

In some examples, the first strand synthesis primer reduces concatenation byproducts compared to a single-stranded first strand synthesis primer.

In some examples, the first strand synthesis primer comprises a region of RNA.

In some examples, the first strand synthesis primer is hybridized to a complementary oligonucleotide, thereby forming a double stranded portion.

Also presented herein is a plurality of beads, wherein each bead comprises a plurality of oligonucleotides, each oligonucleotide comprising: (a) a linker; (b) an amplification primer binding site; (c) optionally a Unique Molecular Identifier which differs for each oligonucleotide; (d) a bead-specific sequence that is the same on each oligonucleotide on the bead but is different on other beads; and (e) a capture sequence for capturing mRNAs and priming reverse transcription.

In some examples, the capture sequence comprises oligo-dT.

In some examples, each bead is in a separate droplet segregated from other beads.

In one aspect, the present disclosure provides a plurality of beads, wherein each bead comprises a plurality of oligonucleotides, each oligonucleotide comprising: (a) a linker; (b) an amplification primer binding site comprising a template switching primer sequence; (c) a bead-specific sequence that is the same on each oligonucleotide on the bead but is different on other beads; and (d) a capture sequence for capturing mRNAs and priming reverse transcription.

The details of one or more examples are set forth in the accompanying drawings and the description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is an exemplary schematic showing a schematic of cDNA synthesis using SMARTer methodology.

Fig. 2A is an exemplary schematic showing first strand synthesis according to one embodiment, primed with oligo dT that has been appended with an optional molecular barcode (UMI), a sample barcode (BC), an amplification primer binding

What is claimed is:

1. A plurality of beads, wherein each bead comprises a plurality of oligonucleotides, each oligonucleotide comprising:
 - (a) a linker;
 - (b) an amplification primer binding site comprising a template switching primer sequence;
 - (c) a bead-specific sequence that is the same on each oligonucleotide on the bead but is different on other beads; and
 - (d) a capture sequence for capturing mRNAs and priming reverse transcription.
2. The plurality of beads of claim 1, wherein each oligonucleotide comprises a Unique Molecular Identifier (UMI) which differs for each oligonucleotide.
3. The plurality of beads of claim 1 or claim 2, wherein the capture sequence comprises an oligo-dT sequence.
4. The plurality of beads of claim 3, wherein each bead comprises a plurality of oligonucleotides, each oligonucleotide comprising:
 - (a) a linker;
 - (b) an amplification primer binding site comprising a template switching primer sequence;
 - (c) a tag comprising a UMI which differs for each oligonucleotide and a bead-specific sequence that is the same on each oligonucleotide on the bead but is different on other beads; and
 - (d) an oligo-dT sequence as a capture sequence for capturing mRNAs and priming reverse transcription.
5. The plurality of beads of any one of claims 1 to 4, wherein the beads are magnetically responsive.
6. The plurality of beads of any one of claims 1 to 5, wherein the beads are porous or comprise a resin or polymer material; optionally wherein the resin or polymer material is methylstyrene, polystyrene, an acrylic polymer, or silica.

7. The plurality of beads of any one of claims 1 to 6, wherein the beads comprise a material selected from plastic, ceramic, glass, a paramagnetic material, thoria sol, carbon graphite, titanium dioxide, latex, cross-linked dextrans, cellulose, nylon, cross-linked micelle, and Teflon.

8. The plurality of beads of any one of claims 1 to 7, wherein the beads comprise polymer material, coatings, or moieties which permit attachment of an assay reagent.

9. The plurality of beads of any one of claims 1 to 8, wherein the beads are microspheres or nanoparticles.

10. The plurality of beads of any one of claims 1 to 9, wherein the amplification primer binding site further comprises a transposome adapter sequence.

11. The plurality of beads of any one of claims 1 to 10, wherein the plurality of beads are segregated in droplets; optionally wherein each bead of the plurality of beads is in a separate droplet segregated from other beads; further optionally wherein the droplets are segregated in an emulsion.

12. The plurality of beads of claim 11, wherein the droplets are formed using a droplet actuator.

13. The plurality of beads of claim 12, wherein the beads are magnetically responsive and the droplet actuator comprises a magnet; optionally wherein the magnetically responsive beads are capable of being restrained in position in a droplet or in a filler fluid on a droplet actuator.