dT) sequence, and wherein said engineered reverse transcription enzyme performs a template switching reaction, thereby generating said complementary DNA molecule, wherein said complementary DNA molecule comprises said barcode sequence. In some instances, said plurality of nucleic acid barcode molecules are attached to a support. In some instances, said nucleic acid barcode molecules are releasably attached to said support. In some instances, said support is a bead. In some instances, said bead is a gel bead. In some instances, said nucleic acid barcode molecules are covalently attached to said bead. In some instances, said nucleic acid barcode molecules are releasably attached to said bead. In some instances, said nucleic acid barcode molecules are released upon application of a stimulus. In some instances, said stimulus is a chemical stimulus. In some instances, said chemical stimulus is a reducing agent. In some instances, said gel bead is a degradable gel bead. In some instances, said degradable gel bead comprises chemically cleavable cross-linking. In some instances, said chemically cleavable cross-linking comprises disulfide cross-linking. In some instances, said reaction volume comprises a cell comprising said RNA molecule. In some instances, the method further comprises releasing said RNA molecule from said cell.

[0007] Disclosed herein, in some embodiments, are kits for performing a reverse transcription reaction, comprising: (a) an engineered reverse transcription enzyme comprising (i) a truncation of at least 15 amino acids from the N-terminus relative to SEQ ID NO: 3; and (ii) one or more mutations selected from the group consisting of an E69 mutation, an L139 mutation, a D200 mutation, an E302 mutation, a T306 mutation, a W313 mutation, a T330 mutation, an

L435 mutation, a P448 mutation, a D449 mutation, an N454 mutation, a D524 mutation, an L603 mutation, and an E607 mutation relative to SEQ ID NO: 3; and (b) instructions for using said engineered reverse transcription enzyme to perform a reverse transcription reaction. In some instances, said kit further comprises a reaction buffer for performing said reverse transcription reaction. In some instances, said kit further comprises dNTPs. In some instances, said engineered reverse transcription enzyme, said buffer, and said dNTPs are provided together in a master mix solution. In some instances, said master mix is present at a concentration at least two times the working concentration indicated in said instructions for use in said reverse transcription reaction. In some instances, said kit further comprises a primer for priming said reverse transcription reaction. In some instances, said primer is a poly-dT primer, a random N-mer primer, or a target-specific primer.

[0007a] In one aspect, provided herein is a kit for performing a reverse transcription reaction, comprising: an engineered reverse transcription enzyme comprising (i) a truncation of 15 amino acids to 40 amino acids from the N-terminus relative to SEQ ID NO: 3; and (ii) a plurality of mutations selected from the group consisting of an E69 mutation, an Ll39 mutation, a D200 mutation, an E302 mutation, a T306 mutation, a W313 mutation, a T330 mutation, an L435 mutation, a P448 mutation, a D449 mutation, an N454 mutation, a D524 mutation, an L603 mutation, and an E607 mutation relative to SEQ ID NO: 3; and instructions for using said engineered reverse transcription enzyme to perform a reverse transcription reaction.

INCORPORATION BY REFERENCE

[0008] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also “Figure” and “FIG.” herein), of which:

[0010] **FIG. 1** shows an example of a microfluidic channel structure for partitioning individual or small groups of cells.

[0011] **FIG. 2** shows an example of a microfluidic channel structure for co-partitioning cells and beads or microcapsules comprising additional reagents.

[0012] **FIG. 3** schematically illustrates an example process for amplification and barcoding of cell's nucleic acids.

[0013] **FIG. 4** provides a schematic illustration of use of barcoding of cell's nucleic acids in attributing sequence data to individual cells or groups of cells for use in their characterization.

[0014] **FIG. 5** provides a schematic illustrating cells associated with labeled cell-binding ligands.

[0015] **FIG. 6** provides a schematic illustration of an example workflow for performing RNA analysis using the methods described herein.

[0016] **FIG. 7** provides a schematic illustration of an example barcoded oligonucleotide structure for use in analysis of ribonucleic (RNA) using the methods described herein.

[0017] **FIG. 8** provides an image of individual cells co-partitioned along with individual barcode bearing beads

[0018] **FIG. 9A-FIG. 9E** provides schematic illustration of example barcoded oligonucleotide structures for use in analysis of RNA and example operations for performing RNA analysis.

CLAIMS

WHAT IS CLAIMED IS:

1. An engineered reverse transcription enzyme, comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 3, wherein said amino acid sequence comprises:

(i) a truncation of 15 amino acids to 40 amino acids from the N-terminus relative to SEQ ID NO: 3; and

(ii) wherein said amino acid sequence comprises a plurality of mutations selected from the group consisting of an E69 mutation, an Ll39 mutation, a D200 mutation, an E302 mutation, a T306 mutation, a W313 mutation, a T330 mutation, an L435 mutation, a P448 mutation, a D449 mutation, an N454 mutation, a D524 mutation, an L603 mutation, and an E607 mutation relative to SEQ ID NO: 3.

2. The engineered reverse transcription enzyme of claim 1, wherein said engineered reverse transcription enzyme comprises: (i) three or more mutations selected from the group consisting of an Ll39 mutation, a D200 mutation, a T330 mutation, a P448 mutation, a D449 mutation, a D524 mutation, and a L603 mutation relative to SEQ ID NO: 3; and (ii) three or more mutations selected from the group consisting of an E69 mutation, an E302 mutation, a T306 mutation, a W313 mutation, an L435 mutation, and an N454 mutation relative to SEQ ID NO: 3.

3. The engineered reverse transcription enzyme of claim 2, wherein said engineered reverse transcription enzyme comprises: (i) three or more mutations selected from the group consisting of an L139P mutation, a D200N mutation, a T330P mutation, a P448A mutation, a D449G mutation, a D524N or D524A mutation, and a L603W mutation relative to SEQ ID NO: 3; and

(ii) three or more mutations selected from the group consisting of an E69K mutation, an E302R mutation, a T306K mutation, a W313F mutation, an L435G or L435K mutation, and an N454K mutation relative to SEQ ID NO: 3.

4. The engineered reverse transcription enzyme of claim 1, wherein said engineered reverse transcription enzyme comprises: an E69 mutation, an Ll39 mutation, a D200 mutation, an E302 mutation, a T306 mutation, a W313 mutation, a T330 mutation, an L435 mutation, a P448 mutation, a D449 mutation, an N454 mutation, a D524 mutation, an L603 mutation, and an E607 mutation relative to SEQ ID NO: 3.

5. The engineered reverse transcription enzyme of claim 4, wherein said engineered reverse transcription enzyme comprises: an E69K mutation, an Ll39P mutation, a D200N mutation, an E302R mutation, a T306K mutation, a W313F mutation, a T330P mutation, an L435G or

L435K mutation, a P448A mutation, a D449G mutation, an N454K mutation, a D524N or D524A mutation, an L603W mutation, and an E607K mutation relative to SEQ ID NO: 3.

6. The engineered reverse transcription enzyme of claim **1,** wherein said truncation comprises a truncation of at least 20 amino acids from said N-terminus relative to SEQ ID NO: 3.

7. The engineered reverse transcription enzyme of claim **1,** wherein said truncation comprises a truncation of 23 amino acids from said N-terminus relative to SEQ ID NO: 3.

8. The engineered reverse transcription enzyme of claim 1, wherein said engineered reverse transcription enzyme further comprises an affinity tag at said N-terminus or at a C-terminus of said amino acid sequence.

9. The engineered reverse transcription enzyme of claim 1, wherein said amino acid sequence comprises a MRSSHHHHHHSSGLVPRGS (SEQ ID NO: 7) amino acid sequence at said N-terminus.

10. The engineered reverse transcription enzyme of claim **1,** wherein said engineered reverse transcription enzyme comprises an amino acid sequence according to SEQ ID NO: 6.

11. The engineered reverse transcription enzyme of claim 1, wherein said engineered reverse transcription enzyme comprises an amino acid sequence according to SEQ ID NO: 5.

12. The engineered reverse transcription enzyme of claim **1,** wherein said engineered reverse transcription enzyme has improved ability to capture full-length transcripts as compared to a reverse transcriptase enzyme consisting of SEQ ID NO: 3.

13. The engineered reverse transcription enzyme of claim **1,** wherein said engineered reverse transcription enzyme has higher resistance to cell lysate as compared to a reverse transcriptase enzyme consisting of SEQ ID NO: 3.

14. The engineered reverse transcription enzyme of claim **1,** wherein said engineered reverse transcription enzyme has higher activity in a reaction volume of less than **1** nanoliter as compared to a reverse transcriptase enzyme consisting of SEQ ID NO: 3.

15. The engineered reverse transcription enzyme of claim **1,** wherein said engineered reverse transcription enzyme has increased thermal stability and reverse transcription processivity as compared to a reverse transcriptase enzyme consisting of SEQ ID NO: 3.

16. The engineered reverse transcription enzyme of claim 11, wherein said engineered reverse transcription enzyme comprises terminal transferase activity and template switching ability.

17. A kit for performing a reverse transcription reaction, comprising:

a. an engineered reverse transcription enzyme comprising (i) a truncation of 15

amino acids to 40 amino acids from the N-terminus relative to SEQ ID NO: 3; and (ii) a plurality of mutations selected from the group consisting of an E69 mutation, an Ll39 mutation, a D200 mutation, an E302 mutation, a T306 mutation, a W313 mutation, a T330 mutation, an L435 mutation, a P448 mutation, a D449 mutation, an N454 mutation, a D524 mutation, an L603 mutation, and an E607 mutation relative to SEQ ID NO: 3; and

b. instructions for using said engineered reverse transcription enzyme to perform a reverse transcription reaction.

18. The engineered reverse transcription enzyme of claim **1,** wherein said amino acid sequence is at least 90% identical to SEQ ID NO:3.