

METHODS FOR ENHANCING OF POLYMERASE ACTIVITY**STATEMENT OF GOVERNMENT SUPPORT**

[0001] This invention was made with government support under HG011868 awarded by National Institutes of Health. The government has certain rights in the invention.

CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application claims priority to U.S. Provisional Application No. 63/550,686, filed February 7, 2024 which is hereby incorporated by reference herein in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0003] This application contains a Sequence Listing, which is submitted electronically via EFS-Web as an XML Document formatted sequence listing with a file name "047162-5366-00WO Sequence Listing.xml," having a creation date of February 6, 2025, and having a size of 18,195 bytes. The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND

[0004] Polymerase enzymes, such as reverse transcriptases, are useful in a variety of commercial settings for synthesizing complementary deoxyribonucleic acids (cDNAs) from ribonucleic acid (RNA) templates. For example, polymerases are used in methods of assessing gene expression, such as RNA sequencing or reverse transcription coupled with quantitative polymerase chain reaction (qRT-PCR). While polymerases (e.g., reverse transcriptases) are critical to these methods, accurate measurement of gene expression is limited by the ability of the reverse transcriptase to reverse transcribe long RNAs (i.e., the processivity of the reverse transcriptase) and RNAs with low abundance in a sample (i.e., detection of low input RNAs by the reverse transcriptase). Thus, there is a need in the art to improve the processivity and detection of low input RNAs (i.e., improve the cDNA yield) by reverse transcriptases.

SUMMARY

[0005] The present disclosure features compositions of polymerases, e.g., reverse transcriptases, and an anionic excipient, e.g., a plurality of anionic excipients. Polymerases, e.g.,

reverse transcriptases, have the ability to synthesize polynucleotides, e.g., DNA or RNA, which is referred to as polymerase activity. This polymerase activity may be enhanced by the addition of an anionic excipient, e.g., a plurality of anionic excipients, to a polymerase reaction, e.g., by providing an anionic excipient to a polymerase under conditions that allow the polymerase to carry out its activity. In one aspect, the present disclosure comprises methods of acquiring a value for the presence of a target ribonucleic acid (RNA) in a mixture, comprising contacting the mixture with (i) a polymerase, e.g., reverse transcriptase; (ii) an oligonucleotide primer; (iii) a deoxynucleotide triphosphate (dNTP) solution, and/or (iv) an anionic polymer. In some embodiments, a value for the presence of the target RNA is acquired. For example, acquiring a value for the presence of the target RNA may comprise detecting the presence of the target RNA. In some embodiments, acquiring a value for the presence of the target RNA comprises producing a cDNA product that comprises a complementary DNA sequence to the target RNA.

[0006] In some embodiments, the target RNA is a low abundance RNA. For example, the level of the target RNA in the mixture can be less than 1 μ g, 500 ng, 250 ng, 100 ng, 50 ng, 25 ng, 10 ng, 5 ng, 1 ng, 500 pg, 250 pg, 100 pg, 50 pg, 25 pg, 10 pg, 5 pg, 1 pg, 500 fg, 250 fg, 100 fg, 50 fg, 25 fg, 10 fg, or less. In some embodiments, the level of the target RNA in the mixture is between about 1 μ g to about 0.1 pg.

[0007] In some embodiments, the polymerase is DNA polymerase or an RNA polymerase. In some embodiments, the polymerase is a reverse transcriptase. For example, the reverse transcriptase may be derived from a virus, an intron, a telomerase, a retrotransposon, a polymerase with reverse transcriptase activity, or an engineered polymerase with reverse transcriptase activity. In some embodiments, the reverse transcriptase is a group II intron reverse transcriptase, a telomerase reverse transcriptase, a viral reverse transcriptase or a retroviral reverse transcriptase. In some embodiments, the reverse transcriptase comprises MarathonRT, UltraMarathonRT, Induro, Maxima H Minus, SuperScript II, SuperScript III, SuperScript IV, PrimeScript, Transcriptor, GoScript, ProtoScript II, SMARTScribe, Avian Myeloblastosis Virus (AMV) reverse transcriptase, Moloney Murine Leukemia Virus (MMLV) reverse transcriptase, Bombyx Morii RT, telomerase RT, TGIRT, or a fragment, variant, mutant, or derivative thereof. In some embodiments, the reverse transcriptase comprises UltraMarathonRT.

[0008] In another aspect, the present disclosure comprises compositions of oligonucleotide primers. In some embodiments, the oligonucleotide primer is between 5 and 200

nucleotides in length. In some embodiments, the composition comprises at least two oligonucleotide primers.

[0009] In yet another aspect, the present disclosure comprises compositions of anionic polymers. For example, the anionic polymer comprises a naturally occurring polymer or a non-naturally occurring polymer. In some embodiments, the anionic polymer comprises an oligonucleotide, peptide, polypeptide, or oligosaccharide. In some embodiments, the anionic polymer comprises an oligosaccharide. In some embodiments, the anionic polymer comprises a glycosaminoglycan. In some embodiments, the anionic polymer comprises an alginate, hyaluronate, dextran, or heparin. In some embodiments, the anionic polymer comprises heparin. In some embodiments, the anionic polymer comprises poly(acrylic acid). In some embodiments, the concentration of the anionic polymer in the mixture is between about 0.5 ng/μL to about 10 μg/μL. For example, the concentration of the anionic polymer in the mixture may be between about 5 ng/μL to about 30 ng/μL. In some embodiments, the concentration of the anionic polymer in the mixture is about 0.5 ng/μL, 1 ng/μL, 2.5 ng/μL, 5 ng/μL, 10 ng/μL, 15 ng/μL, 20 ng/μL, 30 ng/μL, 50 ng/μL, 100 ng/μL, 250 ng/μL, 500 ng/μL, 750 ng/μL, 1 μg/μL, 2.5 μg/μL, 5 μg/μL, or 10 μg/μL.

[0010] In another aspect, the present disclosure comprises compositions of RNA molecules, e.g., input RNA molecules or target RNA molecules. In some embodiments, the composition comprises a plurality of input RNA molecules (e.g., non-target RNA).

[0011] In one aspect, the present disclosure comprises methods for improving polymerase, e.g., reverse transcriptase, activity comprising (i) detecting the level, identity or concentration of a target RNA; (ii) increasing the signal to noise ratio of a target RNA; and/or (iii) increasing the processivity of the reverse transcriptase reaction, compared to a reference standard.

BRIEF DESCRIPTION OF DRAWINGS

[0012] **FIG. 1A** is an image showing agarose gel electrophoresis of the DNA product resulting from reverse transcription and PCR amplification (RT-PCR) of a 4 kilobase (kb) low-input target RNA in the presence of exemplary carrier RNAs or various concentrations of heparin during the reverse transcription step.

[0013] **FIG. 1B** is a graph showing quantification of the bands in each lane of gel in FIG. 1A.

[0014] **FIG. 2A** is an image showing agarose gel electrophoresis of the DNA product resulting from reverse transcription using UltraMarathonRT and PCR amplification (RT-PCR) of an 8 kilobase (kb) ultra low-input target RNA in the presence of heparin during the reverse transcription step.

[0015] **FIG. 2B** is a graph showing quantification of the bands in each lane of gel in FIG. 2A.

[0016] **FIG. 3A** is an image showing agarose gel electrophoresis of the DNA product resulting from reverse transcription using UltraMarathonRT and PCR amplification (RT-PCR) of a 4 kilobase (kb) ultra low-input target RNA in the presence of poly(acrylic acid) during the reverse transcription step.

[0017] **FIG. 3B** is a graph showing quantification of the bands in each lane of gel in FIG. 3A.

[0018] **FIG. 4A** is an image showing agarose gel electrophoresis of the DNA product resulting from reverse transcription using Maxima H Minus reverse transcriptase and PCR amplification (RT-PCR) of a 4 kilobase (kb) ultra low-input target RNA in the presence of heparin during the reverse transcription step.

[0019] **FIG. 4B** is a graph showing quantification of the bands in each lane of gel in FIG. 4A.

[0020] **FIG. 5A** is an image showing agarose gel electrophoresis of the DNA product resulting from reverse transcription using Induro reverse transcriptase and PCR amplification (RT-PCR) of an 8 kilobase (kb) ultra low-input target RNA in the presence of heparin during the reverse transcription step.

[0021] **FIG. 5B** is a graph showing quantification of the bands in each lane of gel in FIG. 5A.

[0022] **FIG. 6A** is an image showing agarose gel electrophoresis of the DNA product resulting from reverse transcription using SuperScript III reverse transcriptase and PCR amplification (RT-PCR) of a 4 kilobase (kb) ultra low-input target RNA in the presence of heparin during the reverse transcription step.

[0023] **FIG. 6B** is a graph showing quantification of the bands in each lane of gel in FIG. 6A.

[0024] **FIG. 7** is an image of a bioanalyzer electrophoresis profile of the DNA product resulting from reverse transcription of total cellular RNA in the presence of various heparin concentrations.

[0025] **FIG. 8** is an image showing agarose gel electrophoresis of the DNA product resulting from reverse transcription using UltraMarathonRT (uMRT; top row), Induro RT (second row), SuperScript III (third row), Maxima H minus (Maxima H -; fourth row), or AMV RT (bottom row) reverse transcriptases and PCR amplification of a 1.8 kilobase (kb) target RNA in the presence of no anionic polymer (left column), heparin (second column), polyacrylic acid (third column), MS2 phage RNA (fourth column), or iota-carrageenan (right column). The target RNA was present in a mixture of total HeLa cell RNA at 10 ng, 1 ng, 100 pg, 10 pg, or 1 pg input.

[0026] **FIG. 9** is a set of graphs showing quantification of the bands in each lane of the gels in FIG. 8.

DESCRIPTION

[0027] The present disclosure features methods for improving complementary DNA (cDNA) yield from a ribonucleic acid (RNA) template, e.g., present in a mixture, during reverse transcription by introducing an anionic polymer. In an embodiment, the improving comprises enhancing the ability of the RT to amplify a target low-abundance RNA in a sample, e.g., a complex sample. In other embodiments, the methods described herein provide for improving the processivity of an RT.

Definitions

[0028] So that the disclosure may be more readily understood, certain technical and scientific terms used herein are specifically defined below. Unless specifically defined elsewhere in this document, all other technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this disclosure belongs.

[0029] As used herein, including the appended claims, the singular forms of words such as “a,” “an,” and “the,” include their corresponding plural references unless the context clearly dictates otherwise.

[0030] “About” or “approximately” means when used herein to modify a numerically defined parameter (e.g., yield of cDNA resulting from reverse transcription by a reverse transcriptase), means that the recited numerical value is within an acceptable functional range for the defined parameter as determined by one of ordinary skill in the art, which will depend in part on how the numerical value is measured or determined, e.g., the limitations of the measurement system, including the acceptable error range for that measurement system. For example, “about” can mean a range of 20% above and below the recited numerical value. As a non-limiting example, the concentration of an anionic polymer in a reaction mixture may be about 0.01 pg/ μ L to about 100 ng/ μ L. In some embodiments, the term “about” means that the modified parameter may vary by as much as 15%, 10% or 5% above and below the stated numerical value for that parameter. Alternatively, particularly with respect to certain properties of an anionic polymer in a reaction mixture, such as increasing the yield of cDNA produced from low abundance nucleic acids in a sample, the term “about” can mean within an order of magnitude above and below the recited value, e.g., within 5-fold, 4-fold, 3-fold, 2-fold or 1-fold.

[0031] “Acquire” or “acquiring”, as used herein, refer to obtaining possession of a value, e.g., a numerical value, or image, or a physical entity (e.g., a sample), by “directly acquiring” or “indirectly acquiring” the value or physical entity. “Directly acquiring” means performing a process (e.g., performing an analytical method or protocol) to obtain the value or physical entity. “Indirectly acquiring” refers to receiving the value or physical entity from another party or source (e.g., a third-party laboratory that directly acquired the physical entity or value). Directly acquiring a value or physical entity includes performing a process that includes a physical change in a physical substance or the use of a machine or device. Examples of directly acquiring a value include obtaining a sample from a human subject. Directly acquiring a value includes performing a process that uses a machine or device, e.g., using a fluorescence microscope to acquire fluorescence microscopy data.

[0032] A “nucleotide,” as that term is used herein, refers to an entity comprising a sugar, typically a pentameric sugar; a nucleobase; and a phosphate linking group. In an embodiment, a nucleotide comprises a naturally occurring, e.g., naturally occurring in a human cell, nucleotide, e.g., an adenine, thymine, guanine, cytosine, or uracil nucleotide.

[0033] “Nucleic acid”, as used herein, refers to a polymer comprising a nucleotide linked through phosphodiester bonds. In an embodiment, a nucleic acid comprises at least two, and in

some embodiments, at least 10, 100, 1,000, or 10,000 nucleotides. In some embodiments, a nucleic acid comprises ribonucleotides, e.g., is a ribonucleic acid (RNA). In some embodiments, a nucleic acid comprises deoxyribonucleotides, e.g., is deoxyribonucleic acid (DNA). “Nucleic acid”, as used herein, is interchangeable with “polynucleotide” or “oligonucleotide”. The length of a nucleic acid is referred to herein as a number of bases, e.g., a nucleic acid comprising 1,000 nucleotides has a length of 1,000 bases or 1 kilobase (kb).

[0034] “Complementary DNA” or “cDNA”, as used herein, refers to the deoxyribonucleic acid (DNA) product synthesized by a reverse transcriptase, e.g., DNA synthesized by a reverse transcriptase using a ribonucleic acid (RNA) molecule as a substrate.

[0035] “Polypeptide”, as used herein, refers to a polymer comprising amino acid residues linked through peptide bonds and having at least two, and in some embodiments, at least 10, 50, 75, 100, 150 or 200 amino acid residues.

[0036] The term “template” as used herein, with respect to a polynucleotide, refers to a single-stranded polynucleotide substrate for a nucleic acid polymerase, e.g., a reverse transcriptase. For example, a nucleic acid polymerase, e.g., a reverse transcriptase, can synthesize a polynucleotide strand that is complementary to the template strand. In some embodiments, a single-stranded RNA polynucleotide can be a template for a reverse transcriptase. A “template” polynucleotide, as used herein, may also be referred to as a “target” polynucleotide. In some embodiments, a target polynucleotide is RNA. In some embodiments, a target polynucleotide is DNA. The term “product” as used herein, with respect to a polynucleotide, refers to the polynucleotide strand synthesized by a nucleotide polymerase. In some embodiments, the nucleotide polymerase is a reverse transcriptase. In some embodiments, the product polynucleotide is a deoxyribonucleic acid (DNA) polynucleotide synthesized by a reverse transcriptase using a ribonucleic acid (RNA) polynucleotide as a template.

[0037] The term “target” as used herein, with respect to a polynucleotide, refers to a polynucleotide intended for detection by a reverse transcriptase, e.g., a polynucleotide for which a value is acquired using a method of reverse transcription. For example, the oligonucleotide primers in a reverse transcription reaction mixture are complementary to the target polynucleotide, thereby allowing the target polynucleotide to be reverse transcribed. In some embodiments, the target polynucleotide comprises a deoxyribonucleic acid (DNA). In some embodiments, the target polynucleotide comprises a ribonucleic acid (RNA).

[0038] The term “reverse transcription” as used herein, with respect to a subject molecule, e.g., a RNA polynucleotide, refers to synthesis of a deoxyribonucleic acid (DNA), e.g., cDNA, polynucleotide using a ribonucleic acid (RNA) polynucleotide as a template.

[0039] The term “reverse transcriptase” as used herein refers a nucleic acid polymerase capable of synthesizing a deoxyribonucleic acid (DNA) polynucleotide from a template ribonucleic acid (RNA) polynucleotide. For example, a reverse transcriptase may synthesize a single-stranded complementary DNA (cDNA) polynucleotide product from a messenger RNA (mRNA) expressed in a cell or subject. In some embodiments, an RT may be a template-switching RT. In some embodiments, the RT comprises a MarathonRT reverse transcriptase, an UltraMarathonRT reverse transcriptase, a Moloney Murine Luekemia Virus reverse transcriptase, an Avian Myeloblastosis Virus reverse transcriptase, Bombyx mori R2 RNA element reverse transcriptase, a TGIRT™ reverse transcriptase, Induro reverse transcriptase, Maxima H minus reverse transcriptase, SuperScript II reverse transcriptase, SuperScript III reverse transcriptase, SuperScript IV reverse transcriptase, PrimeScript reverse transcriptase, Transcriptor reverse transcriptase, GoScript reverse transcriptase, ProtoScript II reverse transcriptase, or SMARTScribe reverse transcriptase, as well as variants, fragments, and mutants thereof.

[0040] The term “non-templated nucleotide addition” as used herein refers to the addition of nucleotides to the 3' end of a product polynucleotide synthesized by an enzyme upon reaching the 5' terminus of a template polynucleotide, e.g., addition of nucleotides to the product polynucleotide that are not comprised in the template polynucleotide. For example, non-templated nucleotide addition can result in a product polynucleotide that comprises a 3' end which extends beyond the 5' end of the template polynucleotide and is non-complementary to the template polynucleotide. Typically, non-templated nucleotide addition results in a 1-3 nucleotide overhang, e.g., 1, 2, or 3 nucleotide overhang, at the 3' end of the product polynucleotide relative to the template polynucleotide.

[0041] The term “template switching” as used herein refers to the process of a polymerase enzyme switching from a first template polynucleotide to a second template polynucleotide while synthesizing a continuous product polynucleotide. Typically, template switching comprises: (i) non-templated nucleotide addition of nucleotides to the 3' end of the polynucleotide synthesized by the reverse transcriptase upon reaching the 5' terminus of the template polynucleotide; (ii) base pairing between a template switching oligonucleotide (TSO)

and the nucleotide overhang resulting from non-templated addition; and (iii) continued synthesis of the product polynucleotide by the reverse transcriptase using the TSO as the template polynucleotide.

[0042] The term “concatemerization” as used herein refers to the linkage of a plurality of the same polynucleotide sequence in series, e.g., the linkage of a plurality of template switching oligonucleotide (TSO) sequences. In some embodiments, concatemerization of a plurality of a TSO can be a result of repeated cycles of non-templated nucleotide addition by a reverse transcriptase followed by template switching by the reverse transcriptase.

[0043] “Carrier RNA” as used herein refers to a supplemental ribonucleic acid (RNA) molecule that improves the yield of enzymatic reactions on nucleic acids, e.g. cDNA produced during reverse transcription, e.g., an RNA molecule that is present in the reaction mixture but is not a target RNA. Typically, a carrier RNA stabilizes template RNA. Alternatively, carrier RNA may improve the activity of the reverse transcriptase.

[0044] “Polymerase” or “polymerase enzyme”, as used herein, refers to an enzyme capable of forming phosphodiester linkages between nucleotides, e.g., ribonucleotides or deoxyribonucleotides, in a manner that is directed by a template polynucleotide, e.g., a template ribonucleic acid (RNA) or template deoxyribonucleic acid (DNA), thereby generating a polynucleotide strand that is complementary to the template polynucleotide. Polymerases may use RNA templates, e.g., be RNA-dependent, or DNA templates, e.g., be DNA-dependent. Additionally, polymerases may utilize ribonucleotides to synthesize RNA polynucleotides or utilize deoxyribonucleotides to synthesize DNA polynucleotides. In some embodiments, a polymerase is a reverse transcriptase.

Polymerase Enzymes

[0045] In some embodiments, the present disclosure relates to methods of enhancing the activity of a polymerase by adding an additive, such as an anionic polymer, to a reaction mixture. Polymerases are enzymes that catalyze phosphodiester bond formation between nucleotides, e.g., ribonucleotides or deoxyribonucleotides, to generate a polynucleotide product, e.g., a ribonucleic acid (RNA) or a deoxyribonucleic acid (DNA), in a sequence-directed manner. For example, polymerases synthesize polynucleotide products that are complementary in sequence to a template nucleic acid, e.g., a template RNA molecule or template DNA molecule. Polymerases are categorized by the type of nucleic acid synthesized and the type of nucleic acid used as a

template, e.g., RNA or DNA. For example, polymerases can be RNA polymerases, e.g., synthesize RNA products, or DNA polymerases, e.g., synthesize DNA products. As a further example, polymerases can be RNA-dependent, e.g., use RNA as a template, or DNA-dependent, e.g., use DNA as a template. Thus, polymerases are generally categorized as being RNA-dependent RNA polymerases, RNA-dependent DNA polymerases, DNA-dependent RNA polymerases, or DNA-dependent DNA polymerases. In some embodiments, the present disclosure relates to RNA-dependent DNA polymerases, e.g., reverse transcriptases.

[0046] Reverse transcriptases are enzymes that synthesize single-stranded complementary DNA (cDNA) from a single-stranded RNA template. Reverse transcriptases are useful for methods of analyzing gene expression by measuring abundance of messenger RNA (mRNA) such as reverse transcription coupled to quantitative polymerase chain reaction (qRT-PCR) or RNA sequencing (RNA-seq). However, the accuracy of these methods can often be limited by the ability of a reverse transcriptase to reverse transcribe long template RNAs or RNAs with complex secondary structures from end-to-end, e.g., the ability of the reverse transcriptase to synthesize full-length cDNAs, e.g., the processivity of the reverse transcriptase. Additionally, methods for measuring gene expression can further be limited by the ability of a reverse transcriptase to reverse transcribe low-abundance RNAs. For example, certain RTs are unable to amplify meaningful amounts of low-abundance RNAs in complex mixtures, which can impact their utility in a research or commercial setting, e.g., in methods of measuring gene expression from samples with low amounts of input RNA, such as single-cell RNA sequencing or in situ RNA sequencing. The present disclosure provides methods for improving the ability of reverse transcriptases to synthesize cDNAs, e.g., full-length cDNAs, from long or complex RNA templates, e.g., improving the processivity of the reverse transcriptase, or enhancing the ability of reverse transcriptase to synthesize cDNA from RNAs with low abundance in a sample, by including an anionic polymer into the RT reaction mixture.

[0047] The methods described herein for enhancing polymerase activity apply to any polymerase, e.g., reverse transcriptase, known in the art. For example, the polymerase may be a reverse transcriptase. In some embodiments, the reverse transcriptase is derived from a virus, an intron, a telomerase, or a retrotransposon. In some embodiments, the reverse transcriptase is derived from a telomerase, e.g., a mammalian telomerase. In some embodiments, the reverse transcriptase is a telomerase reverse transcriptase, e.g., TERT. In some embodiments, the reverse

transcriptase is TERT. In some embodiments, the reverse transcriptase is derived from a mobile genetic element, e.g., a retrotransposon, e.g., a plant or animal retrotransposon, e.g., a non-long terminal repeat (non-LTR) retrotransposon. In some embodiments, the reverse transcriptase is derived from a mobile genetic element, e.g., a self-splicing intron, e.g., a group II intron. In some embodiments, the reverse transcriptase has a sequence listed in Table 1. In some embodiments, the reverse transcriptase has a sequence with at least 60% to 99.9% identity to a sequence listed in Table 1, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to a sequence listed in Table 1. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to a sequence listed in Table 1, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to a sequence listed in Table 1. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SEQ ID NO: 1, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SEQ ID NO: 1. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SEQ ID NO: 2, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SEQ ID NO: 2. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SEQ ID NO: 3, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SEQ ID NO: 3. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SEQ ID NO: 4, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SEQ ID NO: 4. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SEQ ID NO: 5, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SEQ ID NO: 5. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SEQ ID NO: 6, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SEQ ID NO: 6. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SEQ ID NO: 7, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SEQ ID NO: 7. In some embodiments, the reverse transcriptase comprises a sequence

having at least 60% to 99.9% identity to SEQ ID NO: 8, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SEQ ID NO: 8. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SEQ ID NO: 9, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SEQ ID NO: 9. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SEQ ID NO: 10, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SEQ ID NO: 10. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SEQ ID NO: 11, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SEQ ID NO: 11. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SEQ ID NO: 12, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SEQ ID NO: 12.

Table 1. Exemplary reverse transcriptase sequences.

SEQ ID NO.	Sequence	Comment
1	MDTSNLMEQILSSDNLNRAYLQVVRNKGAEV DGMKYTELKEHLAK NGETIKGQLRTRKYKQPARRVEIPKPDGGVRNLGVPTVTDRFIQQAIA QVLTPYEEQFHDHSYGFRPNRCAQQAILTALNIMNDGNDWIVDIDLEK FFDTVNHDKLMTLIGRTIKDGDVISIVRKYLVSIGIMIDDEYEDSIVGTPQ GGNLSPLL ANIMLNELDKEMEKRGLNFVRYADDCIIMVGSEMSANRV MRNISRFIEEKLGLKVNMTKSKVDRPSGLKYLGFYFDPRAHQFKAK PHAKSVAKFKKRMKELTCRSWGSNSYKVEKLNQLIRGWINYFKIGS MKTLCCKELDSRIRYRLRMCIWQWKTPQNQEKNLVVKLGIDRNTARRV AYTGKRIAYVCNKGAVNVAISNKR LASFGLISMLDYYIEKCVTC	MarathonRT full length enzyme
2	MDTSNLMEQILSSDNLNRAYLQVVRNKGAEV DGMKYTELKEHLAK NGETIKGQLRTRKYKQPARRVEIPKPDGGVRNLGVPTVTDRFIQQAIA QVLTPYEEQFHDHSYGFRPNRCAQQAILTALNIMNDGNDWIVDIDLEK FFDTVNHDKLMTLIGRTIKDGDVISIVRKYLVSIGIMIDDEYEDSIVGTPQ GGNLSPLL ANIMLNELDKEMEKRGLNFVRYADDCIIMVGSEMSANRV MRNISRFIEEKLGLKVNMTKSKVDRPSGLKYLGFYFDPRAHQFKAK PHAK	RT domain of MarathonRT (finger and palm subdomains; residues 1-293)

3	MVKSSGTERKERMDTSSLMEQILSNDNLNRAYLQVVRNKGAEGVDG MKYTELKEYLAKNGEIIKEQLRIRKYKQPVRVEIPKPDGGVRNLGVP TVTDRFIQQAIAQVLTPIYEEQFHDHSYGFRPNRCAQQAILTALDMMND GNDWIVDIDLEKFFDTVNHDKLMTHIGRTIKDGDVISIVRKYLVSIMID DEYEDSIVGTPQGGNLSPLLANIMLNELDKEMEKRGLNFVRYADDCII MVGSEMSANRVMRNISRFIEEKLGLKVNMTKSKVDRPRGIKYLGFIFY YDTSAAQQFKAKPHAKSVMKYKKRMRELTCRSWGVSNYSYKVERLNQL IRGWINYFKIGSMKTLCRELDGNIRYRIRMCIWKHWKTPQNKEKNLVK LGVPRWAAHKVANTGNRYAHMCHNGWIQKAISTKRLLTSFGLVSMILY YTERCVTC	Roseburia intestinalis XB6B4 full length enzyme
4	MVKSSGTERKERMDTSSLMEQILSNDNLNRAYLQVVRNKGAEGVDG MKYTELKEYLAKNGEIIKEQLRIRKYKQPVRVEIPKPDGGVRNLGVP TVTDRFIQQAIAQVLTPIYEEQFHDHSYGFRPNRCAQQAILTALDMMND GNDWIVDIDLEKFFDTVNHDKLMTHIGRTIKDGDVISIVRKYLVSIMID DEYEDSIVGTPQGGNLSPLLANIMLNELDKEMEKRGLNFVRYADDCII MVGSEMSANRVMRNISRFIEEKLGLKVNMTKSKVDRPRGIKYLGFIFY YDTSAAQQFKAKPHAK	Roseburia intestinalis XB6B4 RT domain
5	AFPLERPDWDYTTQAGRNHLVHYRQLLAGLQNAAGRSPTNLAKVKGI TQGPNSPSAFLERLKEAYRRYTPYDPEDPGQETNVSMFSIWQSAPDIG RKLGRLEDLKSKTLGDLVREAEKIFNKRETPEEREERIRRETEEKEERR RTVDEQKEKERDRRRHREMSKLLATVVIGQEQRQEGERKRPQLDKD QCAYCKEKGHWAKDCPKKPRGPRGPRPQTSLTLGDXXGGQGDPPPE PRITLKVGQPVTFVLDTGAQHSVLTQNPGLSDKSAWVQGATGGKRY RWTTDRKVHLATGKVTHSFLHVPDCPYPLLGRDLTLKLAKIHFEGSG AQVVGPMGQPLQVLTNLNIEDEYRLHETSKEPDVSLGFTWLSDFPQAWA ESGGMGLAVRQAPLIPLKATSTPVSIKQYPMSQEARLGKPHIQRLLDQ GILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNRVEDIHPTVNP YNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDPEMGI GQLTWTRLPGGFKNSPTLFDEALHRDLADFR	MMLV full length
6	AFPLERPDWDYTTQAGRNHLVHYRQLLAGLQNAAGRSPTNLAKVKGI TQGPNSPSAFLERLKEAYRRYTPYDPEDPGQETNVSMFSIWQSAPDIG RKLGRLEDLKSKTLGDLVREAEKIFNKRETPEEREERIRRETEEKEERR RTVDEQKEKERDRRRHREMSKLLATVVIGQEQRQEGERKRPQLDKD QCAYCKEKGHWAKDCPKKPRGPRGPRPQTSLTLGDXXGGQGDPPPE PRITLKVGQPVTFVLDTGAQHSVLTQNPGLSDKSAWVQGATGGKRY RWTTDRKVHLATGKVTHSFLHVPDCPYPLLGRDLTLKLAKIHFEGSG AQVVGPMGQPLQVLTNLNIEDEYRLHETSKEPDVSLGFTWLSDFPQAWA ESGGMGLAVRQAPLIPLKATSTPVSIKQYPMSQEARLGKPHIQRLLDQ GILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNRVEDIHPTVNP YNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDPEMGI GQLTWTRLPGGFKNSPTLFDEALHRDLADFR	MMLV predicted RT domain
7	MMASTALSLMGRCPDGTGRGHVTAAPMDGPRGPSSLAGTFGWGL AIPAGEPCGRVCSPTAVGFFPVAKKSNKENRPEASGLPLESERTGDNPTV RGSAGADPVGQDAPGWTCQFCERTFSTNRGLGVHKRRRAHPVETNTDA APMMVKRRWHGEEIDLLARTEARLLAERGQCSGGDLFGALPGFGRTL EAIKGQRRREPYPALVQAHLARFGSQPGPSSGGCSAEPDFRRASGAEE AGEERCAEDAAAYDPSAVGQMSPDAAARVISELLEGAGRRRACRAMRP KTAGRNRDLHDDRTASAHKTSRQKRRAEYARVQELYKKCRSRAAAEV IDGACGGVGHSLSEMETYWRPILERSDAPGPTPEALHALGRAEWHG GNRDYTLQWKPISVEEIKASRFDWRTSPGPDGIRSGQWRAPVHLKAE MFNAWMARGEIPEILRQCRTVFVVPKVERPGGPGEYRPIASISPLRHFHSI LARLLACCPDARQRFICADGTLENSAVLDAVLGDSRKKLRECHVA VLDFAKAFDTSHEALVELLRLRGMPEQFCGYIAHLYDTASTTLAVNN EMSSPVKVGRGVQGDPLSPILFNVMDLILASLPERVGYRLEMELVS ALAYADDLVLLAGSKVGMQESISAVDCVGRQMGLRLNCRKSAVLSMIP	Bombyx mori R2 element RT

	DGHRKKHHYLTERTFNIGGKPLRQVSCVERWRYLGVDFEASGCVTLE HSISSALNNISRAPLKPOQRLEILRAHLIPRFQHGFLGNISDDRLRMLD VQIRKAVGQWLRRLPADVPKAYYHAAVQDGGLAIPSVRATIPDLIVRRFG GLDSSPWSVARAAAKSDKIRKKLRWAWKQLRRFSRVDSTTQRPVRLF WREHLHASVDGRELRESTRPTSTKWIRERCAQITGRDFVQFVHTHIN ALPSRIRGSRGRRGGESSLTCRAGCKVRETTAHILQQCHRTHGGRILR HNKIVSFVAKAMEENKWTVELEPRLRTSVGLRKPDIASRDGVGVIVD VQVVSQGQSLDELHREKRKNYGNHGELVELVAGRLGLPKAECVRATS CTISWRGVWSLTSYKELRSIIGLREPTLQIVPILALRGSHMNWTRFNQM TSVMGGGVG	
8	AWMARGEIPEILRQCRTVFVPKVERPGPGGEYRPIASIPLRHFHSILAR RLACCPDARQRFICADGTLENSAVLDAVLGDSRKKLRECHVAVLD FAKAFDTSVSHALVELLRLRGMPEQFCGYIAHLYDTASTTLAVNNEMS SPVKVGRGVQGDPLSPILFNVMMDLILASLPERVGYRLEMELVSALA YADDLVLLAGSKVGMQESISAVDCVGRQMGLRLNCRKSAVLSMIPDG HRKKHHYLTERTFNIGGKPLRQVSCVERWRYLGVD	Bombyx mori R2 element RT domain
9	RATVFTVALHLAIPKWKPDHTPVWIDQWPLPEGKLVALTQLVEKELQ LGHIPELSLSCWNTPVFVIRKASGSYRLLHDLRAVNAKLVPFGAVQQGA PVL SALPRGWPLMVLDLKDCFFSIPLAEQDREAFATLPSVNNQAPAR RFQWKVLPQGMTCSPTICQLIVGOILEPLRLKHPSLRMLHYMDDL ASSHDGLEAAGEEVISTLERAGFTISPDKVQKEPGVQYLGYKLGSTYV APVGLVAEPRIATLWDVQKLVGSLQSVRPALGIPRLMGPFYEQLRGSD PNEAREWNLDMMMAWREIVQLSTTAALERWDPALPLEGAVARCEQG AIGVLGQGLSTHPRPCLWLFSTQPTKAFTAWLEVLTLLITKLASAVRT FGKEVDILLPACFREDLPLPEGILLALRGFAGKIRSSDTPSIFDIARPLH VSLKVRVTDHPVPGPTVFTDASSSTHKGVVWREGPRWEIKEIADLG ASVQQLARAVAMALLWPTTPTNVVTDFAVAKMLLKMGQEGVPS TAAAFILEDALSQRSAMAAVLHVRSHSEVPGFTEGNDVADSQATFQA YPLREAKDLHTALHIGPRALSACNISMQQAREVVQTCPHCNSAPALE AGVNPRGLGPLQIWQDFTLEPRMAPRSWLAVTVDTASSAIVVTQH RVTSVAAQHHWATAIAVLGRPKAIKTDNGSCFTSKSTREWLARWGIAH TTGIPGNSQGGAMVERANRLKDKIRVLAEGDGMKRIPTSKQGELL AKAVYALNHFERGENTKTPIQKHWRPTVLTGPPVKIRIETGEWEKGW NVLVWGRGYAAVKNRDTDKVIWVPSRKVKPDITQKDEVTKRDEASPL FAGISDWAPWEGEQEGLQEETASNKQERPGETLAANES	Avian myeloblastosis virus (AMV) full length
10	LQLGHIPELSLSCWNTPVFVIRKASGSYRLLHDLRAVNAKLVPFGAVQQG APVLSALPRGWPLMVLDLKDCFFSIPLAEQDREAFATLPSVNNQAPA RRFQWKVLPQGMTCSPTICQLIVGOILEPLRLKHPSLRMLHYMDDL AASSHDGLEAAGEEVISTLERAGFTISPDKVQKEPGVQYLGYKL	Avian myeloblastosis virus (AMV) predicted RT domain
11	MVHYRSLSKSRQKAPKIVNSKYNSILNIALKNFRLCKKHKTCKPVQI LALLQEIPKSYFGTTNLKRFYKVVEKILTQSSFECHLSVLHKCYDYD AIPWLQNVENLRPKLLKHNLFLLDNIVKPIAFYKPIKTLNGHEIKF IRKEEYISFESKVFHKLKMKYLVEVQDEVKPRGVLNIPKQDNFRAIV SIFPDSARKPFFKLLTSKIYK VLEEKYKTSGLYTCWSEFTQKTQGQIY GIKVDIRDAYGNVKIPVLCCLIQSIPHTLLDSEKKNFIVDHISNQFVAFR RKIYKWNHGLLQGDPLSGCLCELYMAFMDRLYFSNLDKDAFIHRTVD DYFFCSPHPKVYDFELLIKGVYQVNPTKTRTNLPTHRHPQDEIPYCG KIFNLTTTRQVRTLYKLPPNYEIRHKFKLWNFNQISDDNPARFLQKAM DFPFICNSFTKFEFNTVFNDQRTVFANFYDAMICVAYKFDAAMMALRT SFLVNDGFIWLVLSSTVRAYASRAFKKIVTYKGGKYRKVTFQCLKSIA WRAFLAVLKRRTEIYKGLIDRIKSREKLTMKFHDGEVDA SYFCKLPEK FRFVKINRKASI	Human telomerase (TERT), full enzyme sequence
12	MKYLVEVQDEVKPRGVLNIPKQDNFRAIVSIFPDSARKPFFKLLTSKIY KVLEEKYKTSGLYTCWSEFTQKTQGQIYGIKVDIRDAYGNVKIPVLC KLIQSIPHTLLDSEKKNFIVDHISNQFVAFRRKIYKWNHGLLQGDPLSG	Human telomerase (TERT), predicted RT domain

	CLCELYMAFMDRLYFSNLDKDAFIHRTVDDYFFCSPHPHKVYDFELLI KGVYQVNPTKTRTNLPTHRHPQDEIPYCGKIF	
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[0048] In some embodiments, the reverse transcriptase is derived from a mobile genetic element. For example, the reverse transcriptase may be derived from a non-long terminal repeat (non-LTR) retrotransposon or a group II intron. In some embodiments, the reverse transcriptase is a non-LTR retrotransposon reverse transcriptase. In some embodiments, the non-LTR retrotransposon reverse transcriptase is a *Bombyx mori* R2 RNA element reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to *Bombyx mori* R2 RNA element reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to *Bombyx mori* R2 RNA element reverse transcriptase. In some embodiments, the non-LTR retrotransposon reverse transcriptase is a human L1 element reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to human L1 element reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to human L1 element reverse transcriptase. In some embodiments, the reverse transcriptase is a group II intron reverse transcriptase, e.g., a maturase reverse transcriptase. In some embodiments, the group II intron reverse transcriptase is MarathonRT reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to MarathonRT reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to MarathonRT reverse transcriptase. In some embodiments, the group II intron reverse transcriptase is UltraMarathonRT. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to UltraMarathonRT reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to UltraMarathonRT reverse transcriptase. In some embodiments, the group II intron reverse transcriptase is Induro reverse transcriptase (Induro RT). In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to Induro reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to Induro reverse transcriptase. In some embodiments, the group II intron reverse transcriptase is a TGIRT reverse transcriptase. In some embodiments, the reverse transcriptase

comprises a sequence having at least 60% to 99.9% identity to TGIRT reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to TGIRT reverse transcriptase.

[0049] In some embodiments, the reverse transcriptase is derived from a virus. For example, the reverse transcriptase may be derived from a retrovirus. In some embodiments, the reverse transcriptase is an Avian Myeloblastosis Virus (AMV) reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to AMV reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to AMV reverse transcriptase. In some embodiments, the reverse transcriptase is a Human Immunodeficiency Virus (HIV) reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to HIV reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to HIV reverse transcriptase. In some embodiments, the reverse transcriptase is a Rous Sarcoma Virus reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to Rous sarcoma virus reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to Rous sarcoma virus reverse transcriptase. In some embodiments, the reverse transcriptase is a Moloney Murine Leukemia Virus (MMLV) reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to MMLV reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to MMLV reverse transcriptase. In some embodiments, the MMLV reverse transcriptase is Maxima H Minus reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to Maxima H Minus reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to Maxima H Minus reverse transcriptase. In some embodiments, the MMLV reverse transcriptase is SuperScript reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SuperScript reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to Super Script reverse transcriptase. In some embodiments, the MMLV reverse

transcriptase is SuperScript II reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SuperScript II reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to Super Script II reverse transcriptase. In some embodiments, the MMLV reverse transcriptase is SuperScript III reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SuperScript III reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SuperScript III reverse transcriptase. In some embodiments, the MMLV reverse transcriptase is SuperScript IV reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SuperScript IV reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SuperScript IV reverse transcriptase. In some embodiments, the MMLV reverse transcriptase is PrimeScript reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to PrimeScript reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to PrimeScript reverse transcriptase. In some embodiments, the MMLV reverse transcriptase is GoScript reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to GoScript reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to GoScript reverse transcriptase. In some embodiments, the MMLV reverse transcriptase is ProtoScript II reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to ProtoScript II reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to ProtoScript II reverse transcriptase. In some embodiments, the MMLV reverse transcriptase is SMARTScribe reverse transcriptase. In some embodiments, the reverse transcriptase comprises a sequence having at least 60% to 99.9% identity to SMARTScribe reverse transcriptase, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% or greater identity to SMARTScribe reverse transcriptase.

[0050] In another aspect, the present disclosure features ultraprocessive reverse transcriptases. In some embodiments, the reverse transcriptase is an ultraprocessive reverse transcriptase, e.g., a reverse transcriptase capable of synthesizing cDNAs of 4,000 nucleotides, 5,000 nucleotides, 6,000 nucleotides, 7,000 nucleotides, 8,000 nucleotides, 9,000 nucleotides, 10,000 nucleotides, 11,000 nucleotides, 12,000 nucleotides, 15,000 nucleotides, 20,000 nucleotides, 25,000 nucleotides, or 30,000 nucleotides or more in length. In some embodiments, the ultraprocessive reverse transcriptase is UltraMarathonRT. In some embodiments, the ultraprocessive reverse transcriptase is MarathonRT.

Oligonucleotide Primers

[0051] The methods described herein for enhancing RT activity may further include using an oligonucleotide primer, e.g., a plurality of oligonucleotide primers, that contain many sequence elements. In some embodiments, the reverse transcription reaction mixture comprises at least two oligonucleotide primers, e.g., at least 2, 3, or 4 oligonucleotide primers. In some embodiments, the oligonucleotide primer is between about 5 and about 250 nucleotides in length, e.g., about 5 and about 200, about 5 and about 150, about 5 and about 100, about 5 and about 75, about 5 and about 50, and about 5 and about 25 nucleotides in length. In some embodiments, the oligonucleotide primer is between about 5 and about 100 nucleotides in length. In some embodiments, the oligonucleotide primer is about 5 nucleotides in length. In some embodiments, the oligonucleotide primer is about 10 nucleotides in length. In some embodiments, the oligonucleotide primer is about 20 nucleotides in length. In some embodiments, the oligonucleotide primer is about 25 nucleotides in length. In some embodiments, the oligonucleotide primer is about 35 nucleotides in length. In some embodiments, the oligonucleotide primer is about 40 nucleotides in length. In some embodiments, the oligonucleotide primer is about 45 nucleotides in length. In some embodiments, the oligonucleotide primer is about 50 nucleotides in length. In some embodiments, the oligonucleotide primer is about 55 nucleotides in length. In some embodiments, the oligonucleotide primer is about 60 nucleotides in length. In some embodiments, the oligonucleotide primer is about 65 nucleotides in length. In some embodiments, the oligonucleotide primer is about 70 nucleotides in length. In some embodiments, the oligonucleotide primer is about 75 nucleotides in length. In some embodiments, the oligonucleotide primer is about 80 nucleotides in length. In some

embodiments, the oligonucleotide primer is about 85 nucleotides in length. In some embodiments, the oligonucleotide primer is about 90 nucleotides in length. In some embodiments, the oligonucleotide primer is about 95 nucleotides in length. In some embodiments, the oligonucleotide primer is about 100 nucleotides in length. In some embodiments, the oligonucleotide primer is about 125 nucleotides in length. In some embodiments, the oligonucleotide primer is about 150 nucleotides in length. In some embodiments, the oligonucleotide primer is about 200 nucleotides in length. In some embodiments, the oligonucleotide primer is about 250 nucleotides in length. In some embodiments, the oligonucleotide primer is about 500 nucleotides in length. In some embodiments, the oligonucleotide primer is about 750 nucleotides in length.

[0052] The oligonucleotide primer may be a deoxyribonucleic acid (DNA) or a ribonucleic acid (RNA). In some embodiments, the oligonucleotide primer is DNA. In some embodiments, the oligonucleotide primer is RNA. In some embodiments the oligonucleotide primer comprises a naturally occurring nucleotide or a non-naturally occurring nucleotide. In some embodiments, the oligonucleotide primer comprises one or more naturally occurring nucleotides. In some embodiments, the oligonucleotide primer comprises one or more non-naturally occurring nucleotides.

[0053] In some embodiments, the oligonucleotide primer comprises an adenine (A), thymine (T), guanine (G), cytosine (C), or uracil (U) nucleotide. In some embodiments, the oligonucleotide primer comprises between 1 and about 30 adenine nucleotides, e.g., about 1 and about 25, about 1 and about 20, about 1 and about 15, about 1 and about 10, and about 1 and about 5 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 1 adenine nucleotide. In some embodiments, the oligonucleotide primer comprises 2 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 3 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 4 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 5 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 6 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 7 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 8 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 9 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 10 adenine nucleotides. In some

embodiments, the oligonucleotide primer comprises 11 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 12 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 13 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 14 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 15 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 16 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 17 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 18 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 19 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 20 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 21 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 22 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 23 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 24 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 25 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 26 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 27 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 28 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 29 adenine nucleotides. In some embodiments, the oligonucleotide primer comprises 30 adenine nucleotides.

[0054] In some embodiments, the oligonucleotide primer comprises between 1 and about 30 thymine nucleotides, e.g., about 1 and about 25, about 1 and about 20, about 1 and about 15, about 1 and about 10, and about 1 and about 5 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 1 thymine nucleotide. In some embodiments, the oligonucleotide primer comprises 2 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 3 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 4 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 5 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 6 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 7 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 8 thymine nucleotides. In some embodiments, the

oligonucleotide primer comprises 9 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 10 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 11 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 12 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 13 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 14 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 15 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 16 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 17 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 18 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 19 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 20 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 21 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 22 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 23 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 24 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 25 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 26 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 27 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 28 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 29 thymine nucleotides. In some embodiments, the oligonucleotide primer comprises 30 thymine nucleotides.

[0055] In some embodiments, the oligonucleotide primer comprises between 1 and about 30 cytosine nucleotides, e.g., about 1 and about 25, about 1 and about 20, about 1 and about 15, about 1 and about 10, and about 1 and about 5 cytosine nucleotides. In some embodiments, the oligonucleotide primer has 1 cytosine nucleotide. In some embodiments, the oligonucleotide primer comprises 2 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 3 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 4 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 5 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 6 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 7 cytosine nucleotides. In some

embodiments, the oligonucleotide primer comprises 8 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 9 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 10 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 11 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 12 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 13 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 14 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 15 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 16 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 17 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 18 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 19 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 20 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 21 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 22 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 23 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 24 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 25 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 26 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 27 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 28 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 29 cytosine nucleotides. In some embodiments, the oligonucleotide primer comprises 30 cytosine nucleotides.

[0056] In some embodiments, the oligonucleotide primer comprises between 1 and about 30 uracil nucleotides, e.g., about 1 and about 25, about 1 and about 20, about 1 and about 15, about 1 and about 10, and about 1 and about 5 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 1 uracil nucleotide. In some embodiments, the oligonucleotide primer comprises 2 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 3 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 4 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 5 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 6 uracil nucleotides. In

some embodiments, the oligonucleotide primer comprises 7 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 8 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 9 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 10 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 11 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 12 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 13 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 14 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 15 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 16 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 17 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 18 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 19 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 20 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 21 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 22 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 23 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 24 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 25 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 26 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 27 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 28 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 29 uracil nucleotides. In some embodiments, the oligonucleotide primer comprises 30 uracil nucleotides.

[0057] In some embodiments, the oligonucleotide primer comprises between 1 and about 30 guanosine nucleotides, e.g., about 1 and about 25, about 1 and about 20, about 1 and about 15, about 1 and about 10, and about 1 and about 5 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 1 guanosine nucleotide. In some embodiments, the oligonucleotide primer comprises 2 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 3 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 4 guanosine nucleotides. In some embodiments, the

oligonucleotide primer comprises 5 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 6 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 7 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 8 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 9 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 10 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 11 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 12 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 13 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 14 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 15 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 16 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 17 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 18 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 19 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 20 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 21 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 22 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 23 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 24 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 25 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 26 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 27 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 28 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 29 guanosine nucleotides. In some embodiments, the oligonucleotide primer comprises 30 guanosine nucleotides.

[0058] In an embodiment, the reaction mixture comprises a plurality of oligonucleotide primers, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or more oligonucleotide primers. In some embodiments, the reaction mixture comprises 2 oligonucleotide primers. In some embodiments, the reaction mixture comprises 3 oligonucleotide primers. In some embodiments, the oligonucleotide primer is a template-switching oligonucleotide primer.

Input and Target RNA

[0059] In one aspect, the present disclosure features methods for enhancing a polymerase reaction, e.g., a reverse transcription reaction. The polymerase reaction, e.g., reverse transcription reaction, comprises a reaction mixture, e.g., a reverse transcription reaction mixture and an input polynucleotide, e.g., a plurality of input polynucleotides. An input polynucleotide may be a polynucleotide from a source, e.g., a cell or plurality of cells, that is included in a polymerase reaction, e.g., a reverse transcription reaction. An input polynucleotide may or may not serve as a template for a polymerase reaction, e.g., a reverse transcription reaction.

[0060] The input polynucleotide may be a ribonucleic acid (RNA) or a deoxyribonucleic acid (DNA). In some embodiments, the reaction mixture comprises a plurality of input polynucleotides, e.g., a plurality of RNA or DNA polynucleotides. In some embodiments, the input polynucleotide is RNA. In some embodiments, the input polynucleotide is DNA. In some embodiments, the input polynucleotide is a plurality of input polynucleotides, e.g., a plurality of RNA molecules and/or a plurality of DNA molecules. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules, e.g., 10, 100, 1,000, 10,000, 100,000, 1,000,000, 10,000,000, 100,000,000 or more RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 10 RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 100 RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 1,000 RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 10,000 RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 100,000 RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 1,000,000 RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 10,000,000 RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 100,000,000 or more RNA molecules. In some embodiments, the input polynucleotide is a plurality of RNA molecules, e.g., 10-100, 100-1,000, 1,000-10,000, 10,000-100,000, 100,000-1,000,000, 1,000,000-10,000,000, or 10,000,000-100,000,000 or more RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 10-100 RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 100-1,000 RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 1,000-10,000 RNA molecules. In some embodiments, the plurality of input

polynucleotides comprises about 10,000-100,000 RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 100,000-1,000,000 RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 1,000,000-10,000,000 RNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 10,000,000-100,000,000 RNA molecules.

[0061] In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules, e.g., a plurality of RNA molecules comprising the same sequence and/or a plurality of RNA molecules comprising different sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising the same nucleotide sequence. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising different nucleotide sequences, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000 or more unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 2 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 10 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 50 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 100 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 500 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 1,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 5,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 10,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 20,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 50,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a

plurality of RNA molecules comprising about 100,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising more than 100,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising different nucleotide sequences, e.g., 1-10, 10-100, 100-1,000, 1,000-10,000, or 10,000-100,000 or more unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 10-100 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 100-1,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 1,000-10,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 10,000-100,000 or more unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules comprising about 10-100,000 or more unique nucleotide sequences.

[0062] In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules, e.g., 10, 100, 1,000, 10,000, 100,000, 1,000,000, 10,000,000, 100,000,000 or more DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 10 DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 100 DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 1,000 DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 10,000 DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 100,000 DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 1,000,000 DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 10,000,000 DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 100,000,000 or more DNA molecules. In some embodiments, the input polynucleotide is a plurality of DNA molecules, e.g., 10-100, 100-1,000, 1,000-10,000, 10,000-100,000, 100,000-1,000,000, 1,000,000-10,000,000, or 10,000,000-100,000,000 or more DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 10-100 DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 100-1,000 DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 1,000-

10,000 DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 10,000-100,000 DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 100,000-1,000,000 DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 1,000,000-10,000,000 DNA molecules. In some embodiments, the plurality of input polynucleotides comprises about 10,000,000-100,000,000 DNA molecules.

[0063] In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules, e.g., a plurality of DNA molecules comprising the same sequence and/or a plurality of DNA molecules comprising different sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising the same nucleotide sequence. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising different nucleotide sequences, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000 or more unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 2 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 10 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 50 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 100 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 500 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 1,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 5,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 10,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 20,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 50,000 unique

nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 100,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising more than 100,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising different nucleotide sequences, e.g., 1-10, 10-100, 100-1,000, 1,000-10,000, or 10,000-100,000 or more unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 10-100 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 100-1,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 1,000-10,000 unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 10,000-100,000 or more unique nucleotide sequences. In some embodiments, the plurality of input polynucleotides comprises a plurality of DNA molecules comprising about 10-100,000 or more unique nucleotide sequences.

[0064] In some embodiments, the plurality of input polynucleotides comprises a plurality of RNA molecules and a plurality of DNA molecules. In some embodiments, the plurality of input polynucleotides comprises 1% RNA and 99% DNA. In some embodiments, the plurality of input polynucleotides comprises 10% RNA and 90% DNA. In some embodiments, the plurality of input polynucleotides comprises 20% RNA and 80% DNA. In some embodiments, the plurality of input polynucleotides comprises 30% RNA and 70% DNA. In some embodiments, the plurality of input polynucleotides comprises 40% RNA and 60% DNA. In some embodiments, the plurality of input polynucleotides comprises 50% RNA and 50% DNA. In some embodiments, the plurality of input polynucleotides comprises 60% RNA and 40% DNA. In some embodiments, the plurality of input polynucleotides comprises 70% RNA and 30% DNA. In some embodiments, the plurality of input polynucleotides comprises 80% RNA and 20% DNA. In some embodiments, the plurality of input polynucleotides comprises 90% RNA and 10% DNA. In some embodiments, the plurality of input polynucleotides comprises 95% RNA and 5% DNA. In some embodiments, the plurality of input polynucleotides comprises 99% RNA and 1% DNA. In some embodiments, the plurality of input polynucleotides comprises 99.5% RNA and 0.5% DNA. In some embodiments, the plurality of input polynucleotides

comprises 99.9% RNA and 0.1% DNA. In some embodiments, the plurality of input polynucleotides comprises no DNA.

[0065] In some embodiments, the input polynucleotide is about 5 to about 2,500,000 nucleotides in length, e.g., about 5 nucleotides, 50 nucleotides, 100 nucleotides, 500 nucleotides, 1,000 nucleotides, 5,000 nucleotides, 7,000 nucleotides, 10,000 nucleotides, 15,000 nucleotides, 20,000 nucleotides, 50,000 nucleotides, 100,000 nucleotides, 500,000 nucleotides, 1,000,000 nucleotides, or 2,500,000 nucleotides, or more in length. In some embodiments, the input polynucleotide is about 5 nucleotides in length. In some embodiments, the input polynucleotide is about 50 nucleotides in length. In some embodiments, the input polynucleotide is about 100 nucleotides in length. In some embodiments, the input polynucleotide is about 500 nucleotides in length. In some embodiments, the input polynucleotide is about 1,000 nucleotides in length. In some embodiments, the input polynucleotide is about 5,000 nucleotides in length. In some embodiments, the input polynucleotide is about 7,000 nucleotides in length. In some embodiments, the input polynucleotide is about 10,000 nucleotides in length. In some embodiments, the input polynucleotide is about 15,000 nucleotides in length. In some embodiments, the input polynucleotide is about 20,000 nucleotides in length. In some embodiments, the input polynucleotide is about 50,000 nucleotides in length. In some embodiments, the input polynucleotide is about 100,000 nucleotides in length. In some embodiments, the input polynucleotide is about 500,000 nucleotides in length. In some embodiments, the input polynucleotide is about 1,000,000 nucleotides in length. In some embodiments, the input polynucleotide is about 2,500,000 nucleotides in length.

[0066] In some embodiments, the input polynucleotide is associated with another molecule, e.g., a protein or another polynucleotide. In some embodiments, the input polynucleotide is bound by a protein. In some embodiments, the input polynucleotide is associated with another polynucleotide, e.g., physically interacts with another polynucleotide, e.g., base-pairs with another polynucleotide. In some embodiments, the input polynucleotide physically interacts with itself, e.g., forms double-stranded regions within a single polynucleotide.

[0067] In some embodiments, the input polynucleotide is double stranded. In some embodiments, the input polynucleotide is single stranded. In some embodiments, the input polynucleotide has a structure, e.g., a secondary structure. In some embodiments, the input

polynucleotide has a secondary structure, e.g., a stem-loop, hairpin, helix, or pseudoknot. In some embodiments, the input polynucleotide forms a stem-loop structure. In some embodiments, the input polynucleotide forms a hairpin structure. In some embodiments, the input polynucleotide forms a helix structure. In some embodiments, the input polynucleotide forms a pseudoknot structure.

[0068] In some embodiments, the input polynucleotide is naturally occurring. In some embodiments, the input polynucleotide is synthetic. In some embodiments, the source of the input polynucleotide is a cell or a source other than a cell. In some embodiments, the source of the input polynucleotide is a cell, e.g., a eukaryotic cell or a prokaryotic cell. In some embodiments, the eukaryotic cell is an animal cell, fungal cell, or a plant cell. In some embodiments, the eukaryotic cell is a mammalian cell. In some embodiments, the prokaryotic cell is a bacterial cell or an archaeal cell. In some embodiments, the source of the input polynucleotide is a virus. In some embodiments, the source of the input polynucleotide is a single cell or a plurality of cells. In some embodiments, the source of the input polynucleotide is a single cell. In some embodiments, the source of the input polynucleotide is a plurality of cells.

[0069] In some embodiments, the input polynucleotide is purified from its source, e.g., is separated from protein, lipids, carbohydrates, small molecules, and/or other types of nucleic acids in the source material. In some embodiments, some embodiments, the input polynucleotide is not purified from its source. In some embodiments, the input polynucleotide is extracted from a source, e.g., a cell or tissue. In some embodiments, the input polynucleotide is extracted from a cell. In some embodiments, the input polynucleotide is extracted from a eukaryotic cell. In some embodiments, the input polynucleotide is extracted from an animal cell. In some embodiments, the input polynucleotide is extracted from a plant cell. In some embodiments, the input polynucleotide is extracted from a fungal cell. In some embodiments, the input polynucleotide is extracted from a yeast cell. In some embodiments, the input polynucleotide is extracted from a mammalian cell. In some embodiments, the input polynucleotide is extracted from a prokaryotic cell. In some embodiments, the input polynucleotide is extracted from an archaeal cell. In some embodiments, the input polynucleotide is extracted from a bacterial cell. In some embodiments, the input polynucleotide is extracted from a viral particle. In some embodiments, the input polynucleotide is extracted from a plurality of cells. In some embodiments, the input polynucleotide is extracted from a single cell. In some embodiments, the extracted input

polynucleotide is present in a vial. In some embodiments, the extracted input polynucleotide is present in a plate. In some embodiments, the extracted input polynucleotide is present in a microtube. In some embodiments, the extracted input polynucleotide is present in a reaction vessel.

[0070] In some embodiments, the input polynucleotide is input RNA, e.g., substrate RNA for a reverse transcriptase, e.g., template RNA for cDNA synthesis. For example, the input RNA may comprise target RNA, e.g., RNA for which a value is acquired using a method of reverse transcription. In some embodiments, the input RNA comprises a mixture of a plurality of RNAs, e.g., a mixture of RNAs having identical or non-identical sequences. In some embodiments, the input RNA comprises a mixture of RNAs consisting of identical sequences. In some embodiments, the input RNA comprises a mixture of RNAs consisting of non-identical sequences.

[0071] In some embodiments, the source of the input RNA is a cell or a source other than a cell. In some embodiments, the source of the input RNA is a cell, e.g., a eukaryotic cell or a prokaryotic cell. In some embodiments, the eukaryotic cell is an animal cell, fungal cell, or a plant cell. In some embodiments, the eukaryotic cell is a mammalian cell. In some embodiments, the prokaryotic cell is a bacterial cell or an archaeal cell. In some embodiments, the source of the input RNA is a virus. In some embodiments, the source of the input RNA is a single cell or a plurality of cells. In some embodiments, the source of the input RNA is a single cell. In some embodiments, the source of the input RNA is a plurality of cells. In some embodiments, the input RNA comprises total cellular RNA, e.g., ribosomal RNA (rRNA), transfer RNA (tRNA), messenger RNA (mRNA), non-coding RNA (ncRNA), microRNA (miRNA), or any RNA present in a cell. In some embodiments, the input RNA is rRNA. In some embodiments, the input RNA is tRNA. In some embodiments, the input RNA is mRNA. In some embodiments, the input RNA is ncRNA. In some embodiments, the input RNA is miRNA.

[0072] In some embodiments, the input RNA is purified from its source, e.g., is separated from protein, lipids, carbohydrates, small molecules, and/or other types of nucleic acids in the source material. In some embodiments, the input RNA is purified from its source to remove DNA. In some embodiments, some embodiments, the input RNA is not purified from its source. In some embodiments, the input RNA is extracted from a source, e.g., a cell or tissue. In some embodiments, the input RNA is extracted from a cell. In some embodiments, the input RNA is

extracted from a eukaryotic cell. In some embodiments, the input RNA is extracted from an animal cell. In some embodiments, the input RNA is extracted from a plant cell. In some embodiments, the input RNA is extracted from a fungal cell. In some embodiments, the input RNA is extracted from a yeast cell. In some embodiments, the input RNA is extracted from a mammalian cell. In some embodiments, the input RNA is extracted from a prokaryotic cell. In some embodiments, the input RNA is extracted from an archaeal cell. In some embodiments, the input RNA is extracted from a bacterial cell. In some embodiments, the input RNA is extracted from a viral particle. In some embodiments, the input RNA is extracted from a plurality of cells. In some embodiments, the input RNA is extracted from a single cell. In some embodiments, the extracted input RNA is present in a vial. In some embodiments, the extracted input RNA is present in a plate. In some embodiments, the extracted input RNA is present in a microtube. In some embodiments, the extracted input RNA is present in a reaction vessel.

[0073] In some embodiments, the input RNA is about 5 to about 2,500,000 nucleotides in length, e.g., about 5 nucleotides, 50 nucleotides, 100 nucleotides, 500 nucleotides, 1,000 nucleotides, 5,000 nucleotides, 7,000 nucleotides, 10,000 nucleotides, 15,000 nucleotides, 20,000 nucleotides, 50,000 nucleotides, 100,000 nucleotides, 500,000 nucleotides, 1,000,000 nucleotides, or 2,500,000 nucleotides, or more in length. In some embodiments, the input RNA is about 5 nucleotides in length. In some embodiments, the input RNA is about 50 nucleotides in length. In some embodiments, the input RNA is about 100 nucleotides in length. In some embodiments, the input RNA is about 500 nucleotides in length. In some embodiments, the input RNA is about 1,000 nucleotides in length. In some embodiments, the input RNA is about 5,000 nucleotides in length. In some embodiments, the input RNA is about 7,000 nucleotides in length. In some embodiments, the input RNA is about 10,000 nucleotides in length. In some embodiments, the input RNA is about 15,000 nucleotides in length. In some embodiments, the input RNA is about 20,000 nucleotides in length. In some embodiments, the input RNA is about 50,000 nucleotides in length. In some embodiments, the input RNA is about 100,000 nucleotides in length. In some embodiments, the input RNA is about 500,000 nucleotides in length. In some embodiments, the input RNA is about 1,000,000 nucleotides in length. In some embodiments, the input RNA is about 2,500,000 nucleotides in length.

[0074] In some embodiments, the input RNA is associated with another molecule, e.g., a protein or another polynucleotide. In some embodiments, the input RNA is bound by a protein.

In some embodiments, the input RNA is associated with another polynucleotide, e.g., physically interacts with another polynucleotide, e.g., base-pairs with another polynucleotide. In some embodiments, the input RNA physically interacts with itself, e.g., forms double-stranded regions within a single RNA molecule.

[0075] In some embodiments, the input RNA is double stranded. In some embodiments, the input RNA is single stranded. In some embodiments, the input RNA has a structure, e.g., a secondary structure. In some embodiments, the input RNA has a secondary structure, e.g., a stem-loop, hairpin, helix, or pseudoknot. In some embodiments, the input RNA forms a stem-loop structure. In some embodiments, the input RNA forms a hairpin structure. In some embodiments, the input RNA forms a helix structure. In some embodiments, the input RNA forms a pseudoknot structure.

[0076] In some embodiments, the level of input RNA in the reverse transcription reaction mixture is from about 1 μ g to about 10 fg, e.g., the level of input RNA is about 1 μ g, 500 ng, 250 ng, 100 ng, 50 ng, 25 ng, 10 ng, 5 ng, 1 ng, 500 pg, 250 pg, 100 pg, 50 pg, 25 pg, 10 pg, 5 pg, 1 pg, 500 fg, 250 fg, 100 fg, 50 fg, 25 fg, 10 fg, or less. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 1 μ g. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 500 ng. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 250 ng. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 100 ng. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 50 ng. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 25 ng. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 10 ng. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 5 ng. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 1 ng. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 500 pg. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 250 pg. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 100 pg. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 50 pg. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 25 pg. In some embodiments, the level of input RNA in the reverse

transcription reaction mixture is about 10 pg. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 5 pg. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 1 pg. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 500 fg. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 250 fg. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 100 fg. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 50 fg. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 25 fg. In some embodiments, the level of input RNA in the reverse transcription reaction mixture is about 10 fg.

[0077] In some embodiments, the concentration of input RNA in the reverse transcription reaction mixture is from about 0.001 zeptomolar (zM) to about 1 micromolar (μ M), e.g., 0.001 zM, 0.01 zM, 0.1 zM, 1 zM, 0.01 attomolar (aM), 0.1 aM, 1 aM, 0.01 femtomolar (fM), 0.1 fM, 1 fM, 0.01 picomolar (pM), 0.1 pM, 1 pM, 0.01 nanomolar (nM), 0.1 nM, 1 nM, 0.01 μ M, 0.1 μ M, or 1 μ M or greater. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 0.001 zM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 0.01 zM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 0.1 zM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 1 zM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 0.01 aM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 0.1 aM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 1 aM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 0.01 fM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 0.1 fM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 1 fM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 0.01 pM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 0.1 pM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 1 pM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is

about 0.01 nM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 0.1 nM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 1 nM. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 0.01 μ M. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 0.1 μ M. In some embodiments, the concentration of input RNA in a reverse transcription reaction mixture is about 1 μ M.

[0078] In some embodiments, the input polynucleotide comprises a target polynucleotide, e.g., a polynucleotide detected in a polymerase reaction, e.g., a reverse transcription reaction. A target polynucleotide is a polynucleotide present in the plurality of input polynucleotides that serves as a template for a polymerase reaction, e.g., a reverse transcriptase reaction. For example, some of the input polynucleotides in a plurality of input polynucleotides may be used as templates in a polymerase reaction, e.g., a reverse transcription reaction, while other input polynucleotides may not be used as a template. In some embodiments, an input polynucleotide may also be a target polynucleotide. In some embodiments, an input polynucleotide may not be a target polynucleotide. In some embodiments, the product of a polymerase reaction, e.g., the product of a reverse transcription reaction, e.g., a cDNA product, is complementary to a target polynucleotide, e.g., a target RNA. In some embodiments, the input RNA comprises a target RNA, e.g., an RNA sequence detected in the reverse transcription reaction. A target RNA is an RNA molecule present in the plurality of input polynucleotides that serves as a template for a polymerase reaction, e.g., a reverse transcriptase reaction. In some embodiments, a cDNA product resulting from a reverse transcription reaction is complementary to a target RNA.

[0079] In some embodiments, the target RNA is naturally occurring. In some embodiments, the target RNA is synthetic. In some embodiments, the source of the target RNA is a cell or a source other than a cell. In some embodiments, the source of the target RNA is a cell, e.g., a eukaryotic cell or a prokaryotic cell. In some embodiments, the eukaryotic cell is an animal cell, fungal cell, or a plant cell. In some embodiments, the eukaryotic cell is a mammalian cell. In some embodiments, the prokaryotic cell is a bacterial cell or an archaeal cell. In some embodiments, the source of the target RNA is a virus. In some embodiments, the source of the target RNA is a single cell or a plurality of cells. In some embodiments, the source of the target

RNA is a single cell. In some embodiments, the source of the target RNA is a plurality of cells. In some embodiments, the target RNA comprises total cellular RNA, e.g., ribosomal RNA (rRNA), transfer RNA (tRNA), messenger RNA (mRNA), non-coding RNA (ncRNA), microRNA (miRNA), or any RNA present in a cell. In some embodiments, the target RNA is rRNA. In some embodiments, the target RNA is tRNA. In some embodiments, the target RNA is mRNA. In some embodiments, the target RNA is ncRNA. In some embodiments, the target RNA is miRNA.

[0080] In some embodiments, the target RNA is about 5 to about 2,500,000 nucleotides in length, e.g., about 5 nucleotides, 50 nucleotides, 100 nucleotides, 500 nucleotides, 1,000 nucleotides, 5,000 nucleotides, 7,000 nucleotides, 10,000 nucleotides, 15,000 nucleotides, 20,000 nucleotides, 50,000 nucleotides, 100,000 nucleotides, 500,000 nucleotides, 1,000,000 nucleotides, or 2,500,000 nucleotides, or more in length. In some embodiments, the target RNA is about 5 nucleotides in length. In some embodiments, the target RNA is about 50 nucleotides in length. In some embodiments, the target RNA is about 100 nucleotides in length. In some embodiments, the target RNA is about 500 nucleotides in length. In some embodiments, the target RNA is about 1,000 nucleotides in length. In some embodiments, the target RNA is about 5,000 nucleotides in length. In some embodiments, the target RNA is about 7,000 nucleotides in length. In some embodiments, the target RNA is about 10,000 nucleotides in length. In some embodiments, the target RNA is about 15,000 nucleotides in length. In some embodiments, the target RNA is about 20,000 nucleotides in length. In some embodiments, the target RNA is about 50,000 nucleotides in length. In some embodiments, the target RNA is about 100,000 nucleotides in length. In some embodiments, the target RNA is about 500,000 nucleotides in length. In some embodiments, the target RNA is about 1,000,000 nucleotides in length. In some embodiments, the target RNA is about 2,500,000 nucleotides in length.

[0081] In some embodiments, the level of target RNA in the reverse transcription reaction mixture is from about 1 μ g to about 10 fg, e.g., the level of input RNA is about 1 μ g, 500 ng, 250 ng, 100 ng, 50 ng, 25 ng, 10 ng, 5 ng, 1 ng, 500 pg, 250 pg, 100 pg, 50 pg, 25 pg, 10 pg, 5 pg, 1 pg, 500 fg, 250 fg, 100 fg, 50 fg, 25 fg, 10 fg, or less. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 1 μ g. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 500 ng. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 250

ng. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 100 ng. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 50 ng. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 25 ng. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 10 ng. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 5 ng. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 1 ng. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 500 pg. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 250 pg. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 100 pg. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 50 pg. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 25 pg. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 10 pg. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 5 pg. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 1 pg. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 500 fg. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 250 fg. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 100 fg. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 50 fg. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 25 fg. In some embodiments, the level of target RNA in the reverse transcription reaction mixture is about 10 fg.

[0082] In some embodiments, the concentration of target RNA in the reverse transcription reaction mixture is from about 0.001 zeptomolar (zM) to about 1 micromolar (μ M), e.g., 0.001 zM, 0.01 zM, 0.1 zM, 1 zM, 0.01 attomolar (aM), 0.1 aM, 1 aM, 0.01 femtomolar (fM), 0.1 fM, 1 fM, 0.01 picomolar (pM), 0.1 pM, 1 pM, 0.01 nanomolar (nM), 0.1 nM, 1 nM, 0.01 μ M, 0.1 μ M, or 1 μ M or greater. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 0.001 zM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 0.01 zM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is

about 0.1 zM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 1 zM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 0.01 aM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 0.1 aM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 1 aM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 0.01 fM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 0.1 fM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 1 fM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 0.01 pM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 0.1 pM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 1 pM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 0.01 nM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 0.1 nM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 1 nM. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 0.01 μ M. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 0.1 μ M. In some embodiments, the concentration of target RNA in a reverse transcription reaction mixture is about 1 μ M.

Anionic Polymers

[0083] In one aspect, the present disclosure features a reverse transcription reaction mixture comprising an anionic polymer. An anionic polymer, as described herein, is any naturally occurring or non-naturally occurring polymer, e.g., a plurality of repeating monomer units bearing an overall negative charge. The anionic polymer may comprise a homogenous set of monomeric units, or may comprise a heterogenous set of monomeric units, e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more different monomeric units. The anionic polymer may be a linear polymer, branched polymer, or cross-linked polymer.

[0084] In some embodiments, the anionic polymer is naturally occurring. In some embodiments, the anionic polymer comprises an oligonucleotide, peptide, polypeptide, or oligosaccharide, each of which independently bears a net negative charge. In some embodiments,

the anionic polymer is non-naturally occurring. In some embodiments, the anionic polymer is an oligonucleotide, e.g., a deoxyribonucleic acid (DNA) or a ribonucleic acid (RNA). In some embodiments, the anionic polymer is a polynucleotide. In some embodiments, the anionic polymer is DNA. In some embodiments, the anionic polymer is carrier DNA. In some embodiments, the anionic polymer is genomic DNA. In some embodiments, the anionic polymer is viral DNA. In some embodiments, the anionic polymer is phage DNA. In some embodiments, the anionic polymer is archaeal DNA. In some embodiments, the anionic polymer is prokaryotic DNA. In some embodiments, the anionic polymer is eukaryotic DNA. In some embodiments, the anionic polymer is single stranded DNA. In some embodiments, the anionic polymer is non-coding DNA, e.g., DNA that does not encode an RNA or a polypeptide. In some embodiments, the anionic polymer is intergenic DNA. In some embodiments, the anionic polymer is DNA comprising a sequence that does not occur in nature, e.g., a sequence that does not occur in the source of an input polynucleotide. In some embodiments, the anionic polymer is synthetic DNA. In some embodiments, the anionic polymer is RNA. In some embodiments, the anionic polymer is carrier RNA. In some embodiments, the anionic polymer is a mixture of different RNA molecules. In some embodiments, the anionic polymer is a single RNA molecule. In some embodiments, the anionic polymer is genomic RNA. In some embodiments, the anionic polymer is viral RNA. In some embodiments, the anionic polymer is phage RNA. In some embodiments, the anionic polymer is MS2 phage genomic RNA. In some embodiments, the anionic polymer is archaeal RNA. In some embodiments, the anionic polymer is prokaryotic RNA. In some embodiments, the anionic polymer is eukaryotic RNA. In some embodiments, the anionic polymer is single stranded RNA. In some embodiments, the anionic polymer is non-coding RNA, e.g., RNA that does not encode a polypeptide or a regulatory sequence. In some embodiments, the anionic polymer is intergenic RNA. In some embodiments, the anionic polymer is RNA comprising a sequence that does not occur in nature, e.g., a sequence that does not occur in the source of an input polynucleotide. In some embodiments, the anionic polymer is synthetic RNA.

[0085] In some embodiments, the anionic polymer is a peptide. In some embodiments, the anionic polymer is a polypeptide.

[0086] In some embodiments, the anionic polymer is an oligosaccharide, e.g., a glycosaminoglycan, e.g., alginate, hyaluronate, dextran, or heparin. Exemplary anionic polymers

include glycosaminoglycan, alginate, hyaluronate, heparin, unfractionated heparin, low molecular weight heparin, heparin-mimicking polymer, modified dextran, carboxymethyl benzylamide sulfonate dextran, sulfated glycopolymer, poly(GEMA)-sulfate, sulfated mannose polymer, sulfated lactose polymer, polyaromatic anionic compound, polyionomer, sulfonated ionomer, pectin, fucoidan, gum arabic, polysulfonated compound, carrageenan, iota-carrageenan, kappa-carrageenan, lambda-carrageenan, mu-carrageenan, nu-carrageenan, theta-carrageenan, xi-carrageenan, alpha-carrageenan, beta-carrageenan, gamma-carrageenan, omega-carrageenan, delta-carrageenan, or psi-carrageenan. In some embodiments, the anionic polymer is a glycosaminoglycan. In some embodiments, the anionic polymer is alginate. In some embodiments, the anionic polymer is hyaluronate. In some embodiments, the anionic polymer is heparin (e.g., unfractionated heparin or low molecular weight heparin). In some embodiments, the anionic polymer is a heparin-mimicking polymer, e.g., a polymer having similar structure and properties as heparin, e.g., a modified dextran, sulfated glycopolymer, polyaromatic anionic compound, sulfonated ionomer, or a polysulfonated compound. In some embodiments, the anionic polymer is a modified dextran, e.g., a carboxymethyl benzylamide sulfonate dextran. In some embodiments, the anionic polymer is a carboxymethyl benzylamide sulfonate dextran. In some embodiments, the anionic polymer is a polyaromatic anionic compound. In some embodiments, the anionic polymer is a sulfated glycopolymer, e.g., poly(GEMA)-sulfate, sulfated mannose polymer, or sulfated lactose polymer. In some embodiments, the anionic polymer is poly(GEMA)-sulfate. In some embodiments, the anionic polymer is a sulfated mannose polymer. In some embodiments, the anionic polymer is a sulfated lactose polymer. In some embodiments, the anionic polymer is a polysulfonated compound. In some embodiments, the anionic polymer is a polyionomer, e.g., a sulfated ionomer. In some embodiments, the anionic polymer is pectin. In some embodiments, the anionic polymer is fucoidan. In some embodiments, the anionic polymer is gum arabic. In some embodiments, the anionic polymer is carrageenan, e.g., iota-carrageenan, kappa-carrageenan, lambda-carrageenan, mu-carrageenan, nu-carrageenan, theta-carrageenan, xi-carrageenan, alpha-carrageenan, beta-carrageenan, gamma-carrageenan, omega-carrageenan, delta-carrageenan, or psi-carrageenan. In some embodiments, the anionic polymer is iota-carrageenan.

[0087] In other embodiments, the anionic polymer is non-naturally occurring. The anionic polymer may comprise polystyrene, polyethylene, polypropylene, polyacetylene,

poly(vinyl chloride) (PVC), polyolefin copolymers, poly(urethane)s, polyacrylic acid (PAA), polymethacrylates, polyacrylamides and polymethacrylamides, poly(methyl methacrylate), poly(2-hydroxyethyl methacrylate), polyesters, polysiloxanes, polydimethylsiloxane (PDMS), polyethers, poly(orthoester), poly(carbonates), poly(hydroxyalkanoate)s, polyfluorocarbons, polyethylene glycol, nylon, polyalkenes, phenolic resins, natural and synthetic elastomers, adhesives and sealants, polyolefins, polysulfones, polyacrylonitrile, poly(glycolic acid), poly(lactic acid) (PLA), poly(lactic glycolic acid) (PLGA), a polydioxanone (PDA), polycarbonates, (e.g., polyamides (e.g., nylon)), fluoroplastics, carbon fiber, and blends or copolymers thereof. In an embodiment, the anionic polymer comprises PLA or PLGA. In an embodiment, the anionic polymer comprises PAA. In some embodiments, the anionic polymer is polyacrylic acid.

[0088] The anionic polymer may comprise a single negatively charged repeating monomer, or comprise a set of positively and negatively charged monomers (wherein the polymer in its entirety bears a net negative charge). The anionic polymer may comprise about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 750, 1000, 1250, 1500, 2000, 2500, 3000, 4000, 5000, or more monomers bearing a net negative charge. The anionic polymer may comprise a net negative charge of about -1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, -12, -13, -14, -15, -16, -17, -18, -19, -20, -21, -22, -23, -24, -25, -26, -27, -28, -29, -30, -31, -32, -33, -34, -35, -36, -37, -38, -39, -40, -41, -42, -43, -44, -45, -46, -47, -48, -49, -50, -51, -52, -53, -54, -55, -56, -57, -58, -59, -60, -61, -62, -63, -64, -65, -66, -67, -68, -69, -70, -71, -72, -73, -74, or -75, or less.

[0089] In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is between about 0.5 ng/μL to about 10 μg/μL, e.g., a concentration of about 0.5 ng/μL, 1 ng/μL, 1.5 ng/μL, 2 ng/μL, 2.5 ng/μL, 3 ng/μL, 3.5 ng/μL, 4 ng/μL, 4.5 ng/μL, 5 ng/μL, 5.5 ng/μL, 6 ng/μL, 6.5 ng/μL, 7 ng/μL, 7.5 ng/μL, 8 ng/μL, 8.5 ng/μL, 9 ng/μL, 9.5 ng/μL, 10 ng/μL, 11 ng/μL, 12 ng/μL, 13 ng/μL, 14 ng/μL, 15 ng/μL, 16 ng/μL, 17 ng/μL, 18 ng/μL, 19 ng/μL, 20 ng/μL, 21 ng/μL, 22 ng/μL, 23 ng/μL, 24 ng/μL, 25 ng/μL, 26 ng/μL, 27 ng/μL, 28 ng/μL, 29 ng/μL, 30 ng/μL, 40 ng/μL, 50 ng/μL, 100 ng/μL, 250 ng/μL, 500 ng/μL, 750 ng/μL, 1 μg/μL, 2 μg/μL, 3 μg/μL, 4 μg/μL, 5 μg/μL, 6 μg/μL, 7 μg/μL, 8 μg/μL, 9 μg/μL, 10 μg/μL or greater. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 0.5 ng/μL. In some embodiments,

[illegible]

[illegible]

reverse transcription reaction mixture is about 100 ng/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 200 ng/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 300 ng/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 400 ng/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 500 ng/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 600 ng/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 700 ng/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 800 ng/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 900 ng/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 1 μ g/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 2 μ g/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 3 μ g/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 4 μ g/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 5 μ g/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 6 μ g/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 7 μ g/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 8 μ g/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 9 μ g/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is about 10 μ g/ μ L. In some embodiments, the concentration of the anionic polymer in the reverse transcription reaction mixture is greater than 10 μ g/ μ L.

[0090] In some embodiments, the anionic polymer in the reverse transcription reaction mixture is an alginate. Alginate is a polysaccharide made up of β -D-mannuronic acid (M) and α -L-guluronic acid (G). In some embodiments, the alginate is a high guluronic acid (G) alginate, and comprises greater than about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or more

guluronic acid (G). In some embodiments, the alginate is a high mannuronic acid (M) alginate, and comprises greater than about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or more mannuronic acid (M). In some embodiments, the ratio of M:G is about 1. In some embodiments, the ratio of M:G is less than 1. In some embodiments, the ratio of M:G is greater than 1. In an embodiment, the amount of alginate in the RT reaction mixture (e.g., by % weight of the particle, actual weight of the alginate) can be at least 5%, e.g., at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more, e.g., w/w; less than 20%, e.g., less than 20%, 15%, 10%, 5%, 1%, 0.5%, 0.1%, or less.

[0091] In some embodiments, the anionic polymer in the reverse transcription reaction mixture is heparin. Heparin is a glycosaminoglycan polysaccharide made up of repeating disaccharide units bearing negatively charged sulfate groups. Heparin most commonly comprises disaccharide units such as IsoA(2S)-GlcNS(6S), but less commonly may comprise one or more of the disaccharides IdoA(2S)-GlcNS, IdoA-GlcNS(6S), GlcA-GlcNAc, GlcA-GlcNS, and IdoA-GlcNS. In an embodiment, heparin may be unfractionated heparin or low molecular weight heparin (e.g., fractionated heparin). In an embodiment, the amount of heparin in the RT reaction mixture (e.g., by % weight of the particle, actual weight of the heparin) can be at least 5%, e.g., at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more, e.g., w/w; less than 20%, e.g., less than 20%, 15%, 10%, 5%, 1%, 0.5%, 0.1%, or less.

[0092] In some embodiments, the anionic polymer in the reverse transcription reaction mixture is polyacrylic acid (PAA). Polyacrylic acid comprises the chemical formula $(CH_2-CH-COOH)_n$, wherein n denotes the total number of repeating acrylic acid monomer units. Polyacrylic acid may be a homogenous polymer, comprising only PAA units, or a heterogenous polymer, comprising additional monomers of a different polymer. In an embodiment, the amount of polyacrylic acid in the RT reaction mixture (e.g., by % weight of the particle, actual weight of the polyacrylic acid) can be at least 5%, e.g., at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more, e.g., w/w; less than 20%, e.g., less than 20%, 15%, 10%, 5%, 1%, 0.5%, 0.1%, or less. As used herein, the terms “poly(acrylic acid)” and “polyacrylic acid” are interchangeable.

Reaction Mixture

[0093] The present disclosure further provides for reaction mixtures for reverse transcribing nucleic acid molecules, as well as reverse transcription methods employing such

reaction solutions and product nucleic acid molecules produced using such methods, which comprise an anionic polymer for enhancing the activity of the polymerase, e.g., reverse transcriptase. In many instances, reaction mixtures described herein may contain one or more of the following components: (1) one or more buffering agent (e.g., sodium phosphate, sodium acetate, 2-(N-morpholino)-ethanesulfonic acid (MES), tris-(hydroxymethyl)aminomethane (Tris), 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPS), citrate, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), acetate, 3-(N-morpholino)propanesulfonic acid (MOPS), N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), etc.), (2) one or more monovalent cationic salt (e.g., LiCl, NaCl, KCl, NH₄Cl, RbCl, CsCl, etc.), (3) one or more divalent cationic salt (e.g., MnCl₂, MgCl₂, MgSO₄, CaCl₂, etc.), (4) one or more reducing agent (e.g., dithiothreitol, 2-mercaptoethanol, etc.), (5) one or more ionic or non-ionic detergent (e.g., TRITON X-100™, NONIDET P40™, sodium dodecyl sulphate, etc.), (6) one or more crowding or stabilizing agents (e.g., trehalose, betaine, BSA, glycerol, PEG8000, etc.) (7) one or more DNA polymerase inhibitor (e.g., Actinomycin D, etc.), (8) nucleotides (e.g., dNTPs, such as dGTP, dATP, dCTP, dTTP, etc.), (9) RNA to be reverse transcribed and/or amplified, (10) one or more RNase inhibitor (e.g., RNASEOUT™, Invitrogen Corporation, Carlsbad, Calif., etc.), (11) a reverse transcriptase, and/or (12) one or more diluent (e.g., water). Other components and/or constituents (e.g., an oligonucleotide primer, e.g., an RT primer or TSO primer) may also be present in reaction mixtures described herein.

[0094] In some embodiments, the reaction mixture comprises an optimized reaction buffer that enhances the RT activity of a reverse transcriptase, e.g., UltraMarathonRT or MarathonRT. In some embodiments, the optimized reaction buffer comprises a buffering agent, e.g., sodium phosphate, sodium acetate, 2-(N-morpholino)-ethanesulfonic acid (MES), tris-(hydroxymethyl)aminomethane (Tris), 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPS), citrate, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), acetate, 3-(N-morpholino)propanesulfonic acid (MOPS), N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS). In some embodiments, the buffering agent in the optimized reaction buffer is sodium phosphate. In some embodiments, the buffering agent in the optimized reaction buffer is sodium acetate. In some embodiments, the buffering agent in the optimized reaction buffer is 2-(N-morpholino)-ethanesulfonic acid (MES). In some embodiments, the

buffering agent in the optimized reaction buffer is tris-(hydroxymethyl)aminomethane (Tris). In some embodiments, the buffering agent in the optimized reaction buffer is 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPS). In some embodiments, the buffering agent in the optimized reaction buffer is N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES). In some embodiments, the buffering agent in the optimized reaction buffer is acetate. In some embodiments, the buffering agent in the optimized reaction buffer is 3-(N-morpholino)propanesulfonic acid (MOPS). In some embodiments, the buffering agent in the optimized reaction buffer is N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS). In some embodiments, the optimized reaction buffer comprises a crowding or stabilizing agent, e.g., polyethylene glycol (PEG) or glycerol. In some embodiments, the crowding agent in the optimized reaction buffer is PEG. In some embodiments, the crowding agent in the optimized reaction buffer is PEG8000. In some embodiments, the crowding agent in the optimized reaction buffer is glycerol. In some embodiments, the optimized reaction buffer comprises a monovalent cationic salt, e.g., LiCl, NaCl, KCl, NH₄Cl, RbCl, CsCl. In some embodiments, the monovalent cationic salt in the optimized reaction buffer is LiCl. In some embodiments, the monovalent cationic salt in the optimized reaction buffer is KCl. In some embodiments, the monovalent cationic salt in the optimized reaction buffer is NH₄Cl. In some embodiments, the monovalent cationic salt in the optimized reaction buffer is RbCl. In some embodiments, the monovalent cationic salt in the optimized reaction buffer is CsCl. In some embodiments, the optimized reaction buffer comprises a divalent cationic salt, e.g., MnCl₂, MgCl₂, MgSO₄, CaCl₂, SrCl₂, BaCl₂. In some embodiments, the divalent cationic salt in the optimized reaction buffer is MnCl₂. In some embodiments, the divalent cationic salt in the optimized reaction buffer is MgCl₂. In some embodiments, the divalent cationic salt in the optimized reaction buffer is MgSO₄. In some embodiments, the divalent cationic salt in the optimized reaction buffer is CaCl₂. In some embodiments, the divalent cationic salt in the optimized reaction buffer is SrCl₂. In some embodiments, the divalent cationic salt in the optimized reaction buffer is BaCl₂. In some embodiments, the optimized reaction buffer comprises a crowding agent, e.g., glycerol or PEG8000, at a concentration of about 1% to 50%. In some embodiments, the optimized reaction buffer comprises a buffering agent, e.g., MOPS, MES, HEPES, CAPS, TAPS, acetate, phosphate, or Tris, at a concentration of about 0.01 mM to about 1 M. In some embodiments, the optimized reaction buffer comprises a monovalent cationic salt, e.g., LiCl, NaCl, NH₄Cl, RbCl, CsCl or

KCl, at a concentration of about 1 mM to about 1 M. In some embodiments, the optimized reaction buffer comprises a divalent cationic salt, e.g., MnCl_2 , MgCl_2 , MgSO_4 , CaCl_2 , SrCl_2 , BaCl_2 , at a concentration of about 0.01 mM to about 100 mM. In some embodiments, the optimized reaction buffer comprises DTT at a concentration of about 0.1 mM to about 50 mM. In some embodiments, the pH of the optimized reaction buffer is about 6.5 to about 9. In some embodiments, the optimized reaction buffer comprises a crowding agent, e.g., glycerol or PEG8000, at a concentration of about 1% to 50%; a buffering agent, e.g., MOPS, MES, HEPES, CAPS, TAPS, acetate, phosphate, or Tris, at a concentration of about 0.01 mM to about 1 M; a monovalent cationic salt, e.g., LiCl, NaCl, NH_4Cl , RbCl, CsCl or KCl, at a concentration of about 1 mM to about 1 M; a divalent cationic salt, e.g., MnCl_2 , MgCl_2 , MgSO_4 , CaCl_2 , SrCl_2 , BaCl_2 , at a concentration of about 0.01 mM to about 100 mM; and DTT at a concentration of about 0.1 mM to about 50 mM, and wherein the reaction buffer has a pH of about 6.5 to 9. In one embodiment, the optimized reaction buffer comprises PEG8000 at a concentration of about 1% to 20%, Tris at a concentration of about 10mM to about 100mM; KCl at a concentration of about 20mM to about 500mM, MgCl_2 at a concentration of about 0.1mM to about 20mM, and DTT at a concentration of about 1mM to about 10mM, and wherein the reaction buffer has a pH of about 7.5 to 8.5. In one embodiment, the optimized reaction buffer comprises PEG8000 at a concentration of about 1% to 20%, MOPS at a concentration of about 10mM to about 100mM; KCl at a concentration of about 20mM to about 500mM, MgCl_2 at a concentration of about 0.1mM to about 20mM, and DTT at a concentration of about 1mM to about 10mM, and wherein the reaction buffer has a pH of about 7.5 to 8.5. In one embodiment, the optimized reaction buffer comprises PEG8000 at a concentration of about 1% to 20%, MES at a concentration of about 10mM to about 100mM; KCl at a concentration of about 20mM to about 500mM, MgCl_2 at a concentration of about 0.1mM to about 20mM, and DTT at a concentration of about 1mM to about 10mM, and wherein the reaction buffer has a pH of about 7.5 to 8.5. In one embodiment, the optimized reaction buffer comprises PEG8000 at a concentration of about 1% to 20%, HEPES at a concentration of about 10mM to about 100mM; KCl at a concentration of about 20mM to about 500mM, MgCl_2 at a concentration of about 0.1mM to about 20mM, and DTT at a concentration of about 1mM to about 10mM, and wherein the reaction buffer has a pH of about 7.5 to 8.5. In one embodiment, the optimized reaction buffer comprises glycerol at a concentration of about 1% to 40%, Tris at a concentration of about 10mM to about 100mM; KCl

at a concentration of about 20mM to about 500mM, MgCl₂ at a concentration of about 0.1mM to about 20mM, and DTT at a concentration of about 1mM to about 10mM, and wherein the reaction buffer has a pH of about 7.5 to 8.5. In one embodiment, the optimized reaction buffer comprises PEG8000 at a concentration of about 1% to 20%, MOPS at a concentration of about 10mM to about 100mM; KCl at a concentration of about 20mM to about 500mM, MgCl₂ at a concentration of about 0.1mM to about 20mM, and DTT at a concentration of about 1mM to about 10mM, and wherein the reaction buffer has a pH of about 7.5 to 8.5. In one embodiment, the optimized reaction buffer comprises glycerol at a concentration of about 1% to 40%, MES at a concentration of about 10mM to about 100mM; KCl at a concentration of about 20mM to about 500mM, MgCl₂ at a concentration of about 0.1mM to about 20mM, and DTT at a concentration of about 1mM to about 10mM, and wherein the reaction buffer has a pH of about 7.5 to 8.5. In one embodiment, the optimized reaction buffer comprises glycerol at a concentration of about 1% to 40%, HEPES at a concentration of about 10mM to about 100mM; KCl at a concentration of about 20mM to about 500mM, MgCl₂ at a concentration of about 0.1mM to about 20mM, and DTT at a concentration of about 1mM to about 10mM, and wherein the reaction buffer has a pH of about 7.5 to 8.5. In one embodiment, the optimized reaction buffer comprises about 20% glycerol, about 50 mM Tris, about 200 mM KCl, about 2 mM MgCl₂, about 5 mM DTT, and has a pH of about 8.3.

[0095] In one embodiment, the optimized reaction buffer further comprises a protein stabilizing agent. Exemplary protein stabilizing agents include, but are not limited to, osmolytic stabilizers such as glycerol, erythritol, arabitol, sorbitol, mannitol, xylitol, mannisdemannitol, glucosylglycerol, glucose, fructose, sucrose, trehalose, isofluorosid, dextrans, levans, and polyethylene glycol; amino acids and derivatives thereof such as glycine, alanine, proline, taurine, betaine, octopine, glutamate, sarcosine, γ -aminobutyric acid, trimethylamine, N-oxide (TMAO); ionic stabilizers such as citrate, sulfates, acetate, phosphates, and quaternary amines; and proteins such as bovine serum albumin (BSA).

[0096] In one embodiment, the optimized reaction buffer comprises trehalose at a concentration of about 0.1 M to about 2 M. In one embodiment, the optimized reaction buffer comprises betaine at a concentration of about 0.1 M to about 10 M. In one embodiment, the optimized reaction buffer comprises BSA at a concentration of about 0.5mg/mL to about

2mg/mL. In one embodiment, the optimized reaction buffer comprises glycerol at a concentration of about 1% to about 50%.

[0097] The concentration of the buffering agent in the reaction mixtures described herein may vary with the particular buffering agent used. Typically, the working concentration (i.e., the concentration in the reaction mixture) of the buffering agent will be from about 5 mM to about 500 mM (e.g., about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, about 100 mM, from about 5 mM to about 500 mM, from about 10 mM to about 500 mM, from about 20 mM to about 500 mM, from about 25 mM to about 500 mM, from about 30 mM to about 500 mM, from about 40 mM to about 500 mM, from about 50 mM to about 500 mM, from about 75 mM to about 500 mM, from about 100 mM to about 500 mM, from about 25 mM to about 50 mM, from about 25 mM to about 75 mM, from about 25 mM to about 100 mM, from about 25 mM to about 200 mM, from about 25 mM to about 300 mM, etc.). When Tris (e.g., Tris-HCl) is used, the Tris working concentration will typically be from about 5 mM to about 100 mM, from about 5 mM to about 75 mM, from about 10 mM to about 75 mM, from about 10 mM to about 60 mM, from about 10 mM to about 50 mM, from about 25 mM to about 50 mM, etc.

[0098] The final pH of solutions of the invention will generally be set and maintained by buffering agents present in reaction solutions of the invention. The pH of reaction solutions of the invention, and hence reaction mixtures of the invention, will vary with the particular use and the buffering agent present but will often be from about pH 5.5 to about pH 9.0 (e.g., about pH 6.0, about pH 6.5, about pH 7.0, about pH 7.1, about pH 7.2, about pH 7.3, about pH 7.4, about pH 7.5, about pH 7.6, about pH 7.7, about pH 7.8, about pH 7.9, about pH 8.0, about pH 8.1, about pH 8.2, about pH 8.3, about pH 8.4, about pH 8.5, about pH 8.6, about pH 8.7, about pH 8.8, about pH 8.9, about pH 9.0, from about pH 6.0 to about pH 8.5, from about pH 6.5 to about pH 8.5, from about pH 7.0 to about pH 8.5, from about pH 7.5 to about pH 8.5, from about pH 6.0 to about pH 8.0, from about pH 6.0 to about pH 7.7, from about pH 6.0 to about pH 7.5, from about pH 6.0 to about pH 7.0, from about pH 7.2 to about pH 7.7, from about pH 7.3 to about pH 7.7, from about pH 7.4 to about pH 7.6, from about pH 7.0 to about pH 7.4, from about pH 7.6 to about pH 8.0, from about pH 7.6 to about pH 8.5, from about pH 7.7 to about pH 8.5, from about pH 7.9 to about pH 8.5, from about pH 8.0 to about pH 8.5, from about pH 8.2 to about pH 8.5,

from about pH 8.3 to about pH 8.5, from about pH 8.4 to about pH 8.5, from about pH 8.4 to about pH 9.0, from about pH 8.5 to about pH 9.0, etc.)

[0099] As indicated, one or more monovalent cationic salts (e.g., LiCl, NaCl, KCl, NH₄Cl, RbCl, CsCl, etc.) may be included in reaction solutions of the invention. In many instances, salts used in reaction solutions of the invention will dissociate in solution to generate at least one species which is monovalent (e.g., Li⁺, Na⁺, K⁺, NH₄⁺, Rb⁺, Cs⁺ etc.) When included in reaction solutions of the invention, salts will often be present either individually or in a combined concentration of from about 0.5 mM to about 500 mM (e.g., about 1 mM, about 2 mM, about 3 mM, about 5 mM, about 10 mM, about 12 mM, about 15 mM, about 17 mM, about 20 mM, about 22 mM, about 23 mM, about 24 mM, about 25 mM, about 27 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 64 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, about 100 mM, about 120 mM, about 140 mM, about 150 mM, about 175 mM, about 200 mM, about 225 mM, about 250 mM, about 275 mM, about 300 mM, about 325 mM, about 350 mM, about 375 mM, about 400 mM, from about 1 mM to about 500 mM, from about 5 mM to about 500 mM, from about 10 mM to about 500 mM, from about 20 mM to about 500 mM, from about 30 mM to about 500 mM, from about 40 mM to about 500 mM, from about 50 mM to about 500 mM, from about 60 mM to about 500 mM, from about 65 mM to about 500 mM, from about 75 mM to about 500 mM, from about 85 mM to about 500 mM, from about 90 mM to about 500 mM, from about 100 mM to about 500 mM, from about 125 mM to about 500 mM, from about 150 mM to about 500 mM, from about 200 mM to about 500 mM, from about 10 mM to about 100 mM, from about 10 mM to about 75 mM, from about 10 mM to about 50 mM, from about 20 mM to about 200 mM, from about 20 mM to about 150 mM, from about 20 mM to about 125 mM, from about 20 mM to about 100 mM, from about 20 mM to about 80 mM, from about 20 mM to about 75 mM, from about 20 mM to about 60 mM, from about 20 mM to about 50 mM, from about 30 mM to about 500 mM, from about 30 mM to about 100 mM, from about 30 mM to about 70 mM, from about 30 mM to about 50 mM, etc.).

[0100] As indicated, one or more divalent cationic salts (e.g., MnCl₂, MgCl₂, MgSO₄, CaCl₂, etc.) may be included in reaction solutions of the invention. In many instances, salts used in reaction solutions of the invention will dissociate in solution to generate at least one species which is divalent (e.g., Mg⁺⁺, Mn⁺⁺, Ca⁺⁺, etc.) When included in reaction solutions of the

invention, salts will often be present either individually or in a combined concentration of from about 0.1 mM to about 500 mM (e.g., about 0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM, 0.5 mM, 0.6 mM, 0.7 mM, 0.8 mM, 0.9 mM, 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 12 mM, about 15 mM, about 17 mM, about 20 mM, about 22 mM, about 23 mM, about 24 mM, about 25 mM, about 27 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 64 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, about 100 mM, about 120 mM, about 140 mM, about 150 mM, about 175 mM, about 200 mM, about 225 mM, about 250 mM, about 275 mM, about 300 mM, about 325 mM, about 350 mM, about 375 mM, about 400 mM, from about 1 mM to about 500 mM, from about 5 mM to about 500 mM, from about 10 mM to about 500 mM, from about 20 mM to about 500 mM, from about 30 mM to about 500 mM, from about 40 mM to about 500 mM, from about 50 mM to about 500 mM, from about 60 mM to about 500 mM, from about 65 mM to about 500 mM, from about 75 mM to about 500 mM, from about 85 mM to about 500 mM, from about 90 mM to about 500 mM, from about 100 mM to about 500 mM, from about 125 mM to about 500 mM, from about 150 mM to about 500 mM, from about 200 mM to about 500 mM, from about 10 mM to about 100 mM, from about 10 mM to about 75 mM, from about 10 mM to about 50 mM, from about 20 mM to about 200 mM, from about 20 mM to about 150 mM, from about 20 mM to about 125 mM, from about 20 mM to about 100 mM, from about 20 mM to about 80 mM, from about 20 mM to about 75 mM, from about 20 mM to about 60 mM, from about 20 mM to about 50 mM, from about 30 mM to about 500 mM, from about 30 mM to about 100 mM, from about 30 mM to about 70 mM, from about 30 mM to about 50 mM, etc.).

[0101] When included in reaction mixtures described herein, reducing agents (e.g., dithiothreitol, β -mercaptoethanol, etc.) will often be present either individually or in a combined concentration of from about 0.1 mM to about 50 mM (e.g., about 0.2 mM, about 0.3 mM, about 0.5 mM, about 0.7 mM, about 0.9 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 10 mM, about 12 mM, about 15 mM, about 17 mM, about 20 mM, about 22 mM, about 23 mM, about 24 mM, about 25 mM, about 27 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, from about 0.1 mM to about 50 mM, from about 0.5 mM to about 50 mM, from about 1 mM to about 50 mM, from about 2 mM to about 50 mM, from about 3 mM to about 50 mM, from about 0.5 mM to about 20 mM, from

about 0.5 mM to about 10 mM, from about 0.5 mM to about 5 mM, from about 0.5 mM to about 2.5 mM, from about 1 mM to about 20 mM, from about 1 mM to about 10 mM, from about 1 mM to about 5 mM, from about 1 mM to about 3.4 mM, from about 0.5 mM to about 3.0 mM, from about 1 mM to about 3.0 mM, from about 1.5 mM to about 3.0 mM, from about 2 mM to about 3.0 mM, from about 0.5 mM to about 2.5 mM, from about 1 mM to about 2.5 mM, from about 1.5 mM to about 2.5 mM, from about 2 mM to about 3.0 mM, from about 2.5 mM to about 3.0 mM, from about 0.5 mM to about 2 mM, from about 0.5 mM to about 1.5 mM, from about 0.5 mM to about 1.1 mM, from about 5.0 mM to about 10 mM, from about 5.0 mM to about 15 mM, from about 5.0 mM to about 20 mM, from about 10 mM to about 15 mM, from about 10 mM to about 20 mM, etc.).

[0102] Reaction mixtures described herein may also contain one or more ionic or non-ionic detergent (e.g., TRITON X-100™, NONIDET P40™, sodium dodecyl sulfate, etc.). When included in reaction solutions of the invention, detergents will often be present either individually or in a combined concentration of from about 0.01% to about 5.0% (e.g., about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, about 0.15%, about 0.2%, about 0.3%, about 0.5%, about 0.7%, about 0.9%, about 1%, about 2%, about 3%, about 4%, about 5%, from about 0.01% to about 5.0%, from about 0.01% to about 4.0%, from about 0.01% to about 3.0%, from about 0.01% to about 2.0%, from about 0.01% to about 1.0%, from about 0.05% to about 5.0%, from about 0.05% to about 3.0%, from about 0.05% to about 2.0%, from about 0.05% to about 1.0%, from about 0.1% to about 5.0%, from about 0.1% to about 4.0%, from about 0.1% to about 3.0%, from about 0.1% to about 2.0%, from about 0.1% to about 1.0%, from about 0.1% to about 0.5%, etc.). For example, reaction solutions of the invention may contain TRITON X-100™ at a concentration of from about 0.01% to about 2.0%, from about 0.03% to about 1.0%, from about 0.04% to about 1.0%, from about 0.05% to about 0.5%, from about 0.04% to about 0.6%, from about 0.04% to about 0.3%, etc.

[0103] Reaction mixtures described herein may also contain one or more stabilizing agents (e.g., PEG8000, trehalose, betaine, BSA, glycerol, etc.). In some embodiments, when included in reaction solutions of the invention, stabilizing agents are present either individually or in a combined concentration from 0.01 M to about 50 M (e.g., about 0.05M, about 0.1 M, 0.2 M, about 0.3 M, about 0.5 M, about 0.6 M, about 0.7 M, about 0.9 M, about 1 M, about 2 M,

about 3 M, about 4 M, about 5 M, about 6 M, about 10 M, about 12 M, about 15 M, about 17 M, about 20 M, about 22 M, about 23 M, about 24 M, about 25 M, about 27 M, about 30 M, about 35 M, about 40 M, about 45 M, about 50 M, from about 0.1 M to about 1 M, from about 0.5 M to about 5 M, from about 0.2 M to about 2 M, from about 0.3 M to about 3 M, from about 0.4 M to about 4 M, from about 0.5 M to about 5 M, from about 0.2 M to about 0.8 M, from about 0.5 M to about 1 M, from about 0.05 M to about 1 M, from about 0.05 M to about 10 M, from about 0.05 M to about 20M, etc.). In some embodiments, when included in reaction mixtures described herein, such stabilizing agents are present either individually or in a combined concentration of from about 0.01 mg/ml to about 100 mg/ml (e.g., about 0.01 mg/ml, about 0.02 mg/ml, about 0.03 mg/ml, about 0.04 mg/ml, about 0.05 mg/ml, about 0.06 mg/ml, about 0.07 mg/ml, about 0.08 mg/ml, about 0.09 mg/ml, about 0.1 mg/ml, about 0.11 mg/ml, about 0.12 mg/ml, about 0.15 mg/ml, about 0.17 mg/ml, about 0.2 mg/ml, about 0.25 mg/ml, about 0.35 mg/ml, about 0.5 mg/ml, about 0.75mg/ml, about 1.0 mg/ml, about 1.5 mg/ml, about 2.0 mg/ml, about 2.5 mg/ml, about 3.0 mg/ml, about 3.5 mg/ml, about 4.0 mg/ml, about 5.0 mg/ml, about 6.0 mg/ml, about 7.0 mg/ml, about 8.0 mg/ml, about 9.0 mg/ml, about 10.0 mg/ml, from about 0.05 mg/ml to about 3.0 mg/ml, from about 0.1 mg/ml to about 5.0 mg/ml, from about 0.2 mg/ml to about 2.0 mg/ml, etc.). In some embodiments, when included in reaction mixtures described herein, such stabilizing agents are be present either individually or in a combined concentration of from about 0.1% to about 50% (e.g., about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0%, about 1.5%, about 2.0%, about 3.0%, about 5.0%, about 7.0%, about 9.0%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 22%, about 25%, about 27%, about 30%, about 35%, about 40%, about 45%, about 50%, from about 0.1% to about 50%, from about 0.1% to about 40%, from about 0.1% to about 30%, from about 0.0% to about 20%, from about 0.1% to about 10%, etc.

[0104] Reaction mixtures described herein may also contain one or more DNA polymerase inhibitor (e.g., Actinomycin D, etc.). When included in reaction solutions of the invention, such inhibitors will often be present either individually or in a combined concentration of from about 0.1 µg/ml to about 100 µg/ml (e.g., about 0.1 µg/ml, about 0.2 µg/ml, about 0.3 µg/ml, about 0.4 µg/ml, about 0.5 µg/ml, about 0.6 µg/ml, about 0.7 µg/ml, about 0.8 µg/ml, about 0.9 µg/ml, about 1.0 µg/ml, about 1.1 µg/ml, about 1.3 µg/ml, about 1.5 µg/ml, about 1.7 µg/ml, about 2.0 µg/ml, about 2.5 µg/ml, about 3.5 µg/ml, about 5.0 µg/ml, about 7.5 µg/ml,

about 10 µg/ml, about 15 µg/ml, about 20 µg/ml, about 25 µg/ml, about 30 µg/ml, about 35 µg/ml, about 40 µg/ml, about 50 µg/ml, about 60 µg/ml, about 70 µg/ml, about 80 µg/ml, about 90 µg/ml, about 100 µg/ml, from about 0.5 µg/ml to about 30 µg/ml, from about 0.75 µg/ml to about 30 µg/ml, from about 1.0 µg/ml to about 30 µg/ml, from about 2.0 µg/ml to about 30 µg/ml, from about 3.0 µg/ml to about 30 µg/ml, from about 4.0 µg/ml to about 30 µg/ml, from about 5.0 µg/ml to about 30 µg/ml, from about 7.5 µg/ml to about 30 µg/ml, from about 10 µg/ml to about 30 µg/ml, from about 15 µg/ml to about 30 µg/ml, from about 0.5 µg/ml to about 20 µg/ml, from about 0.5 µg/ml to about 10 µg/ml, from about 0.5 µg/ml to about 5 µg/ml, from about 0.5 µg/ml to about 2 µg/ml, from about 0.5 µg/ml to about 1 µg/ml, from about 1 µg/ml to about 10 µg/ml, from about 1 µg/ml to about 5 µg/ml, from about 1 µg/ml to about 2 µg/ml, from about 1 µg/ml to about 100 µg/ml, from about 10 µg/ml to about 100 µg/ml, from about 20 µg/ml to about 100 µg/ml, from about 40 µg/ml to about 100 µg/ml, from about 30 µg/ml to about 80 µg/ml, from about 30 µg/ml to about 70 µg/ml, from about 40 µg/ml to about 60 µg/ml, from about 40 µg/ml to about 70 µg/ml, from about 40 µg/ml to about 80 µg/ml, etc.).

[0105] Reaction mixtures described herein may also contain one or more additional additives that improve RT activity, including agents that improve primer utilization efficiency and improve product yield. In one embodiment, the reaction solution comprises an agent that reduces non-specific binding of primers to the MarathonRT surface. The agent may comprise any protein, nucleic acid molecule, or small molecule that prevents or reduces non-specific binding. In certain embodiments, the agent comprises D4A RNA or variant thereof. D4A and variants of D4A that can be included in the reverse transcription assay of the invention include, but are not limited to, those described in detail in International Patent Publication WO2019005955A1, which is incorporated by reference herein in its entirety.

[0106] When included in reaction mixtures described herein, D4A, or variant thereof, may be present at ratio of D4A (or variant thereof) concentration to MarathonRT concentration from about 0.1:1 to about 100:1. For example, in some embodiments, D4A, or variant thereof, may be present at ratio of D4A (or variant thereof) concentration to MarathonRT concentration of about 0.1:1, 0.2:1, 0.3:1, 0.4:1, 0.5:1, 0.6:1, 0.7:1, 0.8:1, 0.9:1, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1, or 100:1.

[0107] In many instances, nucleotides (e.g., dNTPs, such as dGTP, dATP, dCTP, dTTP, etc.) will be present in reaction mixtures described herein. Typically, individual nucleotides will be present in concentrations of from about 0.001 mM to about 50 mM (e.g., about 0.001 mM, 0.01 mM, 0.07 mM, about 0.1 mM, about 0.15 mM, about 0.18 mM, about 0.2 mM, about 0.3 mM, about 0.5 mM, about 0.7 mM, about 0.9 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 10 mM, about 12 mM, about 15 mM, about 17 mM, about 20 mM, about 22 mM, about 23 mM, about 24 mM, about 25 mM, about 27 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, from about 0.1 mM to about 50 mM, from about 0.5 mM to about 50 mM, from about 1 mM to about 50 mM, from about 2 mM to about 50 mM, from about 3 mM to about 50 mM, from about 0.5 mM to about 20 mM, from about 0.5 mM to about 10 mM, from about 0.5 mM to about 5 mM, from about 0.5 mM to about 2.5 mM, from about 1 mM to about 20 mM, from about 1 mM to about 10 mM, from about 1 mM to about 5 mM, from about 1 mM to about 3.4 mM, from about 0.5 mM to about 3.0 mM, from about 1 mM to about 3.0 mM, from about 1.5 mM to about 3.0 mM, from about 2 mM to about 3.0 mM, from about 0.5 mM to about 2.5 mM, from about 1 mM to about 2.5 mM, from about 1.5 mM to about 2.5 mM, from about 2 mM to about 3.0 mM, from about 2.5 mM to about 3.0 mM, from about 0.5 mM to about 2 mM, from about 0.5 mM to about 1.5 mM, from about 0.5 mM to about 1.1 mM, from about 5.0 mM to about 10 mM, from about 5.0 mM to about 15 mM, from about 5.0 mM to about 20 mM, from about 10 mM to about 15 mM, from about 10 mM to about 20 mM, etc.). The combined nucleotide concentration, when more than one nucleotide is present, can be determined by adding the concentrations of the individual nucleotides together. When more than one nucleotide is present in reaction mixtures described herein, the individual nucleotides may not be present in equimolar amounts. Thus, a reaction solution may contain, for example, 1 mM dGTP, 1 mM dATP, 0.5 mM dCTP, and 1 mM dTTP.

[0108] RNA will typically be present in reaction mixtures described herein. In most instances, RNA will be added to the reaction mixture shortly prior to reverse transcription. Thus, reaction solutions may be provided without RNA. This will typically be the case when reaction solutions are provided in kits. RNA, when present in reaction solutions will often be present in a concentration of 0.01 picogram to 100 µg/20 µl reaction mixture (e.g., about 0.01 picogram/20 µl, about 0.1 picogram/20 µl, about 0.5 picogram/20 µl, about 1 picogram/20 µl, about 10 picograms/20 µl, about 50 picograms/20 µl, about 100 picograms/20 µl, about 200 picograms/20

μl , about 10 picograms/20 μl , about 500 picograms/20 μl , about 800 picograms/20 μl , about 1.0 nanogram/20 μl , about 5.0 nanograms/20 μl , about 10 nanograms/20 μl , about 25 nanograms/20 μl , about 50 nanograms/20 μl , about 75 nanograms/20 μl , about 100 nanograms/20 μl , about 150 nanograms/20 μl , about 250 nanograms/20 μl , about 400 nanograms/20 μl , about 500 nanograms/20 μl , about 750 nanograms/20 μl , about 1.0 μg /20 μl , about 5.0 μg /20 μl , about 10 μg /20 μl , about 20 μg /20 μl , about 30 μg /20 μl , about 40 μg /20 μl , about 50 μg /20 μl , about 70 μg /20 μl , about 85 μg /20 μl , about 100 μg /20 μl , from about 10 picograms/20 μl to about 100 μg /20 μl , from about 10 picograms/20 μl to about 100 μg /20 μl , from about 100 picograms/20 μl to about 100 μg /20 μl , from about 1.0 nanograms/20 μl to about 100 μg /20 μl , from about 100 nanograms/20 μl to about 100 μg /20 μl , from about 10 picograms/20 μl to about 10 μg /20 μl , from about 10 picograms/20 μl to about 5 μg /20 μl , from about 100 nanograms/20 μl to about 5 μg /20 μl , from about 1 μg /20 μl to about 10 μg /20 μl , from about 1 μg /20 μl to about 5 μg /20 μl , from about 100 nanograms/20 μl to about 1 μg /20 μl , from about 500 nanograms/20 μl to about 5 μg /20 μl , etc.). As one skilled in the art would recognize, different reverse transcription reactions may be performed in volumes other than 20 μl . In such instances, the total amount of RNA present will vary with the volume used. Thus, the above amounts are provided as examples of the amount of RNA/20 μl of reaction solution.

[0109] A reverse transcriptase may also be present in reaction mixtures described herein. When present, the reverse transcriptase will often be present in a concentration which results in about 0.01 to about 1,000 units of reverse transcriptase activity/ μl (e.g., about 0.01 unit/ μl , about 0.05 unit/ μl , about 0.1 unit/ μl , about 0.2 unit/ μl , about 0.3 unit/ μl , about 0.4 unit/ μl , about 0.5 unit/ μl , about 0.7 unit/ μl , about 1.0 unit/ μl , about 1.5 unit/ μl , about 2.0 unit/ μl , about 2.5 unit/ μl , about 5.0 unit/ μl , about 7.5 unit/ μl , about 10 unit/ μl , about 20 unit/ μl , about 25 unit/ μl , about 50 unit/ μl , about 100 unit/ μl , about 150 unit/ μl , about 200 unit/ μl , about 250 unit/ μl , about 350 unit/ μl , about 500 unit/ μl , about 750 unit/ μl , about 1,000 unit/ μl , from about 0.1 unit/ μl to about 1,000 unit/ μl , from about 0.2 unit/ μl to about 1,000 unit/ μl , from about 1.0 unit/ μl to about 1,000 unit/ μl , from about 5.0 unit/ μl to about 1,000 unit/ μl , from about 10 unit/ μl to about 1,000 unit/ μl , from about 20 unit/ μl to about 1,000 unit/ μl , from about 50 unit/ μl to about 1,000 unit/ μl , from about 100 unit/ μl to about 1,000 unit/ μl , from about 200 unit/ μl to about 1,000 unit/ μl , from about 400 unit/ μl to about 1,000 unit/ μl , from about 500 unit/ μl to about 1,000 unit/ μl , from about 0.1 unit/ μl to about 300 unit/ μl , from about 0.1 unit/ μl to about 200 unit/ μl , from about 0.1

unit/ μ l to about 100 unit/ μ l, from about 0.1 unit/ μ l to about 50 unit/ μ l, from about 0.1 unit/ μ l to about 10 unit/ μ l, from about 0.1 unit/ μ l to about 5.0 unit/ μ l, from about 0.1 unit/ μ l to about 1.0 unit/ μ l, from about 0.2 unit/ μ l to about 0.5 unit/ μ l, etc. In certain embodiments, the reaction solution comprises a lower concentration of the reverse transcriptase described herein, as compared to what would be necessary to produce equivalent product from other reverse transcriptases.

[0110] Reaction mixtures described herein may be prepared as concentrated solutions (e.g., 5 $\times$ solutions) which are diluted to a working concentration for final use. With respect to a 5 $\times$ reaction solution, a 5:1 dilution is required to bring such a 5 $\times$ solution to a working concentration. Reaction mixtures described herein may be prepared, for examples, as a 2 $\times$, a 3 $\times$, a 4 $\times$, a 5 $\times$, a 6 $\times$, a 7 $\times$, a 8 $\times$, a 9 $\times$, a 10 $\times$, etc. solutions. One major limitation on the fold concentration of such solutions is that, when compounds reach particular concentrations in solution, precipitation occurs. Thus, concentrated reaction solutions will generally be prepared such that the concentrations of the various components are low enough so that precipitation of buffer components will not occur. As one skilled in the art would recognize, the upper limit of concentration which is feasible for each solution will vary with the particular solution and the components present.

[0111] In many instances, reaction mixtures described herein will be provided in sterile form. Sterilization may be performed on the individual components of reaction solutions prior to mixing or on reaction solutions after they are prepared. Sterilization of such solutions may be performed by any suitable means including autoclaving or ultrafiltration.

Kits

[0112] In one aspect, the present disclosure provides a kit for use in the methods of the disclosure. Such kits can be used for making, sequencing or amplifying nucleic acid molecules (single- or double-stranded), e.g., at the particular temperatures described herein. A kit described herein may be useful for acquiring a value for the presence of a target ribonucleic acid (RNA) in a mixture, detecting the level, identity or concentration of a target RNA, increasing the signal to noise ratio of a target RNA, preparing a library for the target RNA templates, or increasing the processivity of the reverse transcription reaction, e.g., relative to a reference standard. An exemplary reference standard is a reverse transcription reaction carried out in the absence of an anionic polymer.

[0113] The kit may comprise components for a polymerase reaction, e.g., a reverse transcription reaction. In some embodiments, the kit comprises components for a reverse transcriptase reaction, e.g., a reverse transcriptase, oligonucleotide primer, deoxyribonucleoside triphosphates (dNTPs), an anionic polymer, and/or a buffer. In some embodiments, the kit comprises a polymerase, e.g., a reverse transcriptase. In some embodiments, the kit comprises a reverse transcriptase, e.g., MarathonRT, UltraMarathonRT, or a variant or derivative thereof. In some embodiments, the kit comprises MarathonRT. In some embodiments, the kit comprises UltraMarathonRT. In some embodiments, the kit comprises deoxyribonucleoside triphosphates (dNTPs), e.g., deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxythymidine triphosphate (dTTP), or deoxycytidine triphosphate (dCTP). In some embodiments, the kit comprises deoxyadenosine triphosphate. In some embodiments, the kit comprises deoxyguanosine triphosphate. In some embodiments, the kit comprises deoxythymidine triphosphate. In some embodiments, the kit comprises deoxycytidine triphosphate. In some embodiments, the kit comprises a mixture of deoxyribonucleoside triphosphates, e.g., a mixture of deoxyadenosine, deoxyguanosine, deoxythymidine, and deoxycytidine triphosphates. In some embodiments, the kit comprises a buffer, e.g., an optimized reaction buffer. In some embodiments, the kit comprises an oligonucleotide primer, e.g., a plurality of oligonucleotide primers. In some embodiments, the kit comprises a plurality of oligonucleotide primers. In some embodiments, the kit comprises an anionic polymer. In some embodiments, the kit comprises all of a reverse transcriptase, dNTPs, buffer, an anionic polymer, and an oligonucleotide primer. In some embodiments, the kit comprises a reverse transcriptase and dNTPs. In some embodiments, the kit comprises a reverse transcriptase and an oligonucleotide primer. In some embodiments, the kit comprises a reverse transcriptase and an anionic polymer. In some embodiments, the kit comprises a reverse transcriptase, an oligonucleotide primer, and a buffer. In some embodiments, the kit comprises a reverse transcriptase, an anionic polymer, and a buffer. In some embodiments, the kit comprises a reverse transcriptase, dNTPs, and a buffer. In some embodiments, the kit comprises a reverse transcriptase, an oligonucleotide primer, an anionic polymer, and a buffer.

[0114] Kits of the disclosure may comprise a carrier, such as a box, bag or carton, having in close confinement therein one or more containers, such as vials, tubes, bottles and the like. In one embodiment, kits of the disclosure may also comprise, in the same or different containers, an

optimized reaction buffer as described elsewhere herein, or components used to produce the optimized reaction buffer. Alternatively, the components of the kit may be divided into separate containers. Certain components of the kit may be present in the same container, e.g., the same tube, vial, or bottle. In some embodiments, the buffer is present in the same container as the reverse transcriptase. In some embodiments, the buffer is present in the same container as the oligonucleotide primer. In some embodiments, the buffer is present in the same container as the dNTPs. In some embodiments, the buffer is present in the same container as the anionic polymer. In some embodiments, the buffer is present in the same container as each of the reverse transcriptase and the oligonucleotide primer. In some embodiments, the buffer is present in the same container as each of the reverse transcriptase and the dNTPs. In some embodiments, the buffer is present in the same container as each of the reverse transcriptase and the anionic polymer. In some embodiments, the buffer is present in the same container as each of the reverse transcriptase, the oligonucleotide primer, and the dNTPs. In some embodiments, the buffer is present in the same container as each of the reverse transcriptase, the oligonucleotide primer, and the anionic polymer. In some embodiments, the buffer is present in the same container as each of the reverse transcriptase, the anionic polymer, and the dNTPs. In some embodiments, the buffer is present in the same container as each of the reverse transcriptase, oligonucleotide primer, dNTPs, and the anionic polymer. In some embodiments, the same buffer is present in a container with each of the kit components. In some embodiments, a different buffer is present in a container with each of the kit components. In some embodiments, the anionic polymer is present in the same container as the buffer.

[0115] The components of the kit, e.g., the polymerase, buffer, oligonucleotide primer, or deoxyribonucleoside triphosphate, may be present in a container, e.g., a tube, vial, or bottle. In some embodiments, each kit component is in an individual container, e.g. a tube, vial, or bottle. In some embodiments, a component of the kit is present in an individual container. In some embodiments, the polymerase, e.g., reverse transcriptase, is present in a first container. In some embodiments, the oligonucleotide primer is present in a second container. In some embodiments, the dNTPs are present in a third container. In some embodiments, the buffer is present in a fourth container. In some embodiments, the buffer is present in a container with the polymerase, e.g., reverse transcriptase, the dNTPs, or the oligonucleotide primer.

[0116] Kits of the disclosure also may comprise instructions or protocols for carrying out the methods of the disclosure. In one embodiment, the kit includes instructional material that describes the use of the kit to carry out reverse transcription reactions, wherein the instructional material creates an increased functional relationship between the kit components and the individual using the kit. In some embodiments, the kit is utilized by one person or entity. In some embodiments, the kit is utilized by more than one person or entity. In some embodiments, the kit is used without any additional components or methods. In another embodiment, the kit is used with at least one additional component or method. In some embodiments, the additional component used with the kit is an input polynucleotide, e.g., a plurality of input polynucleotides.

[0117] Kits of the disclosure may comprise a carrier, such as a box or carton, having in close confinement therein one or more (e.g., one, two, three, four, five, ten, twelve, fifteen, etc.) containers, e.g., tubes, bottles, or vials. In some embodiments, the components of the kit are enclosed in a cardboard box. In some embodiments, the components of the kit are enclosed in a Styrofoam box. In some embodiments, the components of the kit are enclosed in an envelope. In some embodiments, the components of the kit are enclosed in a paper box. In some embodiments, the components of the kit are enclosed in a plastic bag. In some embodiments, the components of the kit are enclosed in a single carrier. In some embodiments, the components of the kit are enclosed in multiple carriers.

Methods for Enhancing Reverse Transcription

[0118] In one aspect, the present disclosure provides methods for enhancing reverse transcription, e.g., a reverse transcription reaction, using an anionic excipient, e.g., an anionic polymer, relative to a reference standard. For example, an anionic excipient, e.g., an anionic polymer, may enhance a reverse transcription reaction by enhancing an activity or property of a polymerase, e.g., reverse transcriptase, and/or by enhancing stability of the input polynucleotide, e.g., input RNA. The methods for enhancing reverse transcription described herein, e.g., relative to a reference standard, may be useful for acquiring a value for the presence of a target ribonucleic acid (RNA) in a mixture, detecting the level, identity or concentration of a target RNA, increasing the signal to noise ratio of a target RNA, or increasing the processivity of the reverse transcription reaction. An exemplary reference standard is a reverse transcription reaction carried out in the absence of an anionic polymer.

[0119] In some embodiments, the anionic polymer enhances activity of a polymerase, e.g., reverse transcriptase, relative to the activity of the polymerase in the absence of the anionic polymer. In some embodiments, the anionic polymer increases activity of a reverse transcriptase relative to the activity of the reverse transcriptase in the absence of the anionic polymer. In some embodiments, the anionic polymer increases activity of a reverse transcriptase by about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 500% or more relative to the activity of the reverse transcriptase in the absence of the anionic polymer.

[0120] In some embodiments, the activity of the polymerase, e.g., reverse transcriptase, that is enhanced by the anionic polymer is the sensitivity, processivity, thermostability, primer incorporation efficiency, error rate, turnover, thermocycling ability, and/or product yield of the polymerase. In some embodiments, the anionic polymer enhances the sensitivity of a polymerase, e.g., reverse transcriptase. Sensitivity may comprise the ability of a polymerase to detect a target polynucleotide, e.g., to use a target polynucleotide as a template for polynucleotide synthesis, e.g., to use a target polynucleotide that is present in a mixture of input polynucleotides as a template. In some embodiments, the anionic polymer enhances the sensitivity of a reverse transcriptase, e.g., the ability of the reverse transcriptase to detect a target RNA, e.g., the ability of the reverse transcriptase to synthesize cDNA from a target RNA template. In some embodiments, the anionic polymer increases the sensitivity of a reverse transcriptase, e.g., increases the sensitivity of the reverse transcriptase by about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 500% or more relative to the sensitivity of the reverse transcriptase in the absence of the anionic polymer. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 1%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 5%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 10%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 20%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 30%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 40%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 50%. In some

embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 60%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 70%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 80%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 90%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 100%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 200%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 300%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 400%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by about 500%. In some embodiments, the anionic polymer increases the sensitivity of the reverse transcriptase by greater than 500%.

[0121] In some embodiments, the activity of the polymerase, e.g., reverse transcriptase, that is enhanced by the anionic polymer is the processivity of the polymerase. Processivity may comprise the ability of a polymerase to synthesize a polynucleotide through a single interaction with a template polynucleotide, e.g., the ability of the polymerase to remain bound to a template polynucleotide as the polymerase synthesizes a complementary polynucleotide, e.g., the duration, in nucleotides, that a polymerase remains bound to a template polynucleotide while synthesizing a complementary polynucleotide, e.g., the rate of polymerization (forward rate) compared to the rate of disassociation from a template polynucleotide (off rate). In some embodiments, the anionic polymer enhances the processivity of a reverse transcriptase, e.g., the length of cDNA that is synthesized from a single interaction with a target RNA. In some embodiments, the anionic polymer increases the processivity of a reverse transcriptase, e.g., increases the processivity of the reverse transcriptase by about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 500% or more relative to the processivity of the reverse transcriptase in the absence of the anionic polymer. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 1%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 5%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 10%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by

about 20%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 30%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 40%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 50%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 60%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 70%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 80%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 90%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 100%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 200%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 300%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 400%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by about 500%. In some embodiments, the anionic polymer increases the processivity of the reverse transcriptase by greater than 500%.

[0122] In some embodiments, the anionic polymer enhances stability of an input polynucleotide, e.g., input RNA. Enhancing stability of an input polynucleotide may comprise reducing degradation, e.g., reducing enzymatic or chemical degradation, e.g., degradation by nucleases, hydrolysis, oxidation, or reducing physical instability, e.g., reducing loss of kinetically stable secondary or tertiary structural conformations, aggregation, or precipitation. In some embodiments, the anionic polymer reduces degradation, e.g., enzymatic or chemical degradation, of an input RNA. In some embodiments, the anionic polymer reduces physical instability, e.g., loss of kinetically stable secondary or tertiary structural conformations, aggregation, or precipitation, of an input RNA. In some embodiments, the anionic polymer increases stability of an input polynucleotide, e.g., an input RNA. In some embodiments, the anionic polymer increases stability of an input RNA, e.g., increases stability by about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 500% or more relative to the stability of an input RNA in the absence of the anionic polymer. In some embodiments, the anionic polymer increases stability of

an input RNA by about 1%. In some embodiments, the anionic polymer increases stability of an input RNA by about 5%. In some embodiments, the anionic polymer increases stability of an input RNA by about 10%. In some embodiments, the anionic polymer increases stability of an input RNA by about 20%. In some embodiments, the anionic polymer increases stability of an input RNA by about 30%. In some embodiments, the anionic polymer increases stability of an input RNA by about 40%. In some embodiments, the anionic polymer increases stability of an input RNA by about 50%. In some embodiments, the anionic polymer increases stability of an input RNA by about 60%. In some embodiments, the anionic polymer increases stability of an input RNA by about 70%. In some embodiments, the anionic polymer increases stability of an input RNA by about 80%. In some embodiments, the anionic polymer increases stability of an input RNA by about 90%. In some embodiments, the anionic polymer increases stability of an input RNA by about 100%. In some embodiments, the anionic polymer increases stability of an input RNA by about 200%. In some embodiments, the anionic polymer increases stability of an input RNA by about 300%. In some embodiments, the anionic polymer increases stability of an input RNA by about 400%. In some embodiments, the anionic polymer increases stability of an input RNA by about 500%. In some embodiments, the anionic polymer increases stability of an input RNA by greater than 500%.

[0123] In some embodiments, the anionic polymer enhances stability of a target polynucleotide, e.g., input RNA, e.g., target RNA. Enhancing stability of a target polynucleotide may comprise reducing degradation, e.g., reducing enzymatic or chemical degradation, e.g., degradation by nucleases, hydrolysis, oxidation, or reducing physical instability, e.g., reducing loss of kinetically stable secondary or tertiary structural conformations, aggregation, or precipitation. In some embodiments, the anionic polymer reduces degradation, e.g., enzymatic or chemical degradation, of a target RNA. In some embodiments, the anionic polymer reduces physical instability, e.g., loss of kinetically stable secondary or tertiary structural conformations, aggregation, or precipitation, of a target RNA. In some embodiments, the anionic polymer increases stability of a target polynucleotide, e.g., an input RNA. In some embodiments, the anionic polymer increases stability of a target RNA, e.g., increases stability by about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 500% or more relative to the stability of a target RNA in the absence of the anionic polymer. In some embodiments, the anionic polymer increases

stability of a target RNA by about 1%. In some embodiments, the anionic polymer increases stability of a target RNA by about 5%. In some embodiments, the anionic polymer increases stability of a target RNA by about 10%. In some embodiments, the anionic polymer increases stability of a target RNA by about 20%. In some embodiments, the anionic polymer increases stability of a target RNA by about 30%. In some embodiments, the anionic polymer increases stability of a target RNA by about 40%. In some embodiments, the anionic polymer increases stability of a target RNA by about 50%. In some embodiments, the anionic polymer increases stability of a target RNA by about 60%. In some embodiments, the anionic polymer increases stability of a target RNA by about 70%. In some embodiments, the anionic polymer increases stability of a target RNA by about 80%. In some embodiments, the anionic polymer increases stability of a target RNA by about 90%. In some embodiments, the anionic polymer increases stability of a target RNA by about 100%. In some embodiments, the anionic polymer increases stability of a target RNA by about 200%. In some embodiments, the anionic polymer increases stability of a target RNA by about 300%. In some embodiments, the anionic polymer increases stability of a target RNA by about 400%. In some embodiments, the anionic polymer increases stability of a target RNA by about 500%. In some embodiments, the anionic polymer increases stability of a target RNA by greater than 500%.

[0124] In some embodiments, the anionic polymer enhances the yield of product polynucleotide produced by a polymerase, e.g., a reverse transcriptase. Enhancing the yield of product polynucleotide produced by a polymerase may comprise increasing the amount of product polynucleotide that is produced by a polymerase. In some embodiments, the anionic polymer enhances the yield of cDNA produced by a reverse transcriptase, e.g., enhances the amount of cDNA produced by a reverse transcriptase that is complementary to a target RNA. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase, e.g., increases the yield of cDNA produced by a reverse transcriptase by about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 500% or more relative to the yield of cDNA produced by a reverse transcriptase in the absence of the anionic polymer. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 1%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 5%. In some embodiments, the anionic polymer increases the yield

of cDNA produced by a reverse transcriptase by about 10%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 20%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 30%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 40%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 50%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 60%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 70%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 80%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 90%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 100%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 200%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 300%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 400%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by about 500%. In some embodiments, the anionic polymer increases the yield of cDNA produced by a reverse transcriptase by greater than 500%.

[0125] In some embodiments, the anionic polymer enhances detection of a target polynucleotide, e.g., a target RNA, by a polymerase, e.g., a reverse transcriptase. Enhancing detection of a target polynucleotide may comprise increasing the ability of a polymerase to use a target polynucleotide, e.g., a target polynucleotide that is present in a mixture of input polynucleotides, as a template for polynucleotide synthesis. In some embodiments, the anionic polymer enhances detection of a target RNA by a reverse transcriptase. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase, e.g., increases detection of a target RNA by a reverse transcriptase by about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 500% or more relative to the detection of the target RNA in the absence of the anionic polymer. In some embodiments, the anionic polymer increases detection of a target

RNA by a reverse transcriptase by about 1%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 5%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 10%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 20%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 30%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 40%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 50%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 60%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 70%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 80%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 90%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 100%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 200%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 300%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 400%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by about 500%. In some embodiments, the anionic polymer increases detection of a target RNA by a reverse transcriptase by greater than 500%.

[0126] In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection, e.g., cDNA synthesis of a target RNA, e.g., a target RNA of 200 nucleotides, 500 nucleotides, 1,000 nucleotides, 2,000 nucleotides, 3,000 nucleotides, or longer. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA, e.g., a target RNA of 4,000 nucleotides, 5,000 nucleotides, 6,000 nucleotides, 7,000 nucleotides, 8,000 nucleotides, 9,000 nucleotides, 10,000 nucleotides, 11,000 nucleotides, 12,000 nucleotides, 15,000 nucleotides, 20,000 nucleotides, 25,000 nucleotides, or 30,000 nucleotides or more in length. For example, the presence of an anionic polymer in a reverse transcription reaction mixture may

increase detection of a long target RNA by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 500% or more. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 10%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 20%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 30%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 40%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 50%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 60%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 70%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 80%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 90%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 100%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 110%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 120%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 130%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 140%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 150%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 160%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 170%. In some embodiments, the presence of an anionic polymer

in a reverse transcription reaction mixture increases detection of a long target RNA by about 180%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 190%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 200%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 250%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 300%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 350%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 400%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 450%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by about 500%. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA by greater than 500%.

[0127] In some embodiments, the anionic polymer present in a reverse transcription reaction mixture is heparin. In some embodiments, the presence of heparin in a reverse transcription reaction mixture increases detection, e.g., cDNA synthesis of a target RNA, e.g., a target RNA of 200 nucleotides, 500 nucleotides, 1,000 nucleotides, 2,000 nucleotides, 3,000 nucleotides, or longer. In some embodiments, the presence of heparin in a reverse transcription reaction mixture increases detection of a long target RNA, e.g., a target RNA of 4,000 nucleotides, 5,000 nucleotides, 6,000 nucleotides, 7,000 nucleotides, 8,000 nucleotides, 9,000 nucleotides, 10,000 nucleotides, 11,000 nucleotides, 12,000 nucleotides, 15,000 nucleotides, 20,000 nucleotides, 25,000 nucleotides, or 30,000 nucleotides or more in length. For example, the presence of heparin in a reverse transcription reaction mixture may increase detection of a long target RNA by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 500% or more. In some embodiments, the presence of heparin in a reverse transcription reaction mixture increases detection of a long target RNA by about 10%. In some embodiments, the presence of heparin in a

[illegible]

mixture increases detection of a long target RNA by about 300%. In some embodiments, the presence of heparin in a reverse transcription reaction mixture increases detection of a long target RNA by about 350%. In some embodiments, the presence of heparin in a reverse transcription reaction mixture increases detection of a long target RNA by about 400%. In some embodiments, the presence of heparin in a reverse transcription reaction mixture increases detection of a long target RNA by about 450%. In some embodiments, the presence of heparin in a reverse transcription reaction mixture increases detection of a long target RNA by about 500%. In some embodiments, the presence of heparin in a reverse transcription reaction mixture increases detection of a long target RNA by greater than 500%.

[0128] In some embodiments, the anionic polymer present in a reverse transcription reaction mixture is poly(acrylic acid). In some embodiments, the presence of poly(acrylic acid) in a reverse transcription reaction mixture increases detection, e.g., cDNA synthesis of a target RNA, e.g., a target RNA of 200 nucleotides, 500 nucleotides, 1,000 nucleotides, 2,000 nucleotides, 3,000 nucleotides, or longer. In some embodiments, the presence of an anionic polymer in a reverse transcription reaction mixture increases detection of a long target RNA, e.g., a target RNA of 4,000 nucleotides, 5,000 nucleotides, 6,000 nucleotides, 7,000 nucleotides, 8,000 nucleotides, 9,000 nucleotides, 10,000 nucleotides, 11,000 nucleotides, 12,000 nucleotides, 15,000 nucleotides, 20,000 nucleotides, 25,000 nucleotides, or 30,000 nucleotides or more in length. For example, the presence of poly(acrylic acid) in a reverse transcription reaction mixture may increase detection of a long target RNA by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 500% or more. In some embodiments, the presence of poly(acrylic acid) in a reverse transcription reaction mixture increases detection of a long target RNA by about 10%. In some embodiments, the presence of poly(acrylic acid) in a reverse transcription reaction mixture increases detection of a long target RNA by about 20%. In some embodiments, the presence of poly(acrylic acid) in a reverse transcription reaction mixture increases detection of a long target RNA by about 30%. In some embodiments, the presence of poly(acrylic acid) in a reverse transcription reaction mixture increases detection of a long target RNA by about 40%. In some embodiments, the presence of poly(acrylic acid) in a reverse transcription reaction mixture increases detection of a long target RNA by about 50%. In some embodiments, the presence of poly(acrylic acid) in a reverse transcription reaction mixture increases detection of a long target

[illegible]

transcription reaction mixture increases detection of a long target RNA by about 450%. In some embodiments, the presence of poly(acrylic acid) in a reverse transcription reaction mixture increases detection of a long target RNA by about 500%. In some embodiments, the presence of poly(acrylic acid) in a reverse transcription reaction mixture increases detection of a long target RNA by greater than 500%.

[0129] In some embodiments, using heparin as a substitute for carrier RNA in a reverse transcription reaction mixture improves the cDNA yield, e.g., increases the amount of cDNA synthesized from RNA by a reverse transcriptase. In some embodiments, using heparin as a substitute for carrier RNA in a reverse transcription reaction mixture improves the DNA yield following PCR amplification of the cDNA synthesized during reverse transcription, e.g., increases the yield of DNA amplified from the cDNA synthesized by the reverse transcriptase. In some embodiments, using heparin as a substitute for carrier RNA during the reverse transcription step of a reverse transcription-polymerase chain reaction (RT-PCR) improves the DNA yield from the polymerase chain reaction step, e.g., increases the yield of DNA amplified from the cDNA synthesized by the reverse transcriptase. In some embodiments, using heparin as a substitute for carrier RNA in a reverse transcription reaction mixture improves detection of target RNAs, e.g., increases the cDNA yield, e.g., increases the yield of DNA amplified from cDNA. For example, the yield of the final DNA PCR product from an RT-PCR procedure is increased when heparin is used instead of carrier RNA during reverse transcription of a target RNA molecule into cDNA.

[0130] In some embodiments, using heparin as a substitute for carrier RNA during reverse transcription increases the cDNA yield from a target RNA, e.g., a target RNA of 200 nucleotides, 500 nucleotides, 1,000 nucleotides, 2,000 nucleotides, 3,000 nucleotides, or longer. In some embodiments, using heparin as a substitute for carrier RNA during reverse transcription increases detection of a long target RNA, e.g., a target RNA of 4,000 nucleotides, 5,000 nucleotides, 6,000 nucleotides, 7,000 nucleotides, 8,000 nucleotides, 9,000 nucleotides, 10,000 nucleotides, 11,000 nucleotides, 12,000 nucleotides, 15,000 nucleotides, 20,000 nucleotides, 25,000 nucleotides, or 30,000 nucleotides or more in length. In some embodiments, using heparin as a substitute for carrier RNA during reverse transcription allows for detection of low abundance target RNAs, e.g., detection of nanomolar (nM) target RNAs, picomolar (pM) target RNAs, femtomolar (fM) target RNAs, or attomolar (aM) target RNAs or less in a sample. In

some embodiments, using heparin as a substitute for carrier RNA during the reverse transcription step of RT-PCR increases the amplified DNA product of a long or low abundance target RNA, e.g., increases the yield of cDNA during reverse transcription thereby increasing the yield of DNA amplified from the cDNA. For example, using heparin as a substitute for carrier RNA can increase the DNA yield of a 12 fM 4 kilobase (kb) long target RNA in a complex input RNA mixture. In some embodiments, using heparin as a substitute for carrier RNA, e.g., in the absence of carrier RNA, during the reverse transcription step of RT-PCR results in an approximately equal yield of DNA using carrier RNA, e.g., results in approximately equal DNA yields as shown in **FIGs. 1A-1B**. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR in the absence of carrier RNA or anionic polymers during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR in the presence of 0.5 $\mu\text{g}/\mu\text{L}$ total RNA as carrier RNA during the reverse transcription step is about 1. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR in the presence of 0.3 $\mu\text{g}/\mu\text{L}$ total RNA as carrier RNA during the reverse transcription step is about 0.2. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR in the presence of 0.3 $\mu\text{g}/\mu\text{L}$ MS2 RNA as carrier RNA during the reverse transcription step is about 1. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR in the presence of 50 $\text{ng}/\mu\text{L}$ heparin during the reverse transcription step, e.g., in the absence of carrier RNA, is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR in the presence of 100 $\text{ng}/\mu\text{L}$ heparin during the reverse transcription step, e.g., in the absence of carrier RNA, is about 0.9. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR in the presence of 200 $\text{ng}/\mu\text{L}$ heparin during the reverse transcription step, e.g., in the absence of carrier RNA, is about 0.7. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR in the presence of 300 $\text{ng}/\mu\text{L}$ heparin during the reverse transcription step, e.g., in the absence of carrier RNA, is about 0.1.

[0131] In some embodiments, including heparin in a reverse transcription reaction mixture improves the cDNA yield, e.g., increases the amount of cDNA synthesized from RNA by a reverse transcriptase. In some embodiments, including heparin in a reverse transcription reaction mixture improves the DNA yield following PCR amplification of the cDNA synthesized

during reverse transcription, e.g., increases the yield of DNA amplified from the cDNA synthesized by the reverse transcriptase. In some embodiments, including heparin in a reverse transcription reaction mixture improves detection of target RNAs, e.g., increases the cDNA yield, e.g., increases the yield of DNA amplified from cDNA. In some embodiments, including heparin in a reverse transcription reaction mixture improves detection of a target RNA in a sample, e.g., a target RNA of 200 nucleotides, 500 nucleotides, 1,000 nucleotides, 2,000 nucleotides, 3,000 nucleotides, or longer. In some embodiments, including heparin in a reverse transcription reaction mixture improves detection of a long target RNA in a sample, e.g., increases the yield of cDNA synthesized from long target RNAs, e.g., target RNAs of 4,000 nucleotides, 5,000 nucleotides, 6,000 nucleotides, 7,000 nucleotides, 8,000 nucleotides, 9,000 nucleotides, 10,000 nucleotides, 11,000 nucleotides, 12,000 nucleotides, 15,000 nucleotides, 20,000 nucleotides, 25,000 nucleotides, or 30,000 nucleotides or more in length. In some embodiments, including heparin in a reverse transcription reaction mixture improves detection of ultra-low abundance RNAs, e.g., detection of nanomolar (nM) target RNAs, picomolar (pM) target RNAs, femtomolar (fM) target RNAs, or attomolar (aM) target RNAs or less in a sample. For example, including heparin in a reverse transcription reaction mixture during the reverse transcription step of RT-PCR can increase the DNA yield of a 12 fM 8 kilobase (kb) target RNA in a complex input RNA mixture, e.g., increase the yield of DNA amplified from cDNA synthesized by UltraMarathonRT reverse transcriptase as shown in **FIGs. 2A-2B**. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 10 pg total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 100 pg total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 1 ng total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 10 ng total RNA input in the absence of heparin during the reverse transcription step is about 0.1. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 100 ng total RNA input in the absence of heparin during the reverse transcription step is about 0.5. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 1 µg total RNA

input in the absence of heparin during the reverse transcription step is about 1. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 10 pg total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.6. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 100 pg total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 1 ng total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 10 ng total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 100 ng total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.5. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 1 μg total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.9.

[0132] In some embodiments, including poly(acrylic acid) in a reverse transcription reaction mixture improves the cDNA yield, e.g., increases the amount of cDNA synthesized from RNA by a reverse transcriptase. In some embodiments, including poly(acrylic acid) in a reverse transcription reaction mixture improves the DNA yield following PCR amplification of the cDNA synthesized during reverse transcription, e.g., increases the yield of DNA amplified from the cDNA synthesized by the reverse transcriptase. In some embodiments, including poly(acrylic acid) in a reverse transcription reaction mixture improves detection of target RNAs, e.g., increases the cDNA yield, e.g., increases the yield of DNA amplified from cDNA. In some embodiments, including poly(acrylic acid) in a reverse transcription reaction mixture improves detection of a target RNA, e.g., a target RNA of 200 nucleotides, 500 nucleotides, 1,000 nucleotides, 2,000 nucleotides, 3,000 nucleotides, or longer. In some embodiments, including poly(acrylic acid) in a reverse transcription reaction mixture improves detection of a long target RNAs in a sample, e.g., increases the yield of cDNA synthesized from long target RNAs, e.g., target RNAs of 4,000 nucleotides, 5,000 nucleotides, 6,000 nucleotides, 7,000 nucleotides, 8,000 nucleotides, 9,000 nucleotides, 10,000 nucleotides, 11,000 nucleotides, 12,000 nucleotides, 15,000 nucleotides, 20,000 nucleotides, 25,000 nucleotides, or 30,000 nucleotides or more in

length. In some embodiments, including poly(acrylic acid) in a reverse transcription reaction mixture improves detection of ultra-low abundance RNAs, e.g., detection of nanomolar (nM) target RNAs, picomolar (pM) target RNAs, femtomolar (fM) target RNAs, or attomolar (aM) target RNAs or less in a sample. For example, including poly(acrylic acid) in a reverse transcription reaction mixture during the reverse transcription step of RT-PCR can increase the DNA yield of a 12 fM 4 kilobase (kb) target RNA in a complex input RNA mixture, e.g., increase the yield of DNA amplified from cDNA synthesized by UltraMarathonRT as shown in **FIGs. 3A-3B**. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 10 pg total RNA input in the absence of poly(acrylic acid) during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 100 pg total RNA input in the absence of poly(acrylic acid) during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 1 ng total RNA input in the absence of poly(acrylic acid) during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 10 ng total RNA input in the absence of poly(acrylic acid) during the reverse transcription step is about 0.5. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 100 ng total RNA input in the absence of poly(acrylic acid) during the reverse transcription step is about 1. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 1 µg total RNA input in the absence of poly(acrylic acid) during the reverse transcription step is about 1. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 10 pg total RNA input in the presence of 100 ng/µL poly(acrylic acid) during the reverse transcription step is about 0.9. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 100 pg total RNA input in the presence of 100 ng/µL poly(acrylic acid) during the reverse transcription step is about 0.9. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 1 ng total RNA input in the presence of 100 ng/µL poly(acrylic acid) during the reverse transcription step is about 0.9. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 10 ng total RNA input in the presence of 100 ng/µL poly(acrylic acid) during the reverse transcription step is about 0.9. In some embodiments, the normalized

DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 100 ng total RNA input in the presence of 100 ng/μL poly(acrylic acid) during the reverse transcription step is about 1. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 1 μg total RNA input in the presence of 100 ng/μL poly(acrylic acid) during the reverse transcription step is about 0.7.

[0133] In some embodiments, including heparin in a reverse transcription reaction mixture during the reverse transcription step of RT-PCR can increase the DNA yield of a 12 fM 4 kilobase (kb) target RNA in a complex input RNA mixture, e.g., increase the yield of DNA amplified from cDNA synthesized by Maxima H Minus reverse transcriptase as shown in **FIGS. 4A-4B**. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 10 pg total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 100 pg total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 1 ng total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 10 ng total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 100 ng total RNA input in the absence of heparin during the reverse transcription step is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 1 μg total RNA input in the absence of heparin during the reverse transcription step is about 1. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 10 pg total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 100 pg total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 1 ng total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 10 ng total RNA input in the presence of 100 ng/μL heparin during

the reverse transcription step is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 100 ng total RNA input in the presence of 100 ng/ μ L heparin during the reverse transcription step is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 1 μ g total RNA input in the presence of 100 ng/ μ L heparin during the reverse transcription step is about 0.8.

[0134] In some embodiments, including heparin in a reverse transcription reaction mixture during the reverse transcription step of RT-PCR can increase the DNA yield of a 12 fM 8 kilobase (kb) target RNA in a complex input RNA mixture, e.g., increase the yield of DNA amplified from cDNA synthesized by Induro reverse transcriptase as shown in **FIGs. 5A-5B**. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 10 pg total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 100 pg total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 1 ng total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 10 ng total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 100 ng total RNA input in the absence of heparin during the reverse transcription step is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 1 μ g total RNA input in the absence of heparin during the reverse transcription step is about 1. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 10 pg total RNA input in the presence of 100 ng/ μ L heparin during the reverse transcription step is about 0.5. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 100 pg total RNA input in the presence of 100 ng/ μ L heparin during the reverse transcription step is about 0.5. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 1 ng total RNA input in the presence of 100 ng/ μ L heparin during the reverse transcription step is about 0.6. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 10 ng total RNA input in the presence of 100 ng/ μ L heparin during the reverse transcription

step is about 0.7. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 100 ng total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.6. In some embodiments, the normalized DNA yield of a 12 fM 8 kb target RNA resulting from RT-PCR from 1 μg total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.8.

[0135] In some embodiments, including heparin in a reverse transcription reaction mixture during the reverse transcription step of RT-PCR can increase the DNA yield of a 12 fM 4 kilobase (kb) target RNA in a complex input RNA mixture, e.g., increase the yield of DNA amplified from cDNA synthesized by SuperScript III reverse transcriptase as shown in **FIGS. 6A-6B**. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 10 pg total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 100 pg total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 1 ng total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 10 ng total RNA input in the absence of heparin during the reverse transcription step is about zero. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 100 ng total RNA input in the absence of heparin during the reverse transcription step is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 1 μg total RNA input in the absence of heparin during the reverse transcription step is about 1. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 10 pg total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.7. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 100 pg total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 1 ng total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.8. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 10 ng total RNA input in the presence of 100 ng/μL heparin during

the reverse transcription step is about 0.7. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 100 ng total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.4. In some embodiments, the normalized DNA yield of a 12 fM 4 kb target RNA resulting from RT-PCR from 1 μg total RNA input in the presence of 100 ng/μL heparin during the reverse transcription step is about 0.9.

[0136] In some embodiments, including heparin in a reverse transcription reaction mixture improves the preparation of cDNA libraries, e.g., improves detection of long, low-abundance RNAs in a cDNA library. Total cellular RNA can be reverse transcribed into a cDNA library to be used for methods of measuring global gene expression in a cell, e.g., RNA-seq. In some embodiments, including heparin in a reverse transcription reaction mixture increases the average cDNA length in a cDNA library, e.g., increases the average cDNA length in a cDNA library as shown in **FIG. 7**. In some embodiments, synthesizing a cDNA library from total cellular RNA in the absence of heparin during the reverse transcription step results in an average cDNA length of 1,196 bp. In some embodiments, synthesizing a cDNA library from total cellular RNA in the presence of 4 ng/μL heparin during the reverse transcription step results in an average cDNA length of 1,601 bp. In some embodiments, synthesizing a cDNA library from total cellular RNA in the presence of 8 ng/μL heparin during the reverse transcription step results in an average cDNA length of 1,714 bp. In some embodiments, synthesizing a cDNA library from total cellular RNA in the presence of 20 ng/μL heparin during the reverse transcription step results in an average cDNA length of 1,723 bp.

[0137] In some embodiments, an anionic polymer improves yield of cDNA product produced from a target RNA at low input levels, e.g., as shown in **FIGs. 8-9**. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using UltraMarathonRT in the absence of an anionic polymer is 1. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using UltraMarathonRT in the absence of an anionic polymer is 0.1. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using UltraMarathonRT in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using UltraMarathonRT in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using UltraMarathonRT in the absence of an anionic polymer is 0. In some embodiments, the relative

yield of cDNA product produced from 10 ng of a target RNA using UltraMarathonRT in the presence of heparin is 0.9. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using UltraMarathonRT in the presence of heparin is 0.9. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using UltraMarathonRT in the presence of heparin is 0.9. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using UltraMarathonRT in the presence of heparin is 0.7. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using UltraMarathonRT in the presence of heparin is 0.4. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using UltraMarathonRT in the presence of polyacrylic acid is 0.9. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using UltraMarathonRT in the presence of polyacrylic acid is 1. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using UltraMarathonRT in the presence of polyacrylic acid is 1. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using UltraMarathonRT in the presence of polyacrylic acid is 1. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using UltraMarathonRT in the presence of polyacrylic acid is 0.3. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using UltraMarathonRT in the presence of MS2 RNA is 0.9. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using UltraMarathonRT in the presence of MS2 RNA is 1. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using UltraMarathonRT in the presence of MS2 RNA is 1. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using UltraMarathonRT in the presence of MS2 RNA is 0.9. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using UltraMarathonRT in the presence of MS2 RNA is 0.2. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using UltraMarathonRT in the presence of iota-carrageenan is 1. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using UltraMarathonRT in the presence of iota-carrageenan is 1. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using UltraMarathonRT in the presence of iota-carrageenan is 0.9. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using UltraMarathonRT

in the presence of iota-carrageenan is 0.8. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using UltraMarathonRT in the presence of iota-carrageenan is 0.1. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using InduroRT in the absence of an anionic polymer is 1. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using InduroRT in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using InduroRT in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using InduroRT in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using InduroRT in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using InduroRT in the presence of heparin is 1. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using InduroRT in the presence of heparin is 1.1. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using InduroRT in the presence of heparin is 0.9. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using InduroRT in the presence of heparin is 0.7. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using InduroRT in the presence of heparin is 0.2. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using InduroRT in the presence of polyacrylic acid is 1.1. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using InduroRT in the presence of polyacrylic acid is 0.8. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using InduroRT in the presence of polyacrylic acid is 0.6. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using InduroRT in the presence of polyacrylic acid is 0. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using InduroRT in the presence of polyacrylic acid is 0. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using InduroRT in the presence of MS2 RNA is 0.9. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using InduroRT in the presence of MS2 RNA is 0.9. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using InduroRT in the

presence of MS2 RNA is 0.8. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using InduroRT in the presence of MS2 RNA is 0.5. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using InduroRT in the presence of MS2 RNA is 0.1. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using InduroRT in the presence of iota-carrageenan is 0.9. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using InduroRT in the presence of iota-carrageenan is 1. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using InduroRT in the presence of iota-carrageenan is 0.9. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using InduroRT in the presence of iota-carrageenan is 0.8. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using InduroRT in the presence of iota-carrageenan is 0. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using SuperScript III in the absence of an anionic polymer is 1. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using SuperScript III in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using SuperScript III in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using SuperScript III in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using SuperScript III in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using SuperScript III in the presence of heparin is 0.9. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using SuperScript III in the presence of heparin is 0.8. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using SuperScript III in the presence of heparin is 0.9. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using SuperScript III in the presence of heparin is 0.7. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using SuperScript III in the presence of heparin is 0.4. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using SuperScript III in the presence of polyacrylic acid is 1. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA

using SuperScript III in the presence of polyacrylic acid is 0.9. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using SuperScript III in the presence of polyacrylic acid is 0.9. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using SuperScript III in the presence of polyacrylic acid is 0.6. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using In SuperScript III duroRT in the presence of polyacrylic acid is 0. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using SuperScript III in the presence of MS2 RNA is 1. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using SuperScript III in the presence of MS2 RNA is 1. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using SuperScript III in the presence of MS2 RNA is 0.9. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using SuperScript III in the presence of MS2 RNA is 0.8. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using SuperScript III in the presence of MS2 RNA is 0.2. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using SuperScript III in the presence of iota-carrageenan is 0.8. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using SuperScript III in the presence of iota-carrageenan is 0.9. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using SuperScript III in the presence of iota-carrageenan is 0.9. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using SuperScript III in the presence of iota-carrageenan is 0.7. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using SuperScript III in the presence of iota-carrageenan is 0.4. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using Maxima H minus in the absence of an anionic polymer is 1. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using Maxima H minus in the absence of an anionic polymer is 0.1. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using Maxima H minus in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using Maxima H minus in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using Maxima H minus in the absence of an anionic

polymer is 0. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using Maxima H minus in the presence of heparin is 0.9. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using Maxima H minus in the presence of heparin is 0.7. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using Maxima H minus in the presence of heparin is 0.8. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using Maxima H minus in the presence of heparin is 0.6. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using Maxima H minus in the presence of heparin is 0.2. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using Maxima H minus in the presence of polyacrylic acid is 1. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using Maxima H minus in the presence of polyacrylic acid is 1.1. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using Maxima H minus in the presence of polyacrylic acid is 1. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using Maxima H minus in the presence of polyacrylic acid is 0.8. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using Maxima H minus in the presence of polyacrylic acid is 0.4. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using Maxima H minus in the presence of MS2 RNA is 1. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using Maxima H minus in the presence of MS2 RNA is 1. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using Maxima H minus in the presence of MS2 RNA is 1. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using Maxima H minus in the presence of MS2 RNA is 0.9. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using Maxima H minus in the presence of MS2 RNA is 0.5. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using Maxima H minus in the presence of iota-carrageenan is 1. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using Maxima H minus in the presence of iota-carrageenan is 1. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using Maxima H minus in the presence of iota-carrageenan is 1. In some embodiments, the relative yield of cDNA product produced from 10 pg

of a target RNA using Maxima H minus in the presence of iota-carrageenan is 0.9. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using Maxima H minus in the presence of iota-carrageenan is 0.5. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using AMV RT in the absence of an anionic polymer is 1. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using AMV RT in the absence of an anionic polymer is 0.8. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using AMV RT in the absence of an anionic polymer is 0.1. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using AMV RT in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using AMV RT in the absence of an anionic polymer is 0. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using AMV RT in the presence of heparin is 0.5. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using AMV RT in the presence of heparin is 0.5. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using AMV RT in the presence of heparin is 0.1. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using AMV RT in the presence of heparin is 0. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using AMV RT in the presence of heparin is 0. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using AMV RT in the presence of polyacrylic acid is 0.9. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using AMV RT in the presence of polyacrylic acid is 0.8. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using AMV RT in the presence of polyacrylic acid is 0.7. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using AMV RT in the presence of polyacrylic acid is 0.4. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using AMV RT in the presence of polyacrylic acid is 0. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using AMV RT in the presence of MS2 RNA is 0.7. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using AMV RT in the presence of MS2 RNA is 0.6. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using AMV RT in the

presence of MS2 RNA is 0.7. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using AMV RT in the presence of MS2 RNA is 0.7. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using AMV RT in the presence of MS2 RNA is 0.3. In some embodiments, the relative yield of cDNA product produced from 10 ng of a target RNA using AMV RT in the presence of iota-carrageenan is 0.8. In some embodiments, the relative yield of cDNA product produced from 1 ng of a target RNA using AMV RT in the presence of iota-carrageenan is 0.7. In some embodiments, the relative yield of cDNA product produced from 100 pg of a target RNA using AMV RT in the presence of iota-carrageenan is 0.6. In some embodiments, the relative yield of cDNA product produced from 10 pg of a target RNA using AMV RT in the presence of iota-carrageenan is 0. In some embodiments, the relative yield of cDNA product produced from 1 pg of a target RNA using AMV RT in the presence of iota-carrageenan is 0.

EXAMPLES

[0138] 1. Analysis of heparin as a substitute for carrier RNA in reverse transcription of a long, low abundance RNA target molecule.

[0139] 2. Detection of an ultra-low abundance target RNA by reverse transcription using UltraMarathonRT in the presence of heparin.

[0140] 3. Detection of an ultra-low abundance target RNA by reverse transcription using UltraMarathonRT in the presence of poly(acrylic acid).

[0141] 4. Detection of an ultra-low abundance target RNA by reverse transcription using Maxima H Minus in the presence of heparin.

[0142] 5. Detection of an ultra-low abundance target RNA by reverse transcription using Induro in the presence of heparin.

[0143] 6. Detection of an ultra-low abundance target RNA by reverse transcription using SuperScript III in the presence of heparin.

[0144] 7. Effect of heparin on long cDNA synthesis during cDNA library preparation from ultra-low RNA input.

[0145] 8. Detection of an ultra-low abundance target RNA by reverse transcription using various reverse transcriptases in the presence of anionic polymers.

Example 1: Analysis of heparin as a substitute for carrier RNA in reverse transcription of a long, low abundance RNA target molecule.

[0146] This example describes a method of reverse transcription coupled with polymerase chain reaction (RT-PCR) for detection of long, low abundance target RNAs using heparin as a substitute for carrier RNA during the reverse transcription step.

[0147] A reverse transcription reaction was carried out by first combining 1 μ L of a 5 μ M Oligo-dT20 solution (Thermo Fisher), 1 μ L of a 50 pg/ μ L SIRV-Set4 solution (Lexogen), and 0.5 μ L of a 10 mM dNTP solution (New England Biolabs). The reaction components were mixed, heated to 95°C for 30 seconds, then cooled on ice for annealing. Next, 4 μ L of 2.5X buffer, 0.5 μ L of a 5 μ M or 20 U/ μ L UltraMarathonRT reverse transcriptase solution, 2 μ L of purified water, and 1 μ L of the following carrier RNAs: 0.5 μ g/ μ L total cellular RNA (extracted from Huh7.5 cells), 0.3 μ g/ μ L *E. coli* total tRNA (Sigma), 0.3 μ g/ μ L MS2 RNA (Sigma), or heparin (Sigma) at the following concentrations: 50 ng/ μ L, 100 ng/ μ L, 200 ng/ μ L, or 300 ng/ μ L were added to the reaction mixture. The mixture was incubated at 42°C for 30 minutes to allow reverse transcription of the SIRV-Set4 input RNA.

[0148] The cDNA product of the 4 kilobase (kb) SIRV4002 target RNA produced from the reverse transcription reaction was amplified using polymerase chain reaction (PCR). Amplification of the target cDNA was carried out by combining 1 μ L of the total cDNA product of the reverse transcription reaction, 1 μ L of an 8 μ M forward primer specific to the SIRV4002 cDNA, 1 μ L of an 8 μ M reverse primer specific to the SIRV4002 cDNA, 10 μ L of LongAmp® Taq 2X Master Mix (New England Biolabs), and 7 μ L of purified water. The reaction mixture was heated to 94°C for 2 minutes, then cycled 30 times through the following stages: 94°C for 30 seconds, 60°C for 30 seconds, and 65°C for 5 minutes. Finally, the reaction mixture was incubated at 65°C for 10 minutes then cooled to 8°C.

[0149] Reverse transcription of the SIRV4002 RNA was assessed by subjecting the corresponding amplified PCR product to agarose gel electrophoresis and visualizing DNA in the gel. In this assay, thicker DNA bands in the agarose gel indicate greater cDNA yield from the reverse transcription step. **FIGs. 1A-1B** summarize the results of RT-PCR to detect the low-abundance SIRV4002 RNA in a complex mixture of RNAs. The results show that the cDNA yield of SIRV4002 RNA in the presence of heparin was approximately equivalent to the cDNA

yield of SIRV4002 RNA in the presence of various carrier RNAs. Thus, heparin simulated carrier RNA during reverse transcription of a long, low-abundance target RNA.

Example 2: Detection of an ultra-low abundance target RNA by reverse transcription using UltraMarathonRT in the presence of heparin.

[0150] This example describes a method of reverse transcription coupled with polymerase chain reaction (RT-PCR) for detection of ultra-low abundance target RNAs in a sample using heparin during the reverse transcription step.

[0151] A reverse transcription reaction was carried out by first combining 1 μL of a 5 μM Oligo-dT20 solution (Thermo Fisher), 1 μL of a 10 $\text{pg}/\mu\text{L}$ SIRV-Set4 solution (Lexogen), 0.5 μL of a 10 mM dNTP solution (New England Biolabs), and 1 μL of 10 $\text{pg}/\mu\text{L}$, 100 $\text{pg}/\mu\text{L}$, 1 $\text{ng}/\mu\text{L}$, 10 $\text{ng}/\mu\text{L}$, 100 $\text{ng}/\mu\text{L}$ or 1 $\mu\text{g}/\mu\text{L}$ total RNA (extracted from Huh7.5 cells). The reaction components were mixed, heated to 95°C for 30 seconds, then cooled on ice for annealing. Next, 4 μL of 2.5X buffer, 0.5 μL of a 5 μM or 20 $\text{U}/\mu\text{L}$ UltraMarathonRT reverse transcriptase solution, and one of:

- (a) 1 μL of 100 $\text{ng}/\mu\text{L}$ heparin (Sigma Aldrich) and 1 μL purified water; or
- (b) 2 μL purified water

were added to the reaction mixture. The mixture was incubated at 42°C for 30 minutes to allow reverse transcription of the SIRV-Set4 input RNA.

[0152] The cDNA product of an 8 kilobase (kb) SIRV8002 target RNA produced from the reverse transcription reaction was amplified using polymerase chain reaction (PCR). Amplification of the target cDNA was carried out by combining 1 μL of the total cDNA product of the reverse transcription reaction, 1 μL of an 8 μM forward primer specific to the SIRV8002 cDNA, 1 μL of an 8 μM reverse primer specific to the SIRV8002 cDNA, 10 μL of LongAmp® Taq 2X Master Mix (New England Biolabs), and 7 μL of purified water. The reaction mixture was heated to 94°C for 2 minutes, then cycled 36 times through the following stages: 94°C for 30 seconds, 60°C for 30 seconds, and 65°C for 10 minutes. Finally, the reaction mixture was incubated at 65°C for 10 minutes then cooled to 8°C.

[0153] Reverse transcription of the SIRV8002 RNA was assessed by subjecting the corresponding amplified PCR product to agarose gel electrophoresis and visualizing DNA in the gel. In this assay, thicker DNA bands in the agarose gel indicate greater cDNA yield from the

reverse transcription step. The thickness of the 8 kb DNA band was quantified using ImageQuant TL software (GE Healthcare). **FIGs. 2A-2B** summarize the results of RT-PCR to detect the low-abundance SIRV8002 RNA in a complex mixture of RNAs. The results show that the cDNA yield of the SIRV8002 RNA from ultra-low amounts of input RNA is increased in the presence of heparin.

Example 3: Detection of an ultra-low abundance target RNA by reverse transcription using UltraMarathonRT in the presence of poly(acrylic acid).

[0154] This example describes a method of reverse transcription coupled with polymerase chain reaction (RT-PCR) for detection of ultra-low abundance target RNAs in a sample using poly(acrylic acid) during the reverse transcription step.

[0155] A reverse transcription reaction was carried out by first combining 1 μ L of a 5 μ M Oligo-dT20 solution (Thermo Fisher), 1 μ L of a 10 pg/ μ L SIRV-Set4 solution (Lexogen), 0.5 μ L of a 10 mM dNTP solution (New England Biolabs), and 1 μ L of 10 pg/ μ L, 100 pg/ μ L, 1 ng/ μ L, 10 ng/ μ L, 100 ng/ μ L or 1 μ g/ μ L total RNA (extracted from Huh7.5 cells). The reaction components were mixed, heated to 95°C for 30 seconds, then cooled on ice for annealing. Next, 4 μ L of 2.5X buffer (Manufacturer), 0.5 μ L of a 5 μ M or 20 U/ μ L UltraMarathonRT reverse transcriptase solution, and one of:

- (a) 1 μ L of 100 ng/ μ L poly(acrylic acid) (Sigma) and 1 μ L purified water; or
- (b) 2 μ L purified water

were added to the reaction mixture. The mixture was incubated at 42°C for 30 minutes to allow reverse transcription of the SIRV-Set4 input RNA.

[0156] The cDNA product of a 4 kilobase (kb) SIRV4002 target RNA produced from the reverse transcription reaction was amplified using polymerase chain reaction (PCR). Amplification of the target cDNA was carried out by combining 1 μ L of the total cDNA product of the reverse transcription reaction, 1 μ L of an 8 μ M forward primer specific to the SIRV4002 cDNA, 1 μ L of an 8 μ M reverse primer specific to the SIRV4002 cDNA, 10 μ L of LongAmp[®] Taq 2X Master Mix (New England Biolabs), and 7 μ L of purified water. The reaction mixture was heated to 94°C for 2 minutes, then cycled 33 times through the following stages: 94°C for 30 seconds, 60°C for 30 seconds, and 65°C for 5 minutes. Finally, the reaction mixture was incubated at 65°C for 10 minutes then cooled to 8°C.

[0157] Reverse transcription of the SIRV4002 RNA was assessed by subjecting the corresponding amplified PCR product to agarose gel electrophoresis and visualizing DNA in the gel. In this assay, thicker DNA bands in the agarose gel indicate greater cDNA yield from the reverse transcription step. The thickness of the 4 kb DNA band was quantified using ImageQuant TL software (GE Healthcare). **FIGs. 3A-3B** summarize the results of RT-PCR to detect the low-abundance SIRV4002 RNA in a complex mixture of RNAs. The results show that the cDNA yield of the SIRV4002 RNA from ultra-low amounts of input RNA is increased in the presence of poly(acrylic acid).

Example 4: Detection of an ultra-low abundance target RNA by reverse transcription using Maxima H Minus in the presence of heparin.

[0158] This example describes a method of reverse transcription coupled with polymerase chain reaction (RT-PCR) for detection of ultra-low abundance target RNAs in a sample using heparin during the reverse transcription step.

[0159] A reverse transcription reaction was carried out by first combining 1 μ L of a 5 μ M Oligo-dT20 solution (Thermo Fisher), 1 μ L of a 10 pg/ μ L SIRV-Set4 solution (Lexogen), 0.5 μ L of a 10 mM dNTP solution (New England Biolabs), and 1 μ L of 10 pg/ μ L, 100 pg/ μ L, 1 ng/ μ L, 10 ng/ μ L, 100 ng/ μ L or 1 μ g/ μ L total RNA (extracted from Huh7.5 cells). The reaction components were mixed, heated to 95°C for 30 seconds, then cooled on ice for annealing. Next, 4 μ L of 2.5X buffer, 0.5 μ L of a 5 μ M or 20 U/ μ L Maxima H Minus reverse transcriptase (Thermo Fisher) solution, and one of:

- (a) 1 μ L of 100 ng/ μ L heparin (Sigma Aldrich) and 1 μ L purified water; or
- (b) 2 μ L purified water

were added to the reaction mixture. The mixture was incubated at 42°C for 30 minutes to allow reverse transcription of the SIRV-Set4 input RNA.

[0160] The cDNA product of a 4 kilobase (kb) SIRV4002 target RNA produced from the reverse transcription reaction was amplified using polymerase chain reaction (PCR). Amplification of the target cDNA was carried out by combining 1 μ L of the total cDNA product of the reverse transcription reaction, 1 μ L of an 8 μ M forward primer specific to the SIRV4002 cDNA, 1 μ L of an 8 μ M reverse primer specific to the SIRV4002 cDNA, 10 μ L of LongAmp© Taq 2X Master Mix (New England Biolabs), and 7 μ L of purified water. The reaction mixture

was heated to 94°C for 2 minutes, then cycled 33 times through the following stages: 94°C for 30 seconds, 60°C for 30 seconds, and 65°C for 5 minutes. Finally, the reaction mixture was incubated at 65°C for 10 minutes then cooled to 8°C.

[0161] Reverse transcription of the SIRV4002 RNA was assessed by subjecting the corresponding amplified PCR product to agarose gel electrophoresis and visualizing DNA in the gel. In this assay, thicker DNA bands in the agarose gel indicate greater cDNA yield from the reverse transcription step. The thickness of the 4 kb DNA band was quantified using ImageQuant TL software (GE Healthcare). **FIGs. 4A-4B** summarize the results of RT-PCR to detect the low-abundance SIRV4002 RNA in a complex mixture of RNAs. The results show that the cDNA yield of the SIRV4002 RNA from ultra-low amounts of input RNA is increased in the presence of heparin.

Example 5: Detection of an ultra-low abundance target RNA by reverse transcription using Induro in the presence of heparin.

[0162] This example describes a method of reverse transcription coupled with polymerase chain reaction (RT-PCR) for detection of ultra-low abundance target RNAs in a sample using heparin during the reverse transcription step.

[0163] A reverse transcription reaction was carried out by first combining 1 µL of a 5 µM Oligo-dT20 solution (Thermo Fisher), 1 µL of a 10 pg/µL SIRV-Set4 solution (Lexogen), 0.5 µL of a 10 mM dNTP solution (New England Biolabs), and 1 µL of 10 pg/µL, 100 pg/µL, 1 ng/µL, 10 ng/µL, 100 ng/µL or 1 µg/µL total RNA (extracted from Huh7.5 cells). The reaction components were mixed, heated to 95°C for 30 seconds, then cooled on ice for annealing. Next, 4 µL of 2.5X buffer, 0.5 µL of a 5 µM or 20 U/µL Induro reverse transcriptase (New England Biolabs) solution, and one of:

- (a) 1 µL of 100 ng/µL heparin (Sigma Aldrich) and 1 µL purified water; or
- (b) 2 µL purified water

were added to the reaction mixture. The mixture was incubated at 42°C for 30 minutes to allow reverse transcription of the SIRV-Set4 input RNA.

[0164] The cDNA product of an 8 kilobase (kb) SIRV8003 target RNA produced from the reverse transcription reaction was amplified using polymerase chain reaction (PCR). Amplification of the target cDNA was carried out by combining 1 µL of the total cDNA product

of the reverse transcription reaction, 1 μ L of an 8 μ M forward primer specific to the SIRV8003 cDNA, 1 μ L of an 8 μ M reverse primer specific to the SIRV8003 cDNA, 10 μ L of LongAmp[®] Taq 2X Master Mix (New England Biolabs), and 7 μ L of purified water. The reaction mixture was heated to 94°C for 2 minutes, then cycled 36 times through the following stages: 94°C for 30 seconds, 60°C for 30 seconds, and 65°C for 10 minutes. Finally, the reaction mixture was incubated at 65°C for 10 minutes then cooled to 8°C.

[0165] Reverse transcription of the SIRV8003 RNA was assessed by subjecting the corresponding amplified PCR product to agarose gel electrophoresis and visualizing DNA in the gel. In this assay, thicker DNA bands in the agarose gel indicate greater cDNA yield from the reverse transcription step. The thickness of the 8 kb DNA band was quantified using ImageQuant TL software (GE Healthcare). **FIGs. 5A-5B** summarize the results of RT-PCR to detect the low-abundance SIRV8003 RNA in a complex mixture of RNAs. The results show that the cDNA yield of the SIRV8003 RNA from ultra-low amounts of input RNA is increased in the presence of heparin.

Example 6: Detection of an ultra-low abundance target RNA by reverse transcription using SuperScript III in the presence of heparin.

[0166] This example describes a method of reverse transcription coupled with polymerase chain reaction (RT-PCR) for detection of ultra-low abundance target RNAs in a sample using heparin during the reverse transcription step.

[0167] A reverse transcription reaction was carried out by first combining 1 μ L of a 5 μ M Oligo-dT20 solution (Thermo Fisher), 1 μ L of a 10 pg/ μ L SIRV-Set4 solution (Lexogen), 0.5 μ L of a 10 mM dNTP solution (New England Biolabs), and 1 μ L of 10 pg/ μ L, 100 pg/ μ L, 1 ng/ μ L, 10 ng/ μ L, 100 ng/ μ L or 1 μ g/ μ L total RNA (extracted from Huh7.5 cells). The reaction components were mixed, heated to 95°C for 30 seconds, then cooled on ice for annealing. Next, 4 μ L of 2.5X buffer, 0.5 μ L of a 5 μ M or 20 U/ μ L SuperScript III reverse transcriptase (Invitrogen) solution, and one of:

- (a) 1 μ L of 100 ng/ μ L heparin (Sigma Aldrich) and 1 μ L purified water; or
- (b) 2 μ L purified water

were added to the reaction mixture. The mixture was incubated at 42°C for 30 minutes to allow reverse transcription of the SIRV-Set4 input RNA.

[0168] The cDNA product of a 4 kilobase (kb) SIRV4002 target RNA produced from the reverse transcription reaction was amplified using polymerase chain reaction (PCR). Amplification of the target cDNA was carried out by combining 1 μ L of the total cDNA product of the reverse transcription reaction, 1 μ L of an 8 μ M forward primer specific to the SIRV4002 cDNA, 1 μ L of an 8 μ M reverse primer specific to the SIRV4002 cDNA, 10 μ L of LongAmp[®] Taq 2X Master Mix (New England Biolabs), and 7 μ L of purified water. The reaction mixture was heated to 94°C for 2 minutes, then cycled 33 times through the following stages: 94°C for 30 seconds, 60°C for 30 seconds, and 65°C for 5 minutes. Finally, the reaction mixture was incubated at 65°C for 10 minutes then cooled to 8°C.

[0169] Reverse transcription of the SIRV4002 RNA was assessed by subjecting the corresponding amplified PCR product to agarose gel electrophoresis and visualizing DNA in the gel. In this assay, thicker DNA bands in the agarose gel indicate greater cDNA yield from the reverse transcription step. The thickness of the 4 kb DNA band was quantified using ImageQuant TL software (GE Healthcare). **FIGs. 6A-6B** summarize the results of RT-PCR to detect the low-abundance SIRV4002 RNA in a complex mixture of RNAs. The results show that the cDNA yield of the SIRV4002 RNA from ultra-low amounts of input RNA is increased in the presence of heparin.

Example 7. Effect of heparin on long cDNA synthesis during cDNA library preparation from ultra-low RNA input.

[0170] This example describes a method for cDNA library preparation from ultra-low RNA input using template switching and heparin during the reverse transcription step.

[0171] Primers were annealed to the template RNA by combining 0.1 μ L of a 5 μ M MRT_dT18 primer solution, 0.33 μ L of 30 pg/ μ L human universal total cellular RNA (Thermo Fisher), and 0.1 μ L of a 10 mM dNTP solution (New England Biolabs) in a nuclease-free 0.2 mL PCR tube. The mixture was incubated at 95°C for 30 seconds in a PCR thermocycler, then snap cooled on ice to anneal the primer to the template. The mixture was collected in the tube by brief centrifugation.

[0172] A reverse transcription master mix was generated by combining 0.8 μ L of a 2.5X stock MarathonRT reaction buffer, 0.1 μ L of 20U/ μ L of UltraMarathonRT reverse transcriptase, 0.5 μ L of 4 ng/ μ L, 8 ng/ μ L, or 20 ng/ μ L heparin (Sigma Aldrich), 0.025 μ L of 40U/ μ L

RNaseOUT™ (Thermo Fisher), and 0.045 µL RNase-free water. The master mix was then incubated at room temperature for 2 minutes.

[0173] First strand cDNA synthesis was carried out by combining the annealed primer/template mixture with the reverse transcription master mix. The final mixture was incubated at 42°C for 1 hour to carry out reverse transcription.

[0174] A template switching reaction was carried out following cDNA synthesis by adding to the cDNA synthesis mixture 0.8 µL of 5X TS buffer, 0.4 µL of 5 µM UltraMarathonRT reverse transcriptase, 0.4 µL of 10 µM template switching oligonucleotide (5'-CCCTCTCTCTCTTTCTCTCTCTTTT-3' (SEQ ID NO:13)), and 0.4 µL of 10 mM deoxyadenosine triphosphate (dATP). The resulting mixture was mixed thoroughly and incubated at 42°C for 30 minutes in a PCR thermocycler to allow for template switching by the reverse transcriptase.

[0175] Following the template switching reaction, the cDNA was pre-amplified by polymerase chain reaction (PCR). Pre-amplification of the cDNA was carried out by combining 4 µL of the template switching product, 7.5 µL of 8 µM AmpPCR primer (Thermo Fisher), 6 µL of 5X GC enhanced buffer (Roche), 0.9 µL of 10 mM dNTP mix (Roche), 0.6 µL of 1 U/µL KAPA HiFi DNA polymerase (Roche), and 11 µL RNase-free water. The resulting mixture was incubated at 98°C for 2 minutes, then cycled 22 times through the following stages: 98°C for 15 seconds, 62°C for 30 seconds, and 72°C for 6 minutes. Finally, the reaction mixture was incubated at 72°C for 5 minutes then cooled to 4°C.

[0176] The pre-amplified cDNA library was purified using AMPure XP beads (Beckman). The beads were brought to room temperature for 30 minutes and resuspended by vortexing. The pre-amplified cDNA was combined with 24 µL of beads (0.8 volumes), mixed by pipetting, and incubated for 10 minutes at room temperature to allow the DNA to bind to the beads. The samples were then placed on a magnet stand for 5 minutes and the beads were washed with 200 µL of 70% (vol/vol) ethanol. The beads were allowed to incubate with the ethanol solution for 30 seconds before the ethanol solution was removed, then the ethanol was repeated. Trace ethanol was removed, and the beads were allowed to dry. The beads were then resuspended in 12 µL nuclease-free water by mixing and incubated at 37°C for 10 minutes to allow complete elution of long DNA molecules. Following elution of the DNA, the beads were

collected using a magnetic stand for 2 minutes and the supernatant containing the eluted DNA was transferred to a new 0.2 mL PCR tube.

[0177] The length of cDNAs synthesized from ultra-low input RNAs was assessed by subjecting the purified DNA to electrophoresis using a Bioanalyzer instrument (Agilent). The concentration of purified DNA was measured using a Qubit (Thermo Fisher) instrument according to manufacturer's instructions before Bioanalyzer analysis. An electrophoresis profile of the purified DNA was generated on a Bioanalyzer instrument using a high sensitivity DNA chip according to manufacturer's instructions. In this assay, thicker DNA bands in the electrophoresis profile indicate greater cDNA yield from the reverse transcription step. Additionally, bands near the top of the electrophoresis profile indicate longer cDNAs synthesized during the reverse transcription step. **FIG. 7** summarizes the results of the electrophoresis profile and Bioanalyzer analysis. The results show that the average DNA length (e.g., DNA pre-amplified from the cDNA) increased as higher concentrations of heparin were present during the reverse transcription step. Thus, including heparin during the reverse transcription step increased the length of cDNAs synthesized from ultra-low RNA input.

Example 8: Detection of an ultra-low abundance target RNA by reverse transcription using various reverse transcriptases in the presence of anionic polymers.

[0178] This example describes a method of reverse transcription coupled with polymerase chain reaction (RT-PCR) for detection of ultra-low abundance target RNAs in a sample using anionic excipients during the reverse transcription step.

[0179] A reverse transcription reaction was carried out by first combining 0.5 μ L of a 5 μ M Oligo-dT18 primer solution (Thermo Fisher), 0.5 μ L of a 10 mM dNTP solution (New England Biolabs), and 1 μ L of 1 pg, 10 pg/ μ L, 100 pg/ μ L, 1 ng/ μ L or 10 ng/ μ L HeLa cell total RNA. The reaction components were mixed, heated to 95°C for 30 seconds, then cooled on ice for Oligo-dT18 primer annealing to mRNA poly(rA) tails. Next, 5 μ L of 2X uMRT buffer or 2 μ L of 5X buffer for other RTs, 0.5 μ L of UltraMarathonRT (5 μ M or 20 U/ μ L), Induro RT, SuperScript III, Maxima H minus, or AMV RT reverse transcriptase solution, and one of:

- (a) purified water to make a total volume of 10 μ L;
- (b) 0.5 μ L of 200 ng/ μ L heparin (Sigma Aldrich) and purified water to make a total volume of 10 μ L;

- (c) 0.5 μ L of 200 ng/ μ L polyacrylic acid and purified water to make a total volume of 10 μ L;
- (d) 0.5 μ L of 200 ng/ μ L MS2 phage RNA and purified water to make a total volume of 10 μ L; or
- (e) 0.5 μ L of 200 ng/ μ L iota-carrageenan and purified water to make a total volume of 10 μ L;

were added to the reaction mixture. The mixture was incubated at 30°C for 15 minutes for uMRT, 55°C for 10 minutes for Induro RT, 50°C for 20 minutes for SuperScript III, 50°C for 30 minutes for Maxima H minus and 42°C for 40 minutes for AMV RT, to allow reverse transcription of the total HeLa cell mRNA.

[0180] The cDNA product of the 1.8 kilobase (kb) beta-actin (ACTB) target RNA produced from the reverse transcription reaction was amplified using polymerase chain reaction (PCR). Amplification of the target cDNA was carried out by combining 1 μ L of the total cDNA product of the reverse transcription reaction, 1 μ L of an 8 μ M forward primer specific to the ACTB cDNA, 1 μ L of an 8 μ M reverse primer specific to the ACTB cDNA, 10 μ L of KOD ONE 2X Master Mix (Toyobo), and 7 μ L of purified water. The reaction mixture was heated to 98°C for 2 minutes, then cycled 40 times through the following stages: 98°C for 10 seconds, 60°C for 15 seconds, and 68°C for 45 sec. Finally, the reaction mixture was incubated at 68°C for 10 minutes then cooled to 8°C.

[0181] Reverse transcription of the ACTB mRNA was assessed by subjecting the corresponding amplified PCR product to agarose gel electrophoresis and visualizing DNA in the gel. In this assay, thicker DNA bands in the agarose gel indicate greater cDNA yield from the reverse transcription step. The thickness of the 1.8 kb DNA band was quantified using ImageJ. **FIGs. 8-9** summarize the results of RT-PCR to detect ACTB mRNA in a complex mixture of RNAs. The results show that the cDNA yield of the ACTB RNA from 1 pg to 1 ng of input RNA is increased in the presence of several anionic polymers. The results additionally show that the effect of improved detection of 1 pg to 1 ng of target RNA by addition of an anionic excipient is observed with various types of reverse transcriptases.

CLAIMS

1. A method for acquiring a value for the presence of a target ribonucleic acid (RNA) in a mixture, comprising contacting the mixture with:
 - (i) a polymerase, e.g., a reverse transcriptase;
 - (ii) an oligonucleotide primer;
 - (iii) a deoxynucleotide triphosphate (dNTP) solution, and
 - (iv) an anionic polymer,thereby acquiring a value for the presence of the target RNA.
2. The method of claim 1, wherein the acquiring a value for the presence of the target RNA comprises detecting the presence of a target RNA.
3. The method of any one of claims 1-2, wherein acquiring a value for the presence of a target RNA comprises quantifying the total amount of a target RNA.
4. The method of any one of claims 1-3, wherein the acquiring a value for the presence of the target RNA comprises producing a cDNA product that comprises a complementary DNA sequence to the target RNA.
5. The method of any one of the preceding claims, wherein the target RNA is a low abundance RNA.
6. The method of any one of the preceding claims, wherein the level of the target RNA in the mixture is less than 1 μ g, 500 ng, 250 ng, 100 ng, 50 ng, 25 ng, 10 ng, 5 ng, 1 ng, 500 pg, 250 pg, 100 pg, 50 pg, 25 pg, 10 pg, 5 pg, 1 pg, 500 fg, 250 fg, 100 fg, 50 fg, 25 fg, 10 fg, or less.
7. The method of any one of the preceding claims, wherein the level of the target RNA in the mixture is between about 1 μ g to about 0.1 pg.

8. The method of any one of the preceding claims, wherein the polymerase is a DNA polymerase or an RNA polymerase.
9. The method of any one of the preceding claims, wherein the polymerase is a reverse transcriptase.
10. The method of any one of the preceding claims, wherein the reverse transcriptase is derived from a virus, an intron, a telomerase, a retrotransposon, a polymerase with reverse transcriptase activity, or an engineered polymerase with reverse transcriptase activity.
11. The method of any one of the preceding claims, wherein the reverse transcriptase is a group II intron reverse transcriptase, a retrotransposon reverse transcriptase, a telomerase reverse transcriptase, a viral reverse transcriptase or a retroviral reverse transcriptase.
12. The method of any one of the preceding claims, wherein the reverse transcriptase comprises MarathonRT, UltraMarathonRT, Roseburia intestinalis reverse transcriptase, Induro RT, Maxima H Minus, SuperScript, SuperScript II, SuperScript III, SuperScript IV, PrimeScript, Transcriptor, GoScript, ProtoScript II, SMARTScribe, Avian Myeloblastosis Virus (AMV) reverse transcriptase, Moloney Murine Leukemia Virus (MMLV) reverse transcriptase, human immunodeficiency virus (HIV) reverse transcriptase, Rous sarcoma virus reverse transcriptase, Bombyx morii R2 RNA element reverse transcriptase, human L1 element reverse transcriptase, human telomerase reverse transcriptase (TERT), TGIRT, or a fragment, variant, mutant, or derivative thereof.
13. The method of any one of the preceding claims, wherein the reverse transcriptase is MarathonRT, UltraMarathonRT, Induro RT, Maxima H minus, SuperScript III, or Avian myeloblastosis virus (AMV) reverse transcriptase.
14. The method of any one of the preceding claims, wherein the reverse transcriptase is MarathonRT.

15. The method of any one of the preceding claims, wherein the reverse transcriptase is UltraMarathonRT.
16. The method of any one of the preceding claims, wherein the reverse transcriptase is Induro RT.
17. The method of any one of the preceding claims, wherein the reverse transcriptase is SuperScript III.
18. The method of any one of the preceding claims, wherein the reverse transcriptase is Maxima H minus.
19. The method of any one of the preceding claims, wherein the reverse transcriptase is AMV reverse transcriptase.
20. The method of any one of the preceding claims, wherein the oligonucleotide primer is between 5 and 200 nucleotides in length.
21. The method of any one of the preceding claims, comprising at least two oligonucleotide primers.
22. The method of any one of the preceding claims, wherein the anionic polymer comprises a naturally occurring polymer or a non-naturally occurring polymer.
23. The method of any one of the preceding claims, wherein the anionic polymer comprises a polynucleotide, peptide, polypeptide, or oligosaccharide.
24. The method of any one of the preceding claims, wherein the anionic polymer comprises an oligosaccharide.

25. The method of any one of the preceding claims, wherein the anionic polymer comprises a glycosaminoglycan.
26. The method of any one of the preceding claims, wherein the anionic polymer comprises an alginate, hyaluronate, dextran, heparin, a heparin-mimicking polymer, or a carrageenan.
27. The method of any one of the preceding claims, wherein the anionic polymer comprises unfractionated heparin, low molecular weight heparin, modified dextran, carboxymethyl benzylamide sulfonate dextran, sulfated glycopolymer, poly(GEMA)-sulfate, sulfated mannose polymer, sulfated lactose polymer, a polyaromatic anionic compound, a polyionomer, a sulfonated ionomer, pectin, fucoidan, gum arabic, a polysulfonated compound, iota-carrageenan, kappa-carrageenan, lambda-carrageenan, mu-carrageenan, nu-carrageenan, theta-carrageenan, xi-carrageenan, alpha-carrageenan, beta-carrageenan, gamma-carrageenan, omega-carrageenan, delta-carrageenan, psi-carrageenan, or poly(acrylic acid).
28. The method of any one of the preceding claims, wherein the anionic polymer comprises heparin.
29. The method of any one of the preceding claims, wherein the anionic polymer comprises iota-carrageenan.
30. The method of any one of the preceding claims, wherein the anionic polymer comprises poly(acrylic acid).
31. The method of any one of the preceding claims, wherein the concentration of the anionic polymer in the mixture is between about 0.5 ng/ μ L to about 10 μ g/ μ L in the mixture.
32. The method of any one of the preceding claims, wherein the concentration of the anionic polymer in the mixture is between about 5 ng/ μ L to about 30 ng/ μ L in the mixture.

33. The method of any one of the preceding claims, wherein the concentration of the anionic polymer in the mixture is about 0.5 ng/uL, 1 ng/uL, 2.5 ng/uL, 5 ng/uL, 10 ng/uL, 15 ng/uL, 20 ng/uL, 30 ng/μL, 50 ng/μL, 100 ng/μL, 250 ng/μL, 500 ng/μL, 750 ng/μL, 1 μg/μL, 2.5 μg/μL, 5 μg/μL, or 10 μg/μL in the mixture.
34. The method of any one of the preceding claims, wherein mixture further comprises a plurality of input RNA sequences (e.g., non-target RNA).
35. The method of any one of the preceding claims, wherein the method comprises:
- (i) detecting the level, identity or concentration of a target RNA;
 - (ii) increasing the signal to noise ratio of a target RNA; and/or
 - (iii) increasing the processivity of the reverse transcription reaction, compared to a reference standard.
36. The method of any one of the preceding claims, wherein the method comprises detecting the level, identity or concentration of a target RNA.
37. The method of any one of the preceding claims, wherein the method comprises increasing the signal to noise ratio of a target RNA.
38. The method of any one of the preceding claims, wherein the method comprises increasing the processivity of the reverse transcription reaction.
39. The method of any one of the preceding claims, wherein the reference standard is a method for acquiring a value for the presence of a target RNA in the absence of an anionic polymer.
40. A kit comprising:
- (v) a reverse transcriptase;
 - (vi) an oligonucleotide primer;
 - (vii) a deoxynucleotide triphosphate (dNTP) solution, and

- (viii) an anionic polymer or a buffer containing an anionic polymer.
41. The kit of claim 40, wherein the reverse transcriptase is derived from a virus, an intron, a telomerase, a retrotransposon, a polymerase with reverse transcriptase activity, or an engineered polymerase with reverse transcriptase activity.
42. The kit of any one of claims 40-41, wherein the reverse transcriptase is a group II intron reverse transcriptase, a retrotransposon reverse transcriptase, a telomerase reverse transcriptase, a viral reverse transcriptase or a retroviral reverse transcriptase.
43. The kit of any one of claims 40-42, wherein the reverse transcriptase comprises MarathonRT, UltraMarathonRT, Roseburia intestinalis reverse transcriptase, Induro RT, Maxima H Minus, SuperScript, SuperScript II, SuperScript III, SuperScript IV, PrimeScript, Transcriptor, GoScript, ProtoScript II, SMARTScribe, Avian Myeloblastosis Virus (AMV) reverse transcriptase, Moloney Murine Leukemia Virus (MMLV) reverse transcriptase, human immunodeficiency virus (HIV) reverse transcriptase, Rous sarcoma virus reverse transcriptase, Bombyx morii R2 RNA element reverse transcriptase, human L1 element reverse transcriptase, human telomerase reverse transcriptase (TERT), TGIRT, or a fragment, variant, mutant, or derivative thereof.
44. The kit of any one of claims 40-43, wherein the reverse transcriptase is MarathonRT, UltraMarathonRT, Induro RT, Maxima H minus, SuperScript III, or Avian myeloblastosis virus (AMV) reverse transcriptase.
45. The kit of any one of claims 40-44, wherein the reverse transcriptase comprises a group II intron RT, e.g., MarathonRT or UltraMarathonRT.
46. The kit of any one of claims 40-45, wherein the reverse transcriptase comprises MarathonRT.

47. The kit of any one of claims 40-46, wherein the reverse transcriptase comprises UltraMarathonRT.
48. The kit of any one of claims 40-47, wherein the reverse transcriptase is Induro RT.
49. The kit of any one of claims 40-48, wherein the reverse transcriptase is SuperScript III.
50. The kit of any one of claims 40-49, wherein the reverse transcriptase is Maxima H minus.
51. The kit of any one of claims 40-50, wherein the reverse transcriptase is AMV reverse transcriptase.
52. The kit of any one of claims 40-51, wherein the oligonucleotide primer is between 5 and 200 nucleotides in length.
53. The kit of any one of claims 40-52, comprising at least two oligonucleotide primers.
54. The kit of any one of claims 40-53, wherein the anionic polymer comprises a naturally occurring polymer or a non-naturally occurring polymer.
55. The kit of any one of claims 40-54, wherein the anionic polymer comprises a polynucleotide, peptide, polypeptide, or oligosaccharide.
56. The kit of any one of claims 40-55, wherein the anionic polymer comprises an oligosaccharide.
57. The kit of any one of claims 40-56, wherein the anionic polymer comprises a glycosaminoglycan.

58. The kit of any one of claims 40-57, wherein the anionic polymer comprises an alginate, hyaluronate, dextran, heparin, a heparin-mimicking polymer, or a carrageenan.
59. The kit of any one of claims 40-58, wherein the anionic polymer comprises unfractionated heparin, low molecular weight heparin, modified dextran, carboxymethyl benzylamide sulfonate dextran, sulfated glycopolymer, poly(GEMA)-sulfate, sulfated mannose polymer, sulfated lactose polymer, a polyaromatic anionic compound, a polyionomer, a sulfonated ionomer, pectin, fucoidan, gum arabic, a polysulfonated compound, iota-carrageenan, kappa-carrageenan, lambda-carrageenan, mu-carrageenan, nu-carrageenan, theta-carrageenan, xi-carrageenan, alpha-carrageenan, beta-carrageenan, gamma-carrageenan, omega-carrageenan, delta-carrageenan, psi-carrageenan, or poly(acrylic acid).
60. The kit of any one of claims 40-59, wherein the anionic polymer comprises heparin.
61. The kit of any one of claims 40-60, wherein the anionic polymer comprises iota-carrageenan.
62. The kit of any one of claims 40-61, wherein the anionic polymer comprises poly(acrylic acid).
63. The kit of any one of claims 40-62, wherein the kit further comprises a container, e.g., a tube, vial, or bottle, or a plurality of containers.
64. The kit of any one of claims 40-63, wherein the reverse transcriptase, oligonucleotide primer, dNTP solution, and anionic polymer or buffer containing an anionic polymer are present in the same or individual containers, e.g., tubes, vials, or bottles.
65. The kit of any one of claims 40-64, wherein the reverse transcriptase and anionic polymer or buffer containing an anionic polymer are present in the same or individual containers, e.g., tubes, vials, or bottles.

66. The kit of any one of claims 40-65, wherein the kit is useful for:
- (i) acquiring a value for the presence of a target ribonucleic acid (RNA) in a mixture;
 - (ii) detecting the level, identity or concentration of a target RNA;
 - (iii) increasing the signal to noise ratio of a target RNA;
 - (iv) preparing a library for the target RNA templates; and/or
 - (v) increasing the processivity of the reverse transcription reaction, relative to a reference standard, e.g., relative to a kit not comprising an anionic polymer.
67. The kit of any one of claims 40-66, wherein the kit is useful for acquiring a value for the presence of a target ribonucleic acid (RNA) in a mixture.
68. The kit of any one of claims 40-67, wherein the kit is useful for detecting the level, identity or concentration of a target RNA.
69. The kit of any one of claims 40-68, wherein the kit is useful for increasing the signal to noise ratio of a target RNA.
70. The kit of any one of claims 40-69, wherein the kit is useful for preparing a library for the target RNA templates.
71. The kit of any one of claims 40-70, wherein the kit is useful for increasing the processivity of the reverse transcriptase reaction.
72. The kit of any one of claims 40-71, wherein the reference standard is a kit not comprising an anionic polymer.