

Claims

1. A method for isolating RNA from a sample comprising,
 - (a) contacting total RNA isolated from the sample with polynucleotide kinase (PNK) to modify 3' phosphate to a hydroxyl group to generate end repaired total RNA;
 - (b) contacting the end repaired total RNA with polyadenylate polymerase (PAP) and adenosine nucleotides to generate polyadenylated total RNA;
 - (c) capturing the polyadenylated total RNA on a substrate comprising one or more oligonucleotides comprising poly T sequences; and
 - (d) eluting the polyadenylated total RNA from the substrate.
2. The method of claim 1, further comprising quantifying the total RNA.
3. A method for preparing an RNA library from a tissue sample comprising,
 - (a) contacting total RNA isolated from the sample with polynucleotide kinase (PNK) to modify 3' phosphate to a hydroxyl group to generate end repaired total RNA;
 - (b) contacting the end repaired total RNA with polyadenylate polymerase (PAP) and adenosine nucleotides to generate polyadenylated total RNA;
 - (c) releasing the polyadenylated total RNA from the tissue sample;
 - (d) capturing the polyadenylated total RNA on a substrate comprising one or more oligonucleotides comprising a poly T sequence; and
 - (e) generating an RNA library from the polyadenylated total RNA using a RNA library prep kit.
4. The method of any one of claims 1 to 3, wherein the RNA comprises rRNA and/or mRNA.
5. A method for preparing an mRNA transcriptome library from a tissue sample comprising,
 - (a) contacting total RNA isolated from the sample with polynucleotide kinase (PNK) to modify 3' phosphate to a hydroxyl group to generate end repaired total RNA;
 - (b) contacting the end repaired total RNA with polyadenylate polymerase (PAP) and adenosine nucleotides to generate polyadenylated total RNA;
 - (c) releasing the polyadenylated total RNA from the tissue sample;
 - (d) capturing the polyadenylated total RNA on a substrate comprising one or more oligonucleotides comprising a poly T sequence;
 - (e) depleting ribosomal RNA from the total RNA to leave polyadenylated mRNA; and

(f) generating an mRNA library from the polyadenylated mRNA using a mRNA library prep kit.

6. The method of any one of the preceding claims, wherein the substrate is a bead, a bead array, a spotted array, a flow cell, clustered particles arranged on a surface of a chip, a film, and a plate.

7. The method of any one of the preceding claims, wherein the sample is a fresh frozen tissue sample or a formalin-fixed paraffin embedded (FFPE) sample.

8. The method of any one of claims 3 to 7, wherein releasing comprises contacting the sample with a lysis buffer, a permeabilization buffer and/or a reagent to deparaffinize a FFPE sample.

9. The method of any one of claims 3 to 8, wherein when the sample is a FFPE sample on a slide, the method comprises permeabilization and collagenase treatment of the sample on the slide prior to contacting the RNA with PNK.

10. The method of any one of claims 3 to 9, wherein generating the RNA library comprises the steps of eluting the polyadenylated total RNA from the substrate and generating the RNA library from the eluted polyadenylated RNA library using a RNA library prep kit.

11. The method of any one of claims 3 to 10, wherein generating the RNA library comprises,

i) contacting the isolated RNA with a reverse transcriptase (RT) to generate a first cDNA strand complementary to the RNA;

ii) contacting the first cDNA strand with a reverse transcriptase (RT) or DNA polymerase to generate a second cDNA strand complementary to the first cDNA strand;

iii) amplifying the second strand cDNA to form a PCR template and isolating the PCR template; and

iv) generating an RNA library from the PCR templates.

12. A method for improving capture efficiency of mRNA transcripts for *in situ* mRNA transcript library preparation comprising,

(a) capturing mRNA transcripts from a sample on a substrate;

(b) contacting the substrate with a high processivity reverse transcriptase (RT) to generate a first cDNA strand complementary to the mRNA transcripts;

(c) contacting the first cDNA strand with a DNA polymerase to generate a second cDNA strand complementary to the first cDNA strand; and

(d) amplifying the second strand cDNA to form a PCR template and isolating the PCR template.

13. A method for improving capture efficiency of mRNA transcripts for in situ mRNA transcript library preparation comprising,

(a) capturing mRNA transcripts from a sample on a substrate;

(b) contacting the substrate with a reverse transcriptase (RT) to generate a first cDNA strand complementary to the mRNA transcripts;

(c) contacting the first cDNA strand with a high processivity reverse transcriptase (RT) or high processivity DNA polymerase to generate a second cDNA strand complementary to the first cDNA strand; and

(d) amplifying the second strand cDNA to form a PCR template and isolating the PCR template.

14. A method for improving capture efficiency of mRNA transcripts for in situ mRNA transcript library preparation comprising,

(a) capturing mRNA transcripts from a sample on a substrate;

(b) contacting the substrate with a high processivity reverse transcriptase (RT) to generate a first cDNA strand complementary to the mRNA transcripts;

(c) contacting the first cDNA strand with the high processivity reverse transcriptase (RT) or high processivity DNA polymerase to generate a second cDNA strand complementary to the first cDNA strand; and

(d) amplifying the second strand cDNA to form a PCR template and isolating the PCR template.

15. A method for improving the nucleotide length of polynucleotides used in generating an *in situ* transcriptome library comprising,

(a) capturing mRNA transcripts from a sample on a substrate;

(b) contacting the substrate with a high processivity reverse transcriptase (RT) to generate a first cDNA strand complementary to the mRNA transcripts;

(c) contacting the first cDNA strand with a high processivity reverse transcriptase (RT) or high processivity DNA polymerase to generate a second cDNA strand complementary to the first cDNA strand;

(d) amplifying the second strand cDNA to form a PCR template and isolating the PCR template.

16. The method of any one of claims 18 to 21, wherein the high processivity RT is Superscript IV, thermostable group II intron RT (TGIRT), or marathon RT.

17. The method of any one of claims 12 to 16, wherein the high processivity DNA polymerase is Klenow exo -, Bst 3.0, or phi29.

18. A method for preparing an mRNA transcriptome library from a tissue sample comprising,

(a) contacting total RNA isolated from the sample with polynucleotide kinase (PNK) to modify 3' phosphate to a hydroxyl group to generate end repaired total RNA;

(b) contacting the total RNA with polynucleotide kinase (PNK) to modify a 3' phosphate to a hydroxyl group to generate end repaired total RNA;

(c) contacting the end repaired total RNA with polyadenylate polymerase (PAP) and adenosine nucleotides to generate polyadenylated total RNA;

(d) releasing the polyadenylated total RNA from the tissue sample;

(e) capturing the polyadenylated total RNA on a substrate comprising one or more oligonucleotides comprising a poly T sequence;

(f) depleting ribosomal RNA from the total RNA leaving polyadenylated mRNA;

(g) contacting the polyadenylated mRNA with a reverse transcriptase (RT) to generate a first cDNA strand complementary to the mRNA transcripts;

(h) contacting the first cDNA strand with a high processivity reverse transcriptase (RT) or high processivity DNA polymerase to generate a second cDNA strand complementary to the first cDNA strand to generate PCR templates;

(i) eluting the PCR templates; and

(j) generating an mRNA library from the PCR templates.

19. The method of any one of claims 12 to 18, wherein the substrate is a bead, a bead array, a spotted array, a flow cell, clustered particles arranged on a surface of a chip, a film, or a plate.

20. The method of any one of claims 12 to 19, wherein the sample is a fresh frozen tissue sample or a formalin-fixed paraffin embedded (FFPE) sample.

21. The method of any one of claims 18 to 20, wherein releasing comprises contacting the sample with a lysis buffer, a permeabilization buffer and/or a reagent to deparaffinize a FFPE sample.

22. The method of any one of claims 18 to 21, wherein when the sample is a FFPE sample on a slide, the method comprises permeabilization and collagenase treatment of the sample on the slide prior to contacting the RNA with PNK.

23. The method of any one of claims 7 to 9 or 20 to 22, further comprising decrosslinking the FFPE sample, optionally wherein the decrosslinking is carried out using TE buffer, pH 9.

24. The method of any one of claims 1 to 11 or 18 to 23, wherein the polyA tail is between 3 and 50 nucleotides.

25. The method of any one of claims 1 to 11 or 18 to 24, wherein generating the RNA library comprises the steps of eluting the polyadenylated total RNA from the substrate and generating the RNA library from the eluted polyadenylated RNA library using a RNA library prep kit.

26. The method of any one of claims 18 to 25, wherein generating the RNA library comprises,

- i) contacting the isolated RNA with a reverse transcriptase (RT) to generate a first cDNA strand complementary to the RNA;
- ii) contacting the first cDNA strand with a reverse transcriptase (RT) or DNA polymerase to generate a second cDNA strand complementary to the first cDNA strand;
- iii) amplifying the second strand cDNA to form a PCR template and isolating the PCR template; and
- iv) generating an mRNA library from the PCR templates.

27. The method of any one of claims 3 to 11 or 18 to 26, wherein the RNA library is an mRNA library.

28. The method of claim 27, wherein the PCR templates are further processed by tagmentation to generate a spatial transcriptomics library.

29. The method of claim 28, wherein the tagmentation comprises on bead tagmentation, wherein the bead comprises a plurality of bead-linked transposomes (BLT).

30. The method of claim 29, wherein the BLT comprises,

i) a plurality of oligonucleotides comprising a first clustering sequence (P7), a first index sequence and a Read 1 sequencing primer (Rd1 SP); and

ii) a plurality of oligonucleotides comprising a second clustering sequence (P5), a second index sequence and a Read 2 sequencing primer (Rd2 SP).

31. The method of any one of claims 24 to 36 wherein reverse transcriptase is a high processivity reverse transcriptase.