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(54) Title: COMPOSITIONS AND METHODS FOR CIRCULAR RNA AFFINITY PURIFICATION

(57) Abstract: The present disclosure provides for circular RNA (circRNA) compositions and methods purification and use of the same. In particular, the disclosure relates to compositions and methods of making and using circRNA comprising one or more aptamers which specifically bind an affinity ligand.

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COMPOSITIONS AND METHODS FOR CIRCULAR RNA AFFINITY PURIFICATION

RELATED APPLICATIONS

[0001] This application is related to EP Priority Application No. 22305884.3, filed June 17, 2022, and EP Priority Application No 22306497.3, filed October 06, 2022, the content of each is incorporated herein by reference.

BACKGROUND OF THE DISCLOSURE

[0002] Exogenous circularized RNAs (circRNAs) containing a protein coding region are emerging as a valuable a molecular tool and an alternative to messenger RNA (mRNA) therapeutics. CircRNAs are single-stranded and characterized by a covalently closed structure. In contrast to linear RNA, circRNAs have elevated stability, a significantly longer half-life, and are resistant to degradation by exonucleases. Uses of exogenous circRNAs include (1) the overexpression of native circRNAs, (2) the engineering of *in vitro* produced circRNA as a substitute to existing linear mRNA delivery, and/or (3) as described herein as part of a production and purification method for linear and/or circular RNA.

[0003] Methods for efficiently purifying exogenous circRNA remain a significant obstacle that must be overcome before the protein coding potential of circRNA can be fully realized. This is partly due to the different types and combinations of undesired contaminants in a sample that need to be separated from a pure sample of circRNA. Such contaminants are typically components and by-products of any upstream processes, for example RNA manufacturing and circularization conditions. The sample typically contains the desired circRNA alongside various contaminants such as linear precursor RNA, nicked circular RNA, double stranded RNA, triphosphate-RNA, free nucleotides, endotoxins, and solvents.

[0004] There remains a need for more effective, reliable, and safer methods of purifying circRNA from large scale manufacturing processes for potential therapeutic applications which are also economical in terms of the number of steps, the complexity of the steps, and the resources used in the steps.

BRIEF SUMMARY OF THE DISCLOSURE

[0005] In one aspect, the disclosure provides a circular RNA comprising a protein coding region and at least one RNA aptamer.

[0006] In certain embodiments, an internal ribosome entry site (IRES) is positioned at the 5' end of the protein coding region.

[0007] In certain embodiments, an IRES is positioned at the 3' end of the protein coding region.

[0008] In certain embodiments, the IRES is derived from Coxsackievirus B3 (CVB3), Encephalomyocarditis virus (EMCV), Dicistroviruses, hepatitis C virus (HCV), poliovirus (PV), enterovirus 71 (EV71), human rhinovirus (HRV), foot-and-mouth disease virus (FMDV), or synthetic IRES.

[0009] In certain embodiments, the IRES comprises a polynucleotide sequence of SEQ ID NO: 75.

[0010] In certain embodiments, the protein coding region encodes at least one polypeptide or peptide.

[0011] In certain embodiments, the polypeptide is a biologically active polypeptide, a therapeutic polypeptide, or an antigenic polypeptide.

[0012] In certain embodiments, the circular RNA comprises at least one 5' internal homology arm and at least one 3' internal homology arm.

[0013] In certain embodiments, the 5' internal homology arm is about 5 to about 50 nucleotides in length.

[0014] In certain embodiments, the 5' internal homology arm comprises the nucleotide sequence of SEQ ID NO: 70.

[0015] In certain embodiments, the 3' internal homology arm is about 5 to about 50 nucleotides in length.

[0016] In certain embodiments, the 3' internal homology arm comprises the nucleotide sequence of SEQ ID NO: 71.

[0017] In certain embodiments, the circular RNA comprises at least one 3' exon element.

[0018] In certain embodiments, the 3' exon element comprises the nucleotide sequence of SEQ ID NO: 81.

[0019] In certain embodiments, the circular RNA comprises at least one 5' exon element.

[0020] In certain embodiments, the 5' exon element comprises the nucleotide sequence of SEQ ID NO: 83.

[0021] In certain embodiments, the circular RNA comprises at least one spacer sequence.

[0022] In certain embodiments, the spacer sequence is about 5 to about 75 nucleotides in length.

[0023] In certain embodiments, the spacer sequence comprises the nucleotide sequence of SEQ ID NO: 78 or 79.

[0024] In certain embodiments, the spacer sequence is positioned at one or both of a 5' end and 3' end of any one of the following elements: the protein coding region, the IRES, the 5' internal homology arm, the 3' internal homology arm, the 5' exon element, and the 3' exon element.

[0025] In certain embodiments, the circular RNA comprises the following elements, from 5' to 3': a) the 3' exon element, b) the 5' internal homology arm, c) the spacer sequence, d) the IRES, e) the protein coding region, f) the spacer sequence, g) the 3' internal homology arm, and h) the 5' exon element.

[0026] In certain embodiments, the circular RNA comprises the following elements, from 5' to 3': a) the 3' exon element, b) the 5' internal homology arm, c) the spacer sequence, d) the protein coding region, e) the IRES, f) the spacer sequence, g) the 3' internal homology arm, and h) the 5' exon element.

[0027] In certain embodiments, the at least one RNA aptamer is positioned at a 5' end or a 3' end of any one of elements a)-h).

[0028] In certain embodiments, the circular RNA contains at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or at least one polyadenylation (polyA) sequence.

[0029] In certain embodiments, the 5' UTR, the 3' UTR, and/or the polyA sequence are spacer sequences.

[0030] In certain embodiments, the RNA aptamer is embedded in an RNA scaffold.

[0031] In certain embodiments, the RNA scaffold comprises at least one secondary structure motif.

[0032] In certain embodiments, the secondary structure motif is a tetraloop, a pseudoknot, or a stem-loop.

[0033] In certain embodiments, the RNA scaffold comprises at least one tertiary structure.

[0034] In certain embodiments, the secondary structure motif and/or tertiary structure are nuclease resistant.

[0035] In certain embodiments, the RNA scaffold comprises a transfer RNA (tRNA).

[0036] In certain embodiments, the RNA aptamer is embedded in a tRNA hairpin loop of the tRNA.

[0037] In certain embodiments, the RNA aptamer is embedded in a tRNA anticodon loop of the tRNA.

[0038] In certain embodiments, the RNA aptamer is embedded in a tRNA D loop of the tRNA.

[0039] In certain embodiments, the RNA aptamer is S1m, Sm, or a derivative or fragment thereof.

[0040] In certain embodiments, the circular RNA comprises between one to four RNA aptamers.

[0041] In certain embodiments, the RNA aptamers are identical.

[0042] In certain embodiments, at least one of the RNA aptamers is distinct.

[0043] In certain embodiments, the RNA aptamer is synthetically derived.

[0044] In certain embodiments, the RNA aptamer is a split aptamer or an X-aptamer.

[0045] In certain embodiments, the RNA aptamer is naturally-derived.

[0046] In certain embodiments, the RNA aptamer is derived from a hairpin RNA, a tRNA, or a riboswitch.

[0047] In certain embodiments, the RNA aptamer binds to an affinity ligand.

[0048] In certain embodiments, the affinity ligand comprises protein A, protein G, streptavidin, glutathione, dextran, or a fluorescent molecule.

[0049] In certain embodiments, the affinity ligand comprises streptavidin.

[0050] In certain embodiments, the affinity ligand is immobilized on a chromatography resin.

[0051] In certain embodiments, the at least one RNA aptamer is positioned: a) before the 3' exon element, b) between the 3' exon element and the 5' internal homology arm, c) between the 5' internal homology arm and the 5' spacer sequence, d) between the 5' spacer sequence and the IRES, e) between the protein coding region and the 3' spacer sequence, f) between the 3' spacer sequence and the 3' internal homology arm, g) between the 3' internal homology arm and the 5' exon element, h) after the 5' exon element, i) between the 3' exon and the IRES, and/or j) between the IRES and the 5' exon element.

[0052] In certain embodiments, the at least one RNA aptamer is positioned: a) before the 3' exon element, b) between the 3' exon element and the 5' internal homology arm, c) between the 5' internal homology arm and the 5' spacer sequence, d) between the 5' spacer sequence and the protein coding region, e) between the IRES and the 3' spacer sequence, f) between the 3' spacer sequence and the 3' internal homology arm, g) between the 3' internal homology arm and the 5' exon element, h) after the 5' exon element, i) between the 3' exon and the protein coding region, and/or j) between the protein coding region and the 5' exon element.

[0053] In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 65 or 66.

[0054] In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 84. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 85. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID

NO: 86. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 87. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 88. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 89. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 90. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 91. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 92. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 93.

[0055] In certain embodiments, the RNA aptamer embedded tRNA comprises the nucleotide sequence of SEQ ID NO: 67.

[0056] In certain embodiments, the RNA aptamer is about 30-200 nucleotides in length.

[0057] In certain embodiments, the RNA aptamer is about 50-200 nucleotides in length.

[0058] In certain embodiments, the RNA aptamer is not a histone stem-loop.

[0059] In certain embodiments, the circular RNA comprises at least one chemical modification.

[0060] In certain embodiments, the chemical modification is pseudouridine, N1-methylpseudouridine, 2-thiouridine, 4'-thiouridine, 5- methylcytosine, 2-thio-l-methyl-1-deaza-pseudouridine, 2-thio-l-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-l-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methyluridine, 5-methyluridine, 5-methoxyuridine, 2'-O-methyl uridine, or N6-methyladenosine.

[0061] In certain embodiments, the chemical modification is pseudouridine, N1-methylpseudouridine, 5-methylcytosine, 5- methoxyuridine, N6-methyladenosine or a combination thereof.

[0062] In certain embodiments, the chemical modification is N1-methylpseudouridine.

[0063] In another aspect, the disclosure provides a linear precursor RNA comprising at least a self-splicing ribozyme and a protein coding region, wherein the linear precursor RNA comprises at least one RNA aptamer.

[0064] In certain embodiments, the self-splicing ribozyme comprises at least two catalytic subunits.

[0065] In certain embodiments, the self-splicing ribozyme catalytic subunits derive from either a group I intron or a group II intron RNA transcript or a fragment thereof.

[0066] In certain embodiments, the self-splicing ribozyme catalytic subunits derive from a permuted intron-exon (PIE) sequence from *Cyanobacterium Anabaena* pre-tRNA-Leu gene, T4 phage Td gene, or *Tetrahymena* pre-rRNA.

[0067] In certain embodiments, the catalytic activity of the two subunits results in a circularized RNA.

[0068] In certain embodiments, the linear precursor RNA comprises the following elements, from 5' to 3': a) a 5' external homology arm, b) a 3' self-splicing PIE fragment, c) a 5' internal homology arm, d) a 5' spacer sequence, e) an internal ribosome entry site (IRES) f) a protein coding region, g) a 3' spacer sequence, h) a 3' internal homology arm, i) a 5' self-splicing PIE fragment, and j) a 3' external homology arm, wherein the RNA aptamer is present at one or both of the 5' end or 3' end of any one of elements a)-j).

[0069] In certain embodiments, the linear precursor RNA comprises the following elements, from 5' to 3': a) a 5' external homology arm, b) a 3' self-splicing PIE fragment, c) a 5' internal homology arm, d) a 5' spacer sequence, e) a protein coding region, f) an IRES, g) a 3' spacer sequence, h) a 3' internal homology arm, i) a 5' self-splicing PIE fragment, and j) a 3' external homology arm, wherein the RNA aptamer is present at one or both of the 5' end or 3' end of any one of elements a)-j).

[0070] In certain embodiments, the 5' external homology arm and the 3' external homology arm comprises the nucleotide sequence of SEQ ID NO: 69 or SEQ ID NO: 72.

[0071] In certain embodiments, the 5' external homology arm and the 3' external homology arm are each independently about 5 to about 50 nucleotides in length.

[0072] In certain embodiments, the 5' self-splicing PIE fragment comprises the nucleotide sequence of SEQ ID NO: 74.

[0073] In certain embodiments, the 5' internal homology arm comprises the nucleotide sequence of SEQ ID NO: 70.

[0074] In certain embodiments, the 5' internal homology arm is about 5 to about 50 nucleotides in length.

[0075] In certain embodiments, the 5' spacer and the 3' spacer comprises the nucleotide sequence of SEQ ID NO: 78 or SEQ ID NO: 79.

[0076] In certain embodiments, the 5' spacer and the 3' spacer are each independently about 5 to 75 nucleotides in length

[0077] In certain embodiments, the 3' self-splicing PIE fragment comprises the nucleotide sequence of SEQ ID NO: 73.

[0078] In certain embodiments, the IRES is derived from Coxsackievirus B3 (CVB3), Encephalomyocarditis virus (EMCV), Dicistroviruses, hepatitis C virus (HCV), poliovirus (PV), enterovirus 71 (EV71), human rhinovirus (HRV), foot-and-mouth disease virus (FMDV), or synthetic IRES.

[0079] In certain embodiments, the IRES comprises the nucleotide sequence of SEQ ID NO: 75.

[0080] In certain embodiments, the linear precursor RNA comprises at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or a polyadenylation (polyA) sequence.

[0081] In certain embodiments, the protein coding region encodes at least one polypeptide.

[0082] In certain embodiments, the polypeptide is a biologically active polypeptide, a therapeutic polypeptide, or an antigenic polypeptide.

[0083] In certain embodiments, the RNA aptamer is embedded in an RNA scaffold.

[0084] In certain embodiments, the RNA scaffold comprises at least one secondary structure motif.

[0085] In certain embodiments, the secondary structure motif is a tetraloop, a pseudoknot, or a stem-loop.

[0086] In certain embodiments, the RNA scaffold comprises at least one tertiary structure.

[0087] In certain embodiments, the secondary structure motif and/or tertiary structure are nuclease resistant.

[0088] In certain embodiments, the RNA scaffold comprises a transfer RNA (tRNA).

[0089] In certain embodiments, the RNA aptamer is embedded in a tRNA hairpin loop of the tRNA.

[0090] In certain embodiments, the RNA aptamer is embedded in a tRNA anticodon loop of the tRNA.

[0091] In certain embodiments, the RNA aptamer is embedded in a tRNA D loop of the tRNA.

[0092] In certain embodiments, the RNA aptamer is S1m, Sm, or a derivative or fragment thereof.

[0093] In certain embodiments, the linear precursor RNA comprises between one to four RNA aptamers.

[0094] In certain embodiments, the RNA aptamers are identical.

[0095] In certain embodiments, at least one of the RNA aptamers is distinct.

[0096] In certain embodiments, the RNA aptamer is synthetically derived.

[0097] In certain embodiments, the RNA aptamer is a split aptamer or an X-aptamer.

[0098] In certain embodiments, the RNA aptamer is a split aptamer comprising a 5' portion and a 3' portion.

[0099] In certain embodiments, the 5' portion of the split aptamer is positioned 3' of the 5' exon element and the 3' portion of the split aptamer is positioned 5' of the 3' exon element.

[0100] In certain embodiments, the 5' portion of the split aptamer is positioned 3' of the 3' internal homology arm and the 3' portion of the split aptamer is positioned 5' of the 5' internal homology arm.

[0101] In certain embodiments, the split aptamer is reformed to a functional aptamer upon circularization of the linear precursor RNA.

[0102] In certain embodiments, the RNA aptamer is naturally-derived.

[0103] In certain embodiments, the RNA aptamer is derived from a hairpin RNA, a tRNA, or a riboswitch.

[0104] In certain embodiments, the RNA aptamer binds to an affinity ligand.

[0105] In certain embodiments, the affinity ligand comprises protein A, protein G, streptavidin, glutathione, dextran, or a fluorescent molecule.

[0106] In certain embodiments, the affinity ligand comprises streptavidin.

[0107] In certain embodiments, the affinity ligand is immobilized on a chromatography resin.

[0108] In certain embodiments, the at least one RNA aptamer is positioned: a) before the 5' external homology arm, b) between the 5' external homology arm and the 3' self-splicing PIE fragment, c) between the 3' self-splicing PIE fragment and the 5' internal homology arm, d) between the 5' internal homology arm and the 5' spacer sequence, e) between the 5' space sequence and the IRES, f) after the protein coding region but before the 3' spacer sequence, g) between the 3' spacer sequence and the 3' internal homology arm, h) between the 3' internal homology arm and the 5' self-splicing PIE fragment, i) between the 5' self-splicing PIE fragment and the 3' external homology arm, and/or j) after the 3' external homology arm.

[0109] In certain embodiments, at least one RNA aptamer is positioned: a) before the 5' external homology arm, b) between the 5' external homology arm and the 3' self-splicing PIE fragment, c) between the 3' self-splicing PIE fragment and the 5' internal homology arm, d) between the 5' internal homology arm and the 5' spacer sequence, e) between the 5' space sequence and the protein coding region, f) after the IRES but before the 3' spacer sequence, g) between the 3' spacer sequence and the 3' internal homology arm, h) between the 3' internal homology arm and the 5' self-splicing PIE fragment, i) between the 5' self-splicing PIE fragment and the 3' external homology arm, and/or j) after the 3' external homology arm.

[0110] In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 65 or 66.

[0111] In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 84. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 85. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 86. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 87. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 88. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 89. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 90. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 91. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 92. In certain embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 93.

[0112] In certain embodiments, the RNA aptamer embedded tRNA comprises the nucleotide sequence of SEQ ID NO: 67.

[0113] In certain embodiments, the RNA aptamer is about 30-200 nucleotides in length.

[0114] In certain embodiments, the RNA aptamer is about 50-200 nucleotides in length.

[0115] In certain embodiments, the RNA aptamer is not a histone stem-loop.

[0116] In certain embodiments, the linear precursor RNA comprises at least one chemical modification.

[0117] In certain embodiments, the chemical modification is pseudouridine, N1-methylpseudouridine, 2-thiouridine, 4'-thiouridine, 5- methylcytosine, 2-thio-l-methyl-1-deaza-pseudouridine, 2-thio-l-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-l-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methyluridine, 5-methyluridine, 5-methoxyuridine, 2'-O-methyl uridine, or N6-methyladenosine..

[0118] In certain embodiments, the chemical modification is pseudouridine, N1-methylpseudouridine, 5-methylcytosine, 5- methoxyuridine, N6-methyladenosine, or a combination thereof.

[0119] In certain embodiments, the chemical modification is N1-methylpseudouridine.

[0120] In certain embodiments, the linear precursor RNA is synthesized using *in vitro* transcription (IVT)

[0121] In one aspect, the disclosure provides a circular RNA comprising a protein coding region and at least one RNA aptamer, wherein the circular RNA is formed from the linear precursor RNA described above.

[0122] In one aspect, the disclosure provides a circular RNA comprising a protein coding region, wherein the circular RNA is formed from the linear precursor RNA described above, and wherein the circular RNA lacks an RNA aptamer.

[0123] In one aspect, the disclosure provides a nucleic acid that encodes the linear precursor RNA described above.

[0124] In one aspect, the disclosure provides a vector comprising the nucleic acid described above.

[0125] In one aspect, the disclosure provides a host cell comprising the vector described above.

[0126] In one aspect, the disclosure provides a pharmaceutical composition comprising the circular RNA described above or the linear precursor RNA described above.

[0127] In one aspect, the disclosure provides a method of producing a circular RNA, comprising incubating the linear precursor RNA described above under conditions that result in the circularization of the linear precursor RNA.

[0128] In certain embodiments, the linear precursor RNA is incubated with GTP and Mg2+.

[0129] In certain embodiments, the linear precursor RNA is incubated with GTP and Mg2+ for a time sufficient to circularize the linear precursor RNA.

[0130] In certain embodiments, the GTP is present at a concentration of about 1 mM to about 15 mM.

[0131] In certain embodiments, the GTP is present at a concentration of about 2 mM.

[0132] In certain embodiments, the Mg2+ is present at a concentration of about 1 mM to about 50 mM.

[0133] In certain embodiments, the Mg2+ is present at a concentration of about 10 mM.

[0134] In one aspect, the disclosure provides a method of producing a plurality of circular RNA molecules, comprising incubating a plurality of linear precursor RNA molecules under conditions that result in the circularization of at least a portion of the linear precursor RNA molecules, wherein each linear precursor RNA molecule comprises the linear precursor RNA described above.

[0135] In certain embodiments, at least about 30% (i.e., about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or 100%) of the linear precursor RNA molecules in the plurality are circularized.

[0136] In one aspect, the disclosure provides a method for purifying a circular RNA, comprising the steps of: (a) contacting a sample comprising the circular RNA described above with an affinity ligand

that is immobilized on a chromatography resin, wherein the RNA aptamer comprises binding affinity for the affinity ligand; (b) eluting the circular RNA from the chromatography resin; and (c) purifying the circular RNA from the sample.

[0137] In one aspect, the disclosure provides a method for purifying a linear precursor RNA, comprising the steps of: (a) contacting a sample comprising the linear precursor RNA described above with an affinity ligand that is immobilized on a chromatography resin, wherein the RNA aptamer comprises binding affinity for the affinity ligand; (b) eluting the linear precursor RNA from the chromatography resin; and (c) purifying the linear precursor RNA from the sample.

[0138] In certain embodiments, the method comprises one or more washing steps between the contacting step (a) and the eluting step (b).

[0139] In one aspect, the disclosure provides a method of purifying a circular RNA, comprising the steps of: (a) contacting a sample comprising the circular RNA with an affinity ligand that is immobilized on a chromatography resin; (b) eluting the circular RNA from the chromatography resin; and (c) isolating the circular RNA from the sample, wherein the circular RNA comprises a protein coding region and at least one RNA aptamer, wherein the RNA aptamer comprises binding affinity for the affinity ligand.

[0140] In one aspect, the disclosure provides a method of purifying a linear precursor RNA, comprising the steps of: (a) contacting a sample comprising the linear precursor RNA with an affinity ligand that is immobilized on a chromatography resin; (b) eluting the linear precursor RNA from the chromatography resin; and (c) isolating the linear precursor RNA from the sample, wherein the linear precursor RNA comprises a protein coding region and at least one RNA aptamer, wherein the RNA aptamer comprises binding affinity for the affinity ligand.

[0141] In one aspect, the disclosure provides a method of purifying a circular RNA, comprising the steps of: (a) contacting a sample comprising a plurality of linear precursor RNA molecules and a plurality of circular RNA molecules with an affinity ligand that is immobilized on a chromatography resin; and (b) isolating the circular RNA molecules from the sample, wherein the linear precursor RNA molecules comprise a protein coding region and at least one RNA aptamer and wherein the RNA aptamer comprises binding affinity for the affinity ligand, and wherein the circular RNA molecules lack an RNA aptamer.

[0142] In certain embodiments, the circular RNA molecules do not bind the affinity ligand.

[0143] In certain embodiments, the circular RNA or linear precursor RNA is greater than or equal to 90% pure.

[0144] In one aspect, the disclosure provides a method of treating or preventing a disease or disorder, comprising administering to a subject in need thereof the pharmaceutical composition described above.

[0145] In one aspect, the disclosure provides a pharmaceutical composition comprising a plurality of circular RNA molecules, wherein at least about 90% of the circular RNA comprise a protein coding region and at least one RNA aptamer.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0146] The foregoing and other features and advantages of the present disclosure will be more fully understood from the following detailed description of illustrative embodiments taken in conjunction with the accompanying drawings.

[0147] **FIG. 1** left panel is a schematic diagram of the aptamer tagged linear precursor RNA that becomes circularized to form the aptamer tagged circRNA. The right panel shows streptavidin affinity binding during a purification process can occur with an aptamer tagged to a linear precursor RNA (top) or an aptamer tagged circRNA (bottom).

[0148] **FIG. 2A** depicts the plasmid map encoding the 4xS1m aptamer, the linear precursor RNA, and the PIE sequences used for RNA circularization. The plasmid elements are arranged in the following 5' to 3' order: a T7 promoter, a 5' external homology arm, a 3'' Anabaena intron/exon fragment, a 5' internal homology arm, a 5' polyAC spacer, a CVB3 IRES, a protein coding region, , a 3' polyAC spacer, a 4xS1m aptamer, a 3' internal homology arm, a 5'' Anabaena intron/exon fragment, and a 3' external homology arm.

[0149] **FIG. 2B** depicts the plasmid map encoding the tRNA-S1m aptamer, the linear precursor RNA, and the PIE sequences used for RNA circularization. The plasmid elements are arranged in the following 5' to 3' order: a T7 promoter, a 5' external homology arm, a 3' Anabaena intron/exon fragment, a 5' internal homology arm, a 5' polyAC spacer, a CVB3 IRES, a protein coding region, a 3' polyAC spacer, a 3' internal homology arm, a 5' Anabaena intron/exon fragment, a 3' external homology arm, and a tRNA-S1m aptamer.

[0150] **FIG. 2C** depicts the control plasmid map which encodes the linear precursor RNA and PIE sequences used for RNA circularization but does not encode an aptamer. The plasmid elements are arranged in the following 5' to 3' order: a T7 promoter, a 5' external homology arm, a 3' Anabaena intron/exon fragment, a 5' internal homology arm, a 5' polyAC spacer, a CVB3 IRES, protein coding

region, a 3' polyAC spacer, a 3' internal homology arm, a 5' Anabaena intron/exon fragment, and a 3' external homology arm.

[0151] **FIG. 3** is an image of an agarose gel comparing the amount of RNA species (circular, precursor, or nicked) in the elution, unbound, and wash fractions after streptavidin Sepharose bead affinity purification of a 4xS1m aptamer tagged circRNA, a tRNA-S1m aptamer tagged circRNA, or a circRNA no aptamer control.

[0152] **FIG. 4** is a bar graph that measures the elution, unbound, and wash fractions (wash 1 and wash 2) recovered after streptavidin Sepharose bead affinity purification of a 4xS1m aptamer tagged circRNA, a tRNA-S1m aptamer tagged circRNA, or a circRNA no aptamer control. The amount of recovered RNA measured is expressed as a percent of the input (i.e., the input being the sample of circRNA that did not undergo affinity purification).

[0153] **FIG. 5** illustrates a design strategy to produce an aptamer tagged circRNA (left panel) and subsequent affinity purification (right panel) using a positive selection method. In the positive selection method, the linear precursor RNA will be flanked by a split aptamer which does not undergo affinity purification because the intact aptamer is required for binding to the affinity matrix. Upon circularization of the linear precursor RNA the intact aptamer will form allowing for binding to the affinity matrix.

[0154] **FIG. 6** illustrates a design strategy to produce a circRNA (left panel) and subsequent affinity purification (right panel) using a negative selection method. In the negative selection method, the aptamer is localized outside of the 5' end of 3' intron or the 3' end of 5' intron of the linear precursor RNA such that the linear precursor RNA binds to the affinity matrix. Due to the positioning of the aptamer outside of the 5' end of 3' intron or the 3' end of 5' intron sequence the linear precursor RNA, upon circularization, the circRNA will not contain the aptamer and will not bind to the affinity matrix.

[0155] **FIG. 7** is a bar graph that measures the elution, unbound, and wash recovered after streptavidin Sepharose bead affinity purification of a 4xS1m aptamer tagged linear precursor RNA (pML49), a tRNA-S1m aptamer tagged linear precursor RNA (pML50 and pML51), a no aptamer control (pML47), a 4xS1m aptamer tagged circRNA (pML26), and a tRNA-S1m aptamer tagged circRNA (pML38). The amount of recovered RNA measured is expressed as a percent of the input (i.e., the input being the total RNA in the sample).

[0156] **FIG. 8A – 8D** are images of agarose gels comparing the amount of RNA species (circular, precursor, or nicked) in the elution, unbound, and wash fractions after streptavidin Sepharose bead affinity purification of a 4xS1m aptamer tagged linear precursor RNA (pML49, FIG. 8A), a tRNA-S1m

aptamer tagged linear precursor RNA (pML50, FIG. 8B and pML51, FIG. 8C), and several controls (FIG. 8D).

[0157] **FIG. 9A – 9C** are images of capillary electrophoresis traces comparing the amount of RNA species (circular, precursor, or nicked) in the input, elution, and unbound fractions after streptavidin Sepharose bead affinity purification of a tRNA-S1m aptamer tagged linear precursor RNA (pML50, FIG. 9A and pML51, FIG. 9B), and a 4xS1m aptamer tagged linear precursor RNA (pML49, FIG. 9C).

[0158] **FIG. 10** depicts a bar graph of % linear precursor or circular / nicked RNA in the input, unbound, and wash fractions of a streptavidin Sepharose bead affinity purification.

[0159] **FIG. 11A – 11B** depict % linear precursor or circular / nicked RNA and total yield (mg) in the input, unbound, and wash fractions of a streptavidin Sepharose bead affinity purification.

[0160] **FIG. 12A** depicts % linear precursor, circular / nicked RNA, and introns (combination of bound introns, 5' intron, and 3' intron) in the input, unbound, and wash fractions of a streptavidin Sepharose bead affinity purification. **FIG. 12B** depicts a schematic of a construct for IVT to produce a linear precursor RNA with a 5' end and 3' end aptamer.

[0161] **FIG. 13** depicts % linear precursor or circular / nicked RNA of a large circRNA in the input and purified fractions of a streptavidin Sepharose bead affinity purification.

[0162] **FIG. 14** depicts GFP expression in Hela cells from purified and unpurified circRNA.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0163] The present disclosure is directed to, *inter alia*, novel circRNA compositions and methods for RNA affinity purification. In particular, the disclosure relates to circRNA and linear RNA precursor compositions comprising at least one RNA aptamer. The RNA aptamers associated with the disclosed circRNA compositions enable the use of effective affinity purification. Also disclosed herein are methods of making these circRNA-tagged aptamer compositions.

I. Definitions

[0164] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. In case of conflict, the present specification, including definitions, will control.

Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, virology, immunology, microbiology, genetics, analytical chemistry, synthetic organic chemistry, medicinal and pharmaceutical chemistry, and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Throughout this specification and embodiments, the words "have" and "comprise," or variations such as "has," "having," "comprises," or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. All publications and other references mentioned herein are incorporated by reference in their entirety. Although a number of documents are cited herein, this citation does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

[0165] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a nucleotide sequence," is understood to represent one or more nucleotide sequences. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0166] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0167] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0168] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, may provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0169] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise

indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects of the disclosure. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0170] The term “approximately” or "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" can modify a numerical value above and below the stated value by a variance of, *e.g.*, 10 percent, up or down (higher or lower). In some embodiments, the term indicates deviation from the indicated numerical value by ±10%, ±5%, ±4%, ±3%, ±2%, ±1%, ±0.9%, ±0.8%, ±0.7%, ±0.6%, ±0.5%, ±0.4%, ±0.3%, ±0.2%, ±0.1%, ±0.05%, or ±0.01%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±10%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±5%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±4%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±3%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±2%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±1%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±0.9%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±0.8%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±0.7%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±0.6%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±0.5%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±0.4%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±0.3%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±0.1%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±0.05%. In some embodiments, “about” indicates deviation from the indicated numerical value by ±0.01%.

[0171] Depending on context, the term "polynucleotide" or "nucleotide" may encompass a singular nucleic acid as well as plural nucleic acids. In some embodiments, a polynucleotide is an isolated nucleic acid molecule or construct, *e.g.*, circular RNA (circRNA) or plasmid DNA (pDNA). In some embodiments, a polynucleotide comprises a conventional phosphodiester bond. In some embodiments, a polynucleotide comprises a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). The term "nucleic acid" may refer to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide. By "isolated" nucleic acid

or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a Factor VIII polypeptide contained in a vector is considered isolated for the purposes of the present disclosure. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) from other polynucleotides in a solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides of the present disclosure. Isolated polynucleotides or nucleic acids according to the present disclosure further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid can include regulatory elements such as promoters, enhancers, ribosome binding sites, or transcription termination signals.

[0172] As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" can be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be derived from a natural biological source or produced recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

[0173] An "isolated" polypeptide or a fragment, variant, or derivative thereof refers to a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can simply be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for the purpose of the disclosure, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

[0174] "Administer" or "administering," as used herein refers to delivering to a subject a composition described herein, e.g., a chimeric protein. The composition, e.g., the chimeric protein, can be administered to a subject using methods known in the art. In particular, the composition can be

administered intravenously, subcutaneously, intramuscularly, intradermally, or via any mucosal surface, e.g., orally, sublingually, buccally, nasally, rectally, vaginally or via pulmonary route. In some embodiments, the administration is intravenous. In some embodiments, the administration is subcutaneous. In some embodiments, the administration is self-administration. In some embodiments, a parent administers the chimeric protein to a child. In some embodiments, the chimeric protein is administered to a subject by a healthcare practitioner such as a medical doctor, a medic, or a nurse.

II. Circular RNA and Linear Precursor RNA

[0175] Disclosed herein are circular RNA (circRNA) compositions comprising a protein coding region and at least one RNA aptamer. Also disclosed herein, are linear precursor RNA compositions comprising a self-splicing ribozyme and protein coding region, wherein the linear precursor RNA comprises at least one RNA aptamer.

[0176] As used herein, the term “circular RNA” or “circRNA” refers to an RNA polynucleotide that does not comprise a 5’ end or 3’ end, i.e., a continuous RNA molecule without a 5’ end or 3’ end. Exogenous circRNA constructs containing a protein coding region are previously described and shown to extend the duration of protein expression from full-length RNA. Wesselhoeft et al., (2018), Nat Commun., 9(1):2629; Wesselhoeft et al., (2019), Mol Cell., 74(3):508-520; WO2019236673.

[0177] As used herein, the term “linear RNA precursor” refers to an RNA polynucleotide that is not circular, but that contains sequence motifs to facilitate a circularization reactions, thereby creating a circular RNA. In certain embodiments, the sequence motif that facilitates circularization is a self-splicing ribozyme. The self-splicing ribozyme method orchestrates circularization efficiently in a wide range of RNAs *in vitro,* including RNAs with a protein coding region. Designing the linear precursor RNA with additional auxiliary sequences aid in creating favorable conditions for splicing (i.e., 5’ external homology arm, 5’ internal homology arm, 5’ spacer sequence, 3’ spacer sequence, 3’ internal homology arm, and 3’ external homology arm). *Id.* Functional protein was produced exogenous circRNA constructs in eukaryotic cells and translation was successfully initiated by incorporating an internal ribosome entry sites (IRES) and internal polyadenosine tracts.

[0178] Exogenous circRNA purified by high performance liquid chromatography displayed exceptional protein production qualities in terms of both quantity of protein produced and stability. However, samples retained impurities and unwanted RNA species including linear precursor RNA,

nicked circular RNA, double stranded RNA, triphosphate-RNA, free nucleotides, endotoxins, and solvents.

[0179] Provided herein are methods and compositions that facilitate the use of exogenous circRNA for robust and stable protein expression in eukaryotic cells by improving the efficiency, quality, and reliability of circRNA purification methods.

A. IRES

[0180] The translation of circRNAs can only be initiated in a cap-independent fashion because circRNA lacks a 5′ cap and 3′ poly-A tail. IRES-mediated translation of exogenous circRNA is one of the widely accepted mechanisms of circRNA translation initiation. Pamudurti *et al.*, (2017), 66:9–21 e27; Petkovic (2015), Nucleic Acids Res., 43:2454-2465.

[0181] In some embodiments, the circRNA disclosed herein comprises an internal ribosome entry site (IRES) which is positioned at the 5' end of the protein coding region. In some embodiments, the linear precursor RNA disclosed herein comprises an IRES. In some embodiments, the IRES is positioned at the 3' end of the protein coding region in the linear precursor RNA but shifts to the 5' end of the protein coding region upon circularization.

[0182] In some embodiments, the IRES is derived from Taura syndrome virus, Triatoma virus, Theiler's encephalomyelitis virus, simian Virus 40, Solenopsis invicta virus 1, Rhopalosiphum padi virus, Reticuloendotheliosis virus, fuman poliovirus 1, Plautia stali intestine virus, Kashmir bee virus, Human rhinovirus 2, Homalodisca coagulata virus- 1, Human Immunodeficiency Virus type 1, Homalodisca coagulata virus- 1, Himetobi P virus, Hepatitis C virus (HCV), Hepatitis A virus, Hepatitis GB virus, Equine rhinitis virus, Ectropis obliqua picorna-like virus, Encephalomyocarditis virus (EMCV), Drosophila C Virus, Crucifer tobamo virus, Cricket paralysis virus, Bovine viral diarrhea virus 1, Black Queen Cell Virus, Aphid lethal paralysis virus, Avian encephalomyelitis virus, Acute bee paralysis virus, Hibiscus chlorotic ringspot virus, Classical swine fever virus, Human FGF2, Human SFTPA1, Human AMLURUNX1, Drosophila antennapedia, Human AQP4, Human AT1R, Human BAG-1, Human BCL2, Human BiP, Human c-IAP1 , Human c-myc, Human eIF4G, Mouse NDST4L, Human LEF1, Mouse HIF1 alpha, Human n.myc, Mouse Gtx, Human p27kip1, Human PDGF2/c-sis, Human p53, Human Pim-1, Mouse Rbm3, Drosophila reaper, Canine Scamper, Drosophila Ubx, Human UNR, Mouse UtrA, Human VEGF-A, Human XIAP, Drosophila hairless, *S. cerevisiae* TFIID, *S. cerevisiae* YAP1, Human c-src, Human FGF-1, Simian picomavirus, Turnip crinkle virus, an aptamer to eIF4G, Coxsackievirus B3 (CVB3) or Coxsackievirus A (CVB1/2), Dicistroviruses,

poliovirus (PV), enterovirus 71 (EV71), human rhinovirus (HRV), foot-and-mouth disease virus (FMDV), or synthetic IRES. In some embodiments, the is derived from a CVB3 IRES. In yet another embodiment, the IRES comprises a polynucleotide sequence of SEQ ID NO: 75. In yet another embodiment, the IRES is encoded by a polynucleotide sequence of SEQ ID NO: 51.

B. 5' and 3' homology arms

[0183] As used herein, a "homology arm" is any contiguous sequence that is predicted to form base pairs with at least about 75% (e.g., at least about 80%, at least about 85%, at least about 90%, at least about 95%, or 100%) of another homology arm in the RNA (i.e., the circular RNA or linear RNA precursor). A homology arm sequence is about 5 to about 50 nucleotides in length. The homology arm sequence may be located before and adjacent to, or included within, the 3' intron fragment and/or after and adjacent to, or included within, the 5' intron fragment. The homology arm sequence is predicted to have less than 50% (e.g., less than 45%, less than 40%, less than 35%, less than 30%, less than 25%) base pairing with unintended sequences in the RNA (e.g., non-homology arm sequences). A "strong homology arm" refers to a homology arm with a Tm of greater than 50°C when base paired with another homology arm in the RNA.

[0184] "Internal homology arms" and "external homology arms" refer to the orientation of the homology arms with respect to the self-splicing PIE fragments and the protein coding region. In the linear precursor RNA, internal homology arms are positioned between the self-splicing PIE fragments and the protein coding region. Upon circularization conditions, the internal homology arms remain in the circular RNA. In the linear precursor RNA, the external homology arms flank the self-splicing PIE fragments. Upon circularization conditions, the external homology arms are excised and are not present in the circular RNA.

[0185] In some embodiments, the circRNA disclosed herein comprises a 5' internal homology arm. In some embodiments, the linear precursor RNA disclosed herein comprises a 5' internal homology arm. In some embodiments, the 5' internal homology arm comprises the nucleotide sequence of SEQ ID NO: 70. In some embodiments, the 5' internal homology arm is about 5 to about 50 nucleotides in length.

[0186] In some embodiments, the circRNA disclosed herein comprises a 3' internal homology arm. In some embodiments, the linear precursor RNA disclosed herein comprises a 3' internal homology arm. In some embodiments, the 3' internal homology arm comprises the nucleotide sequence of SEQ

ID NO: 71. In some embodiments, the 3' internal homology arm is about 5 to about 50 nucleotides in length.

[0187] In some embodiments, the linear precursor RNA disclosed herein comprises a 5' external homology arm and a 3' external homology arm. In some embodiments, the 5' external homology arm and the 3' external homology arm comprises the nucleotide sequence of SEQ ID NO: 69 or SEQ ID NO: 72. In some embodiments, the 5' external homology arm and the 3' external homology arm are each independently about 5 to about 50 nucleotides in length.

C. Spacer sequence

[0188] Spacer sequences may be employed to separate different elements in the circular RNA or linear precursor RNA of the disclosure. By separating the different elements, RNA secondary structure may fold better. For example, but in no way limiting, a spacer may be placed at the 5' end of an IRES to allow the IRES to fold into the proper structure. The spacer sequences can be polyA sequences, polyAC sequences, polyC sequences, polyU sequences, or the spacer sequences can be engineered depending on the spatial constraints of secondary structures that are made by the other elements contained in the linear precursor RNA (*e.g.*, the aptamer, the IRES, and the 5' and 3' self-splicing PIE fragments). Spacer sequences may promote circularization by introducing a region of spacer-spacer complementarity to promote the formation of a "splicing bubble" and spacer sequences promote functionality by allowing the highly structured intron portion of the self-splicing PIE fragment and IRES to fold into their correct secondary structures.

[0189] In some embodiments, the circular RNA or linear precursor RNA disclosed herein comprises at least one spacer sequence. In some embodiments, the circular RNA or linear precursor RNA comprises two or more spacer sequences. The two or more spacer sequences may comprise identical nucleotide sequences. In other embodiments, at least one of the two or more spacer sequences comprises a distinct nucleotide sequence. In some embodiments, the spacer sequence is about 5 to about 500 nucleotides in length. In some embodiments, the spacer sequence is about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 150, about 200, about 250, about 300, about 350, about 400, about 450, or about 500 nucleotides in length. In some embodiments, the spacer sequence is longer than about 500 nucleotides in length.

[0190] In some embodiments, the circular RNA or linear precursor RNA disclosed herein comprises a 5' spacer and a 3' spacer sequence. In some embodiments, the 5' spacer and the 3' spacer comprises the nucleotide sequence of SEQ ID NO: 78 or SEQ ID NO: 79.

D. Self-splicing ribozyme elements and circularization of the linear precursor RNA

[0191] The self-splicing ribozyme method of circularization utilizing a permuted group I catalytic intron can circularize long linear precursor RNA and requires only the addition of GTP and Mg2+ as cofactors (i.e., circularization conditions). Petkovic& Muller, (2015) Nucleic Acids Research, 43(4):2454-2465. Permuted intron-exon (PIE) splicing strategy consists of fused partial exons flanked by half-intron sequences (i.e., 3' self-splicing PIE fragment and 5' self-splicing PIE fragment). Puttaraju & Been, (1992) Nucleic Acids Research, 20(20):5357-5364. Upon addition of circularization conditions, linear precursor RNA containing the 3' and 5' self-splicing PIE undergo the double transesterification reactions characteristic of group I catalytic introns. During the reactions, the exon elements are fused resulting in the 5' to 3' linked circles. Petkovic & Muller, (2015) Nucleic Acids Research, 43(4):2454-2465; Wesselhoeft et al., (2018), Nat Commun., 9(1):2629.

[0192] In some embodiments, the linear precursor RNA disclosed herein comprises at least two catalytic subunits. In some embodiments, the self-splicing ribozyme catalytic subunits derive from either a group I intron or a group II intron RNA transcript or a fragment thereof. In some embodiments, the self-splicing ribozyme catalytic subunits derive from a permuted intron-exon (PIE) sequence from *Cyanobacterium Anabaena* pre-tRNA-Leu gene, T4 phage Td gene, or *Tetrahymena* pre-rRNA. In some embodiments, RNA catalytic subunits comprise a 3' self-splicing PIE fragment and a 5' self-splicing PIE fragment. In some embodiments, the 3' self-splicing PIE fragment comprises the nucleotide sequence of SEQ ID NO: 73. In some embodiments, the 5' self-splicing PIE fragment comprises the nucleotide sequence of SEQ ID NO: 74. In some embodiments, the catalytic activity of the two subunits result in a circularized RNA.

[0193] In some embodiments, the circRNA disclosed herein comprises a 3' exon element. In some embodiments, the 3' exon element comprises the nucleotide sequence of SEQ ID NO: 81. In some embodiments, the circRNA comprising the protein coding region and at least one RNA aptamer comprises a 5' exon element. In some embodiments, the 5' exon element comprises the nucleotide sequence of SEQ ID NO: 83.

E. 5' and 3' UTR sequence and polyA sequences

[0194] Previous studies have shown that 5' and 3' UTR sequences do not prevent efficient circularization of RNA and can potentially improve the expression of circRNA by acting as additional spacer sequence (See, e.g., WO2019236673). Polyadenylation (polyA) sequences may also function as spacers.

[0195] In some embodiments the circRNA disclosed herein contains at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or at least one polyadenylation (polyA) sequence. In some embodiments, the linear precursor RNA disclosed herein contains at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or a polyadenylation (polyA) sequence.

[0196] In some embodiments, the 5' UTR comprises the nucleotide sequence of SEQ ID NO: 76. In some embodiments, the 3' UTR comprises the nucleotide sequence of SEQ ID NO: 77.

[0197] In some embodiments, a 5' UTR may be between about 50 and 500 nucleotides in length. In some embodiments, a 3' UTR may be between 50 and 500 nucleotides in length or longer. In some embodiments, the circular RNA and linear precursor RNA disclosed herein comprise a 5' or 3' UTR that is derived from a gene distinct from the gene encoding the polypeptide in the protein coding region. In some embodiments, the circRNA disclosed herein comprise a 5' or 3' UTR that is chimeric. In some embodiments, the linear precursor RNA disclosed herein comprise a 5' or 3' UTR that is chimeric.

F. IVT: Generation of the linear precursor

[0198] The term "in vitro transcription" or "IVT" relates to a process wherein RNA is synthesized in a cell-free system (in vitro). As disclosed herein, linearized plasmid DNA can be used as template for the generation of linear RNA precursors. The promoter for controlling in vitro transcription can be any promoter for any DNA dependent RNA polymerase. Examples of DNA dependent RNA polymerases are the T7, T3, and SP6 RNA polymerases. A DNA template for in vitro RNA transcription may be obtained by cloning of a nucleic acid, in particular cDNA corresponding to the target RNA to be in vitro transcribed and introducing it into an appropriate DNA for in vitro transcription, for example into plasmid DNA. The cDNA may be obtained by reverse transcription of mRNA, chemical synthesis, or oligonucleotide cloning.

[0199] The linear precursor RNA disclosed herein may be synthesized according to any of a variety of known methods. In some embodiments, the linear precursor RNA according to the present

invention may be synthesized via in vitro transcription (IVT). Methods for in vitro transcription are known in the art. See, e.g., Geall et al. (2013) Semin. Immunol. 25(2): 152-159; Brunelle et al. (2013) Methods Enzymol. 530:101-14. Briefly, IVT is typically performed with a linear or circular DNA template containing a promoter, a pool of ribonucleotide triphosphates, a buffer system that may include DTT and magnesium ions, and an appropriate RNA polymerase (e.g., T3, T7 or SP6 RNA polymerase), DNAse I, pyrophosphatase, and/or RNAse inhibitor. The exact conditions will vary according to the specific application. The presence of these reagents is undesirable in a final RNA product and are considered impurities or contaminants which must be purified to provide a clean and homogeneous linear precursor RNA or resulting circRNA that is suitable for therapeutic use.

G. Total length and chemical modifications to circRNA and linear precursor RNA

[0200] The methods disclosed herein may be used to purify circRNA or the linear precursor RNA of a variety of nucleotide lengths. In some embodiments, the disclosed methods may be used to purify circRNA or linear precursor RNA of greater than about 1 kb, 1.5 kb, 2 kb, 2.5 kb, 3 kb, 3.5 kb, 4 kb, 4.5 kb, 5 kb, 6 kb, 7 kb, 8 kb, 9 kb, 10 kb, 11 kb, 12 kb, 13 kb, 14 kb, or 15 kb in length. The circRNA or the linear precursor RNA disclosed herein may be modified or unmodified. In some embodiments, the circRNA or the linear precursor RNA disclosed herein contain one or more modifications that typically enhance RNA stability or regulate translation of circRNA. Tang and Lv, (2021), Int J Biol Sci. 17(9);2262-2277. Exemplary modifications include backbone modifications, sugar modifications, or base modifications. In some embodiments, the disclosed linear precursor RNA may be synthesized from naturally occurring nucleotides and/or nucleotide analogues (modified nucleotides) including, but not limited to, purines (adenine (A), guanine (G)) or pyrimidines (thymine (T), cytosine (C), uracil (U)), and as modified nucleotides analogues or derivatives of purines and pyrimidines, such as e.g. 1-methyl- adenine, 2-methyl-adenine, 2-methylthio-N-6-isopentenyl-adenine, N6-methyl-adenine, N6- isopentenyl-adenine, 2-thio-cytosine, 3-methyl-cytosine, 4-acetyl-cytosine, 5-methyl-cytosine, 2,6-diaminopurine, 1-methyl-guanine, 2-methyl-guanine, 2,2-dimethyl-guanine, 7-methyl- guanine, inosine, 1-methyl-inosine, pseudouracil (5-uracil), dihydro-uracil, 2-thio-uracil, 4-thio-uracil, 5-carboxymethylaminomethyl-2-thio-uracil, 5-(carboxyhydroxymethyl)-uracil, 5-fluoro- uracil, 5-bromo-uracil, 5-carboxymethylaminomethyl-uracil, 5-methyl-2-thio-uracil, 5-methyl- uracil, N-uracil-5-oxy acetic acid methyl ester, 5-methylaminomethyl-uracil, 5- methoxyaminomethyl-2-thio-uracil, 5'-methoxycarbonylmethyl-uracil, 5-methoxy-uracil, uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid (v), 1-methyl-pseudouracil, queosine, β-D-mannosyl-queosine, phosphoramidates,

phosphorothioates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine, N6-methyladenosine, and inosine. In some embodiments, the disclosed circRNA or the linear precursor RNA comprise at least one chemical modification including but not limited to, consisting of pseudouridine, N1-methylpseudouridine, 2-thiouridine, 4'-thiouridine, 5- methylcytosine, 2-thio-l-methyl-1-deaza-pseudouridine, 2-thio-l-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-l-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methyluridine, 5-methyluridine, 5-methoxyuridine, and 2'-O-methyl uridine. In some embodiments, the modified nucleotides comprise N1-methylpseudouridine. The preparation of such analogues is known to a person skilled in the art e.g., from the U.S. Pat. No. 4,373,071, U.S. Pat. No. 4,401,796, U.S. Pat. No. 4,415,732, U.S. Pat. No. 4,458,066, U.S. Pat. No. 4,500,707, U.S. Pat. No. 4,668,777, U.S. Pat. No. 4,973,679, U.S. Pat. No. 5,047,524, U.S. Pat. No. 5,132,418, U.S. Pat. No. 5,153,319, U.S. Pat. No. 5,262,530, and U.S. Pat. No. 5,700,642.

H. Protein coding region

[0201] The circRNA or the linear precursor RNA disclosed herein contains a protein coding region encoding for a protein (e.g., a polypeptide or peptide). In some embodiments, the protein coding region is derived from a single gene or a single synthesis or expression construct. However, in some embodiments, the circRNA or the linear precursor RNA compositions disclosed herein comprise multiple protein coding regions and each can or collectively code for one or more proteins.

[0202] In some embodiments, the circRNA or the linear precursor RNA comprising the RNA aptamer as disclosed herein encodes a therapeutic polypeptide. In some embodiments, the therapeutic polypeptide comprises an antibody heavy chain, an antibody light chain, an enzyme, or a cytokine.

[0203] In some embodiments, the circRNA or the linear precursor RNA encodes a cytokine. Non-limiting examples of cytokines include IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, INF -α, INF-y, GM-CFS, M-CSF, LT-β, TNF-α, growth factors, and hGH.

[0204] In one embodiment, the circRNA or the linear precursor RNA comprising the RNA aptamer encodes a genome-editing polypeptide. In some embodiments, the genome-editing polypeptide is a CRISPR protein, a restriction nuclease, a meganuclease, a transcription activator-like effector protein

(TALE, including a TALE nuclease, TALEN), or a zinc finger protein (ZF, including a ZF nuclease, ZFN). See, e.g., Int'l Pub. No. WO2020139783.

[0205] In some embodiments, the circRNA or the linear precursor RNA encodes an enzyme that is utilized in an enzyme replacement therapy. Examples of enzyme replacement therapy include lysosomal diseases, such as Gaucher disease, Fabry disease, MPS I, MPS II (Hunter syndrome), MPS VI and Glycogen storage disease type II.

[0206] In some embodiments, the circRNA or the linear precursor RNA comprising the RNA aptamer encodes an antigen of interest. The antigen may be a polypeptide derived from a virus, for example, influenza virus, coronavirus (e.g., SARS-CoV-1, SARS-CoV-2, or MERS-related virus), Ebola virus, Dengue virus, human immunodeficiency virus (HIV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus (HSV), respiratory syncytial virus (RSV), rhinovirus, cytomegalovirus (CMV), zika virus, human papillomavirus (HPV), human metapneumovirus (hMPV), human parainfluenza virus type 3 (PIV3), Epstein-Barr virus (EBV), or chikungunya virus.

[0207] The antigen may be derived from a bacterium, for example, Staphylococcus aureus, Moraxella (e.g., Moraxella catarrhalis; causing otitis, respiratory infections, and/or sinusitis), Chlamydia trachomatis (causing chlamydia), borrelia (e.g., Borrelia burgdorferi causing Lyme Disease), Bacillus anthracis (causing anthrax), Salmonella typhi (causing typhoid fever), Mycobacterium tuberculosis (causing tuberculosis), Propionibacterium acnes (causing acne), or non-typeable Haemophilus influenzae.

[0208] Where desired, the circRNA or the linear precursor RNA comprising the RNA aptamer may encode for more than one antigen. In some embodiments, the circRNA or the linear precursor RNA disclosed herein encode for two, three, four, five, six, seven, eight, nine, ten, or more antigens. These antigens can be from the same or different pathogens. For example, a polycistronic protein coding region that can be translated into more than one antigen (e.g., each antigen-coding sequence is separated by a nucleotide linker encoding a self-cleaving peptide such as a 2A peptide) and can be further fused to the aptamer.

[0209] In some embodiments, the circRNA or the linear precursor RNA compositions disclosed herein are used in a vaccine. RNA vaccines provide a promising alternative to traditional subunit vaccines, which contain antigenic proteins derived from a pathogen. Vaccines based on RNA allow *de novo* expression of complex antigens in the vaccinated subject, which in turn allows proper post-translational modification and presentation of the antigens in its natural conformation. Moreover, once established, the manufacturing process for circRNA vaccines can be used for a variety of antigens,

enabling rapid development and deployment of circRNA vaccines. A detailed discussion of RNA vaccines can be found in Pardi, et al. (2018) Nat Rev Drug Discov 17, 261–279.

III. Aptamers

[0210] Widespread use of affinity purification of RNA has been limited due to the lack of efficient RNA fusion tags. Unless the RNA to be purified naturally contains a sequence with strong affinity for a target that can be immobilized on the stationary phase (i.e., a chromatography resin), the RNA may require tagging with a specific sequence to do so, analogous to the polyhistidine tag used in protein science.

[0211] Disclosed herein are circular RNA compositions which comprise a protein coding region and at least one aptamer. Also disclosed herein are linear precursor RNA compositions which comprise at least a self-splicing ribozyme and protein coding region, wherein the linear precursor RNA comprises at least one RNA aptamer. The aptamers associated with these circular RNA and linear precursor RNA compositions enable the use of affinity purification with minimal impact on translation efficiency and immunogenicity. Also disclosed herein are methods of making such circular RNA- and linear precursor RNA-tagged aptamer compositions.

[0212] The term “aptamer” as used herein refers to any nucleic acid sequence that has a non-covalent binding site for a specific target. Exemplary aptamer targets include nucleic acid sequence, protein, peptide, antibody, small molecule, mineral, antibiotic, and others. The aptamer binding site may result from secondary, tertiary, or quaternary conformational structure of the aptamer.

[0213] The term “RNA aptamer” as used herein refers to an aptamer comprised of RNA. In some embodiments, the RNA aptamer is included in the nucleotide sequence of the circRNA or the linear precursor RNA. In other embodiments, the RNA aptamer is separate from the nucleotide sequence of the circRNA or the linear precursor RNA.

[0214] Aptamers are typically capable of binding to specific targets with high affinity and specificity. Aptamers have several advantages over other binding proteins (e.g., antibodies). For example, aptamers can be engineered completely *in vitro* (e.g., via a SELEX aptamer selection method), can be produced by chemical synthesis, possess desirable storage properties, and elicit little or no immunogenicity in therapeutic applications. See, generally, Proske *et al.*, (2005) Appl. Microbiol. Biotechnol 69:367-374.

[0215] Aptamers have historically been used to modulate gene expression by directly binding to ligands. These aptamers act similarly to regulatory proteins, forming highly specific binding pockets for the target, followed by conformational changes.

[0216] In some embodiments, the RNA aptamer is synthetically derived. In some embodiments, the RNA aptamer is naturally derived from prokaryotes and/or eukaryotes. In some embodiments, the RNA aptamer is derived from a hairpin RNA, a tRNA, or a riboswitch.

[0217] In some embodiments the RNA aptamer is derived from a riboswitch. Riboswitches are regulatory RNA elements that act as small molecule sensors to control gene transcription and translation. Several riboswitch classes are known in the art. Exemplary riboswitches include B_{12} riboswitch, TPP riboswitch, SAM riboswitch, guanine riboswitch, FMN riboswitch, lysine riboswitch, and the PreQ1 riboswitch.

[0218] In some embodiments, the RNA aptamer is a split aptamer. Split aptamers are analogs to split-protein systems (e.g., beta-galactosidase) and rely on two or more short nucleic acid strands that assemble into a higher order structure upon the presence of a specific target. Debais *et al.* (2020) Nucleic Acids Res 48(7): 3400-3422. An exemplary split aptamer is the ATP-aptamer. Sassanfar & Szostak (1993) Nature 364(6437)-550-553. The ATP aptamer is an RNA aptamer that was divided into two RNA fragments by removing the loop that closes the stem and by extending each fragment with additional nucleotides to compensate for the loss of stability. Neither of the two RNA fragments bind ATP alone but in the presence of ATP the binding ability is reactivated. Debais *et al.* (2020) Nucleic Acids Res 48(7): 3400-3422.

[0219] In other embodiments, the split aptamer is reformed through the circularization of a linear precursor RNA. In this context, the split aptamer comprises a 5' portion and a 3' portion. Each portion may be of any length that is less than the full, un-split aptamer. The 5' portion and 3' portion together form the full un-split aptamer. For linear precursor RNA that comprise a 3' exon element and a 5' exon element, then the 5' portion of the split aptamer is positioned 3' of the 5'' exon element and the 3' portion of the split aptamer is positioned 5' of the 3'' exon element. For linear precursor RNA that do not comprise a 5' exon element and a 3' exon element, then the 5' portion of the split aptamer is positioned 3' of the 3' internal homology arm and the 3' portion of the split aptamer is positioned 5' of the 5' internal homology arm.

[0220] In certain embodiments, the split aptamer is reformed to a functional aptamer upon circularization of the linear precursor RNA.

[0221] In some embodiments, the RNA aptamer is an X-aptamer. X-aptamers are engineered with a combination of natural and chemically-modified nucleotides to improve binding affinity, specificty,

and versatility. An exemplary embodiment of a X-aptamer is the PS2-aptamer. The PS2-aptamer is an RNA aptamer that contains a phosphorodithioate (i.e., PS2) substitution at a single nucleotide of RNA aptamer which increases the aptamer's binding affinity from a nanomolar to a picomolar range. Abeydeera *et al.* (2016) Nucleic Acids Res. 44(17):8052-8064.

[0222] In some embodiments, the RNA aptamer binds to a ligand. In some embodiments the ligand is utilized in an affinity purification system. In some embodiments, the affinity ligand comprises protein A, protein G, streptavidin, glutathione (GSH), dextran (sephadex), cellulose (e.g., diethylaminoethyl cellulose) or a fluorescent molecule. In some embodiments, the affinity ligand is immobilized on a chromatography resin.

[0223] In some embodiments, the affinity ligand comprises protein A. DNA aptamers have been shown previously to target protein A. See, *e.g.,* Stoltenburg et al. (2016) Sci Rep. 6:33812.

[0224] In some embodiments, the disclosed RNA aptamers bind streptavidin. Streptavidin-binding aptamers are described in, *e.g.,* Srisawat & Engelke (2001) RNA 7(4): 632-641. An exemplary RNA aptamer that binds streptavidin is S1. In some embodiments, the RNA aptamer comprises the nucleotide sequence of UCAUGCAAGUGCGUAAGAUAGUCGCGGGCCGGGGGCGUAU (SEQ ID NO: 90).

[0225] Also disclosed herein are RNA aptamers that bind to sephadex. Sephadex-binding aptamers are described in, *e.g.,* Srisawat *et al.* (2001) Nucleic Acid Res 29(2): e4. An exemplary RNA aptamer that binds sephadex (e.g., Sephadex G-100) is Sephadex D8. In some embodiments, the RNA aptamer comprises the nucleotide sequence of GUCCGAGUAAUUUACGUUUUGAUACGGUUGCGGAACUUGC (SEQ ID NO: 91).

[0226] Also disclosed herein are RNA aptamers that bind to glutathione (GSH). Glutathione-binding aptamers are described in, *e.g.,* Bala, et al. (2011). RNA Biology 8(1): 101-111. In some embodiments, the RNA aptamer is GSHapt 8.17 or GSHapt 5.39.

[0227] Also disclosed herein are RNA aptamers that bind to 6xHis. 6xHis corresponds to amino acid sequence of 6 consecutive histidine residues. The 6xHis sequence may be isolated and optionally immobilized on a chromatography resin. Alternatively, the 6xHis sequence may be present as a N or C-terminal tag on a polypeptide, optionally wherein the 6xHis-tagged polypeptide is immobilized on a chromatography resin. 6xHis-binding aptamers are described in, *e.g.,* Tsuji, et al. (2009). Biochem Biophys Res Commun. 386(1): 227-231. In some embodiments, the RNA aptamer is shot47 or 47s. In some embodiments, the RNA aptamer comprises the nucleotide sequence of GGGUACGCUCAGGUAUAUUGGCGCCUUCGUGGAAUGUCAGUGCCUGGACGUGCAGU (SEQ ID NO: 84). In some embodiments, the RNA aptamer comprises the nucleotide sequence of

GGGACGCUCACGUACGCUCACGUCCGAUCGAUACUGGUAUAUUGGCGCCUUCGUGGAAUG UCAGUGCCUGGACGUGCAGU (SEQ ID NO: 85). In some embodiments, the RNA aptamer comprises the nucleotide sequence of GGGUAUAUUGGCGCCUUCGUGGAAUGUCAGUGCCUGG (SEQ ID NO: 86).

Also disclosed herein are RNA aptamers that bind to a MS2 coat protein (MCP). In some embodiments, the RNA aptamer comprises the nucleotide sequence of GGCCAACAUGAGGAUCACCCAUGUCUGCAGGGCC (SEQ ID NO: 87). In some embodiments, the RNA aptamer comprises the nucleotide sequence of ACAUGAGGAUCACCCAUG (SEQ ID NO: 88). In some embodiments, the RNA aptamer comprises the nucleotide sequence of ACAUGAGGAUCACCCAUGU (SEQ ID NO: 89). In some embodiments, the aptamer-containing circular RNA or linear RNA precursor described herein binds to an MCP immobilized on a chromatography resin. M2 aptamers are described in further detail in Bertrand et al. (1998). Molecular cell, 2(4), 437-445.

[0228] Also disclosed herein are RNA aptamers that bind to a fluorescent molecule. Examples of such aptamers are described in, e.g., Paige *et al.* (2011) Science 333(6042): 642-646. In some embodiments, the RNA aptamer comprises the nucleotide sequence of GAAGGGACGGUGCGGAGAGGAGA (SEQ ID NO: 92). The recited RNA aptamer is designated RNA Mango and binds the fluorescent molecule Thizole Orange (TO), such as TO1-biotin as described in Dolgosheina et al. (2014) ACS Chemical Biology, 9(10): 2412-2420.

[0229] In some embodiments, the RNA aptamer comprises the nucleotide sequence of AGCUUAUCCAUUGCAUCUCGGAUGAGCU (SEQ ID NO: 93). The recited RNA aptamer is designated U1hp and binds the spliceosomal protein U1A as described in Katsamba et al. (2001) J Biol Chem. 276(24): 21476-81.

[0230] In some embodiments, the RNA aptamer comprises a S1m aptamer or a derivative or fragment thereof. In some embodiments, the S1m aptamer used according to the instant disclosure is the aptamer described in Bachler *et al.* (1999) RNA 5(11):1509-1516, Srisawat & Engelke (2001) RNA 7(4): 632-641, or Li & Altman. (2002) Nuc. Acids Res. 30(17): 3706-3711. In some embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 65 or SEQ ID NO: 66. In some embodiments, the RNA adapter is encoded by the nucleotide sequence of SEQ ID NO: 52 or SEQ ID NO: 53.

[0231] In some embodiments, the RNA aptamer comprises a Sm aptamer.

[0232] In some embodiments, the RNA aptamer is about 30-200 nucleotides in length. In some embodiments, the RNA aptamer is about 50-200 nucleotides in length. In some embodiments, the

RNA aptamer is about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 105, about 110, about 115, about 120, about 125, about 130, about 135, about 140, about 145, about 150, about 155, about 160, about 165, about 170, about 175, about 180, about 185, about 190, about 195, or about 200 nucleotides in length.

[0233] In some embodiments, the aptamer (e.g., RNA aptamer) is not a histone stem-loop. As used herein, the term "histone stem-loop" refers to a stem-loop RNA structure that is typically found in histone-encoding mRNA. The histone stem-loop binds the stem-loop binding protein (SLBP) and is used to regulate histone expression during the cell cycle. Histone stem-loops are described in further detail in Lopez et al. (RNA. 14(1): 1-10. 2008) and WO2013120498.

[0234] In some embodiments, the aptamer (e.g., RNA aptamer) is not an internal ribosome entry site (IRES). In some embodiments, the aptamer (e.g., RNA aptamer) does not bind a ribosome or a protein that regulates protein translation. In some embodiments, the aptamer (e.g., RNA aptamer) does not bind the protein eIF4G. In some embodiments, the aptamer (e.g., RNA aptamer) is capable of binding a specific target (e.g., a protein) immobilized on a surface (e.g., a protein immobilized on a surface, such as a crosslinked agarose or crosslinked dextran).

A. Aptamer Location

[0235] Disclosed herein are RNA aptamers which include aptamers at various locations with respect to the other elements present in the linear precursor RNA or the subsequent circRNA. Selection of location of the RNA aptamer on the circRNA or the linear precursor RNA can be evaluated with respect to both the magnitude of regulation of translation and basal expression level.

[0236] In some embodiments, the RNA aptamer in the circRNA is positioned: a) before the 3' exon element, b) between the 3' exon element and the 5' internal homology arm, c) between the 5' internal homology arm and the 5' spacer sequence, d) between the 5' spacer sequence and the IRES, e) between the protein coding region and the 3' spacer sequence, f) between the 3' spacer sequence and the 3' internal homology arm, g) between the 3' internal homology arm and the 5' exon element, h) after the 5' exon element, j) between the 3' exon and the IRES, and/or i) between the IRES and the 5' exon element.

[0237] In some embodiments, the RNA aptamer in the circRNA is positioned: a) before the 3' exon element, b) between the 3' exon element and the 5' internal homology arm, c) between the 5' internal homology arm and the 5' spacer sequence, d) between the 5' spacer sequence and the protein coding

region, e) between the IRES and the 3' spacer sequence, f) between the 3' spacer sequence and the 3' internal homology arm, g) between the 3' internal homology arm and the 5' exon element, h) after the 5' exon element, i) between the 3' exon and the protein coding region, and/or j) between the protein coding region and the 5' exon element.

[0238] In some embodiments, the RNA aptamer in the linear precursor RNA is positioned: a) before the 5' external homology arm, b) between the 5' external homology arm and the 3' self-splicing PIE fragment, c) between the 3' self-splicing PIE fragment and the 5' internal homology arm, d) between the 5' internal homology arm and the 5' spacer sequence, e) between the 5' space sequence and the IRES, f) after the protein coding region but before the 3' spacer sequence, g) between the 3' spacer sequence and the 3' internal homology arm, h) between the 3' internal homology arm and the 5' self-splicing PIE fragment, i) between the 5' self-splicing PIE fragment and the 3' external homology arm, and/or j) after the 3' external homology arm.

[0239] In some embodiments, the RNA aptamer in the linear precursor RNA is positioned: a) before the 5' external homology arm, b) between the 5' external homology arm and the 3' self-splicing PIE fragment, c) between the 3' self-splicing PIE fragment and the 5' internal homology arm, d) between the 5' internal homology arm and the 5' spacer sequence, e) between the 5' space sequence and the protein coding region, f) after the IRES but before the 3' spacer sequence, g) between the 3' spacer sequence and the 3' internal homology arm, h) between the 3' internal homology arm and the 5' self-splicing PIE fragment, i) between the 5' self-splicing PIE fragment and the 3' external homology arm, and/or j) after the 3' external homology arm.

[0240] In some embodiments, the RNA aptamer does not have to be bound directly to the circRNA or the linear precursor RNA. In some embodiments, the RNA aptamer is attached to a linker. See, e.g., Elenko et al. (2009) J Am Chem Soc. 131(29): 9866-9867.

[0241] In some embodiments, the RNA aptamer can be removed from the circRNA or the linear precursor RNA after affinity purification. This may be achieved, for example, using DNA oligonucleotides which hybridize to the RNA aptamer or RNA scaffold. The resulting duplex can then be cleaved with an enzyme such as RNase H. See, e.g, Batey RT. (2014). Curr Opin Struct Biol. 26:1–8.

B. Aptamer Copy Number

[0242] An increase in aptamer copy number may allow aptamers to create a larger three-dimensional structure (*i.e.*, enhancing the number of affinity ligand binding sites available or creating

a unique ligand binding site). A strategic arrangement of aptamer copies may allow for increased avidity with the cognate affinity ligand.

[0243] In some embodiments, the circRNA or the linear precursor RNA used in the disclosed methods and compositions comprises multiple copies of an aptamer. Previous reports have shown that using a single small-molecule binding aptamer in the 5′-UTR enables 8-fold repression of translation upon ligand addition, but using three aptamers causes a 37-fold repression. Kotter *et al.*, (2009). Nucleic Acids Res. 37(18):e120. In some embodiments, the copy number of aptamers introduced into the circRNA or the linear precursor RNA is one, two, three, four, five, six, seven, eight, nine, ten, or more.

[0244] In some embodiments, the RNA aptamer comprises multiple copies of an aptamer sequence. In some embodiments, the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 65.

[0245] In some embodiments, copies of the aptamer are in repeat tandem configuration. The 4XS1m aptamer disclosed herein is an example of a multiple copy aptamer in a repeat tandem configuration.

IV. RNA Scaffolds

[0246] In some embodiments, the circular RNA and linear RNA precursor compositions disclosed herein comprise an RNA aptamer that is embedded in an RNA scaffold. As used herein, the term "RNA scaffold" refers to a noncoding RNA molecule that can assemble to have a predefined structure which creates spatial architecture to organize, protect, or enhance the properties of a functional module of interest. Exemplary functional modules can be nucleic acids (e.g., aptamers) or protein. In some embodiments, the RNA scaffolds suitable for use according to the instant disclosure can be associated with an RNA without disrupting the RNA structure. Furthermore, suitable RNA scaffolds allow for an RNA aptamer to be embedded without disrupting the RNA structure. In some embodiments, the RNA scaffolds used according to the instant disclosure can be any RNA scaffolds which do not have a significant negative impact on RNA expression or translation.

[0247] An RNA scaffold's predefined structure contains RNA-specific sequence motifs for self-assembly such as base-pairing between hairpin stems (kissing loops) and/or chemical modifications, Myhrvold & Silver (2015) Nat Struct Mol Bio 22(1):8-10. RNA-specific sequence motifs can form secondary (i.e., two-dimensional) and/or tertiary (i.e., three-dimensional) structures. In some embodiments, the RNA scaffold comprises at least one secondary structure motif. In some

embodiments, the RNA scaffold comprises at least one tertiary structure motif. Common secondary and/or tertiary RNA structural motifs include open and stacked three-way junctions, four-way junctions, four-way junctions similar to Holliday's structures, stem-loops (i.e., hairpin loops), interior loops (i.e., internal loops), bulges, tetraloops, multibranch loops, pseudoknots and knots, 90° kinks, and pseudo-torsional angles. Shanna et al. (2021) Molecules 26(5):1422.

[0248] RNA scaffolds can either be derived from nature (*e.g.*, attenuators, tRNA, riboswitches, terminators) or artificially engineered to form secondary or tertiary RNA structure. Delebecque *et al.* (2012) Nat Protoc 7(10): 1797-1807. Typically, in order to retain the RNA scaffold predefined structure, the RNA scaffold's RNA loop(s) (e.g., a hairpin loop) are the target regions for embedding the functional module of interest. See, e.g., US 20050282190 A1. The RNA scaffold's predefined structure can be modified, however, to have additional desirable properties. For example, the predefined RNA scaffold structure may be modified to become resistant to one or both of exonuclease digestion and endonuclease digestion.

[0249] In some embodiments, the circular RNA or linear precursor RNA compositions disclosed herein comprise an RNA aptamer that is embedded in a transfer RNA (tRNA). Transfer RNA (tRNA) scaffolds are an attractive tagging candidate in affinity purification systems, as tRNAs fold into canonical, stable clover-leaf structures that are resistant to unfolding and can protect RNA fusions from nuclease degradation. It has been demonstrated that embedding an aptamer in the anticodon loop of a tRNA scaffold promotes proper folding. See generally, Ponchon and Dardel (2007) Nat. Methods 4(7):571-576; Ponchon et al. (2013) Nucleic Acids Res. 41:e150. Use of an RNA aptamer embedded in a tRNA scaffold has been demonstrated to successfully pull down transcript-specific RNA-binding proteins from cell lysates. Iioka H et al. (2011) Nuc. Acids Res. 39(8):e53.

[0250] In some embodiments, the circRNA or the linear precursor RNA compositions disclosed herein comprise an RNA aptamer that is embedded in a tRNA which comprises the nucleotide sequence of SEQ ID NO: 67.

[0251] In some embodiments, the RNA aptamer is embedded in a tRNA hairpin loop of the tRNA. In some embodiments, the RNA aptamer is embedded in a tRNA anticodon loop. In some embodiments, the RNA aptamer is embedded in a tRNA D loop. In some embodiments, the RNA aptamer is embedded in a tRNA T loop.

[0252] Other exemplary RNA scaffolds include ribosomal RNA (rRNA) and ribozymes. In some embodiments, the RNA aptamer is embedded in a ribosomal RNA. In some embodiments, the RNA aptamer is embedded in a ribozyme. In some embodiments, the ribozyme is catalytically inactive.

V. Affinity Purification of RNA

[0253] In one aspect, disclosed herein are methods for purifying a circular RNA sample.

[0254] In some embodiments, the disclosed method for purifying circular RNA, comprises the steps of: (a) contacting a sample comprising the circular RNA disclosed herein with an affinity ligand that is immobilized on a chromatography resin, wherein the RNA aptamer comprises binding affinity for the affinity ligand; (b) eluting the circular RNA from the chromatography resin; and (c) purifying the circular RNA from the sample.

[0255] In some embodiments, the disclosed method for purifying a linear precursor RNA, comprises the steps of: (a) contacting a sample comprising the linear precursor RNA disclosed herein with an affinity ligand that is immobilized on a chromatography resin, wherein the RNA aptamer comprises binding affinity for the affinity ligand; (b) eluting the linear precursor RNA from the chromatography resin; and (c) purifying the linear precursor RNA from the sample.

[0256] In some embodiments, the disclosed methods comprise one or more washing steps between the contacting step (a) and the eluting step (b).

[0257] In some embodiments, the disclosed method for purifying a circular RNA, comprising the steps of: (a) contacting a sample comprising the circular RNA with an affinity ligand that is immobilized on a chromatography resin; (b) eluting the circular RNA from the chromatography resin; and (c) isolating the circular RNA from the sample, wherein the circular RNA comprises a protein coding region and at least one RNA aptamer, wherein the RNA aptamer comprises binding affinity for the affinity ligand.

[0258] In some embodiments, the disclosed method for purifying a linear precursor RNA, comprising the steps of: (a) contacting a sample comprising the linear precursor RNA with an affinity ligand that is immobilized on a chromatography resin; (b) eluting the linear precursor RNA from the chromatography resin; and (c) isolating the linear precursor RNA from the sample, wherein the linear precursor RNA comprises a protein coding region and at least one RNA aptamer, wherein the RNA aptamer comprises binding affinity for the affinity ligand.

[0259] In some embodiments, the disclosed methods result in circular RNA or linear precursor RNA that is greater than or equal to 90% pure. In some embodiments, the disclosed methods result in circular RNA and nicked circular RNA that is greater than or equal to 90% pure.

[0260] Affinity chromatography is one purification method that can be used with the circRNA or the linear precursor RNA compositions and methods disclosed herein. The RNA aptamers disclosed herein comprise binding affinity for the selected affinity ligand. The selected affinity ligand is

immobilized (e.g., crosslinked) on a chromatography resin. The circRNA or the linear precursor RNA comprising the RNA aptamer therefore binds with the resin containing the affinity ligand. The chromatography resin material is preferably present in a column, wherein the sample containing RNA is loaded on the top of the column and the eluent is collected at the bottom of the column.

[0261] The chromatography resin can be any material that is known to be used as a stationary phase in chromatography methods. The type of molecules used as affinity ligands, which interact with the RNA aptamers disclosed herein, can be a variety of types. Non-exhaustive examples of affinity ligands are antibodies, proteins, oligonucleotides, dyes, boronate groups, or chelated metal ions. The stationary phase may be composed of organic and/or inorganic material.

[0262] The most widely used stationary phase materials are hydrophilic carbohydrates such as cross-linked agarose and synthetic copolymer materials. These materials may comprise derivatives of cellulose, polystyrene, synthetic poly amino acids, synthetic polyacrylamide gels, or a glass surface. Further examples of materials that can be used as chromotagraphy resins are polystyrenedivinylbenzenes, silica gel, silica gel modified with non-polar residues, or other materials suitable for gel chromatograpy or other chromatographic methods, such as dextran, sephadex, agarose, dextran/agarose mixtures, and others known in the art.

[0263] The chromotography resin can be functionalized with affinity ligands for which the RNA aptamer has binding affinity. In some embodiments, the resin may be an agarose media or a membrane functionalized with phenyl groups (e.g. , Phenyl Sepharose™ from GE Healthcare or a Phenyl Membrane from Sartorius), Tosoh Hexyl, CaptoPhenyl, Phenyl Sepharose™ 6 Fast Flow with low or high substitution, Phenyl Sepharose™ High Performance, Octyl Sepharose™ High Performance (GE Healthcare); Fractogel™ EMD Propyl or Fractogel™ EMD Phenyl (E. Merck, Germany); Macro-Prep™ Methyl or Macro-Prep™ t-Butyl columns (Bio-Rad, California); WP HI-Propyl (C3)™ (J. T. Baker, New Jersey) or Toyopearl™ ether, phenyl or butyl (TosoHaas, PA). ToyoScreen PPG, ToyoScreen Phenyl, ToyoScreen Butyl, and ToyoScreen Hexyl are based on rigid methacrylic polymer beads. GE HiScreen Butyl FF and HiScreen Octyl FF are based on high flow agarose based beads. Preferred are Toyopearl Ether-650M, Toyopearl Phenyl-650M, Toyopearl Butyl-650M, Toyopearl Hexyl-650C (TosoHaas, PA), POROS-OH (ThermoFisher) or methacrylate based monolithic columns such as CIM-OH, CIM-SO3, CIM-C4 A and CIM C4 HDL which comprise OH, sulfate or butyl ligands, respectively (BIA Separations).

[0264] In some embodiments, the chromatography resin comprises protein A as an affinity ligand. Exemplary protein A resins include Byzen Pro Protein A resin (MilliporeSigma; 18887), Dynabeads Protein A Magnetic Beads (ThermoFisher; 10001D), Pierce Protein A Agarose (ThermoFisher;

20334), Pierce Protein A/G Plus Agarose (ThermoFisher; 20423), Pierce Protein A Plus UltraLink (ThermoFisher; 53142), Pierce Recombinant Protein A Agarose (ThermoFisher), POROS MabCapture A Select (ThermoFisher).

[0265] In some embodiments, the chromatography resin comprises streptavidin as an affinity ligand. Exemplary stretavidin resins include Streptavidin–Agarose from *Streptomyces avidinii* (MilliporeSigma; S1638), Pierce Steptavidin Plus UltaLink Resin (ThermoFisher; 53117), Pierce High Capacity Steptavisin Agarose (ThermoFisher; 20357), Streptavidin 6HC Agarose Resin (ABT; STV6HC-5), Streptavidin Resin – Amintra (Abcam; ab270530).

[0266] In some embodiments, the chromatography resin comprises glutathione (GSH) as an affinity ligand. Exemplary GSH resins include Glutathione Resin (GenScript; L00206), Pierce Glutathione Agarose (ThermoFisher; 16102BID), Glutathione Sepharose 4B GST-tagged Protein Resin 9Cytiva; 17075605); Glutathione Affinity Resin - Amintra (Abcam; ab270237).

VI. Vectors

[0267] In one aspect, disclosed herein are vectors comprising the linear precursor RNA disclosed herein. The nucleic acid sequences encoding a protein of interest (e.g., the protein coding region encoding a therapeutic polypeptide) can be cloned into a number of types of vectors. For example, the nucleic acids can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, sequencing vectors and vectors optimized for in vitro transcription.

[0268] In one embodiment, the vector is used to express the linear precursor RNA in a host cell. In another embodiment, the vector is used as a template for IVT. The construction of optimally translated IVT RNA suitable for therapeutic use is disclosed in detail in Sahin, et al. (2014). Nat. Rev. Drug Discov. 13, 759–780; Weissman (2015). Expert Rev. Vaccines 14, 265–281.

[0269] In some embodiments, the vectors disclosed herein comprise the following, from 5' to 3': a) a 5' external homology arm, b) a 5' self-splicing PIE fragment, c) a 5' internal homology arm, d) a 5' spacer sequence, e) an internal ribosome entry site (IRES), f) a protein coding region, g) a 3' spacer sequence, h) a 3' internal homology arm, i) a 3' self-splicing PIE fragment, and j) a 3' external homology arm, wherein the RNA aptamer is present at one or both of the 5' end or 3' end of any one of elements a)-j).

[0270] In some embodiments, the vectors disclosed herein also comprise a polynucleotide sequence 5' UTR, a polynucleotide sequence 3' UTR, a polynucleotide sequence encoding a polyA sequence and/or a polyadenylation signal.

[0271] A variety of RNA polymerase promoters are known in the art. In one embodiment, the promoter is a T7 RNA polymerase promoter. Other useful promoters include, but are not limited to, T3 and SP6 RNA polymerase promoters. Consensus nucleotide sequences for T7, T3 and SP6 promoters are known in the art.

[0272] Also disclosed herein are host cells (e.g., mammalian cells, e.g., human cells) comprising the vectors or RNA compositions disclosed herein.

[0273] Polynucleotides can be introduced into target cells using any of a number of different methods, for instance, commercially available methods which include, but are not limited to, electroporation (Amaxa Nucleofector-II (Amaxa Biosystems, Cologne, Germany)), (ECM 830 (BTX) (Harvard Instruments, Boston, Mass.) or the Gene Pulser II (BioRad, Denver, Colo.), Multiporator (Eppendort, Hamburg Germany), cationic liposome mediated transfection using lipofection, polymer encapsulation, peptide mediated transfection, biolistic particle delivery systems such as "gene guns" (see, for example, Nishikawa, et al. (2001). Hum Gene Ther. 12(8):861-70, or the TransIT-RNA transfection Kit (Mirus, Madison WI).

[0274] Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (e.g., an artificial membrane vesicle).

[0275] Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present invention, in order to confirm the presence of the circRNA or the linear precursor RNA sequence in the host cell, a variety of assays may be performed. Such assays are well known to those of skill in the art.

VII. Pharmaceutical Compositions

[0276] RNA purified according to this invention is useful as a component in pharmaceutical compositions, for example for use as a vaccine. These compositions will typically include RNA and a pharmaceutically acceptable carrier. A pharmaceutical composition of the invention can also include one or more additional components such as small molecule immunopotentiators (e.g., TLR agonists).

A pharmaceutical composition of the invention can also include a delivery system for the RNA, such as a liposome, an oil-in-water emulsion, or a microparticle. In some embodiments, the pharmaceutical composition comprises a lipid nanoparticle (LNP). In one embodiment, the composition comprises an antigen-encoding nucleic acid molecule encapsulated within a LNP. In some embodiments, the LNP comprises at least one cationic lipid. In some embodiments, the LNP comprises a cationic lipid, a polyethylene glycol (PEG) conjugated (PEGylated) lipid, a cholesterol-based lipid, and a helper lipid.

[0277] In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

EXAMPLES

[0278] The foregoing description of the specific embodiments will so fully reveal the general nature of the disclosure that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present disclosure. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

Example 1: Design of aptamer-tagged circular RNA

[0279] Previous studies had demonstrated that aptamer tagged mRNA could be useful for the purification of linear RNA species. *See* WO2023031856A1 , incorporated herein by reference in its entirety.

[0280] As described herein, the following example discloses the design of aptamer tagged circular RNA (circRNA) or the aptamer tagged linear precursor RNA, which is used to generate the circRNA.

[0281] The work described below utilized the S1m aptamer or a tRNA-S1m aptamer, each capable of binding streptavidin. The DNA nucleotide sequence encoding for the S1m aptamer and the tRNA-S1m aptamer are shown below.

SEQ ID NO: 52_S1m aptamer Tag (60 bp)
ATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCAT
SEQ ID NO: 54_tRNA-S1m aptamer Tag (134 bp)
GCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCATGCAAGTGCGT AAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGGTTCAAGTCCCTGTTCGGGCGC CA

The S1m aptamer and the tRNA-S1m aptamer sequence present in the circular RNA and/or linear precursor RNA are shown below:

SEQ ID NO. 65: S1m aptamer	AUGCGGCCGCCGACCAGAAUCAUGCAAGUGCGUAAGAUAGUCGCGGGUCGGCGGC CGCAU
SEQ ID NO. 66: 4xS1m aptamer	AUGCGGCCGCCGACCAGAAUCAUGCAAGUGCGUAAGAUAGUCGCGGGUCGGCGGC CGCAUCUGCUGGGAAGCUACGAUCCGUAGAAAAUGCGGCCGCCGACCAGAAUCAUG CAAGUGCGUAAGAUAGUCGCGGGUCGGCGGCCGCAUCUGCUGGGUAGCUGUGAAC CGUAGAAAAUGCGGCCGCCGACCAGAAUCAUGCAAGUGCGUAAGAUAGUCGCGGGU CGGCGGCCGCAUCUGCUGGGAAGCUACGAUCCGUAGAAAAUGCGGCCGCCGACCA GAAUCAUGCAAGUGCGUAAGAUAGUCGCGGGUCGGCGGCCGCAU
SEQ ID NO. 67: tRNA-S1m aptamer	GCCCGGAUAGCUCAGUCGGUAGAGCAGCGGCCUAUGCGGCCGCCGACCAGAAUCAU GCAAGUGCGUAAGAUAGUCGCGGGUCGGCGGCCGCAUUCGAGGCCGCGUCCAGGG UUCAAGUCCCUGUUCGGGCGCCA

[0282] **FIG. 1** depicts the experimental schematic of aptamer tagged linear precursor or aptamer tagged circRNA that were tested in streptavidin Sepharose bead affinity purification. The left panel shows the orientation of the aptamer tagged linear precursor RNA with respect to the flanking *Anabaena* PIE sequence. *Anabaena* PIE sequence reacted under group I intron splicing conditions

resulting in synthesis of the aptamer tagged circRNA. The right panel shows that the presence of the intact aptamer in either the linear precursor RNA or the circRNA species enabled binding to the affinity matrix during purification.

[0283] To initially obtain the linear precursor RNA and subsequent circRNA, DNA plasmids were designed.

[0284] **FIG. 2A** depicts the plasmid map encoding the 4xS1m aptamer, the linear precursor RNA, and the *Anabaena* PIE sequences used for RNA circularization. The plasmid elements are arranged in the following 5' to 3' order: a T7 promoter, a 5' external homology arm, a 3' Anabaena intron/exon fragment, a 5' internal homology arm, a 5' polyAC spacer, a CVB3 IRES, a protein coding region, a 3' polyAC spacer, a 4xS1m aptamer, a 3' internal homology arm, a 5' Anabaena intron/exon fragment, and a 3' external homology arm.

[0285] **FIG. 2B** depicts the plasmid map encoding the tRNA-S1m aptamer, the linear precursor RNA, and the *Anabaena* PIE sequences used for RNA circularization. The plasmid elements are arranged in the following 5' to 3' order: a T7 promoter, a 5' external homology arm, a 3' Anabaena intron/exon fragment, a 5' internal homology arm, a 5' polyAC spacer, a CVB3 IRES, a protein coding region, a 3' polyAC spacer, a 3' internal homology arm, a 5' Anabaena intron/exon fragment, a 3' external homology arm, and a tRNA-S1m aptamer.

[0286] **FIG. 2C** depicts the control plasmid map which encodes the linear precursor RNA and PIE sequences used for RNA circularization but does not encode an aptamer. The plasmid elements are arranged in the following 5' to 3' order: a T7 promoter, a 5' external homology arm, a 3' Anabaena intron/exon fragment, a 5' internal homology arm, a 5' polyAC spacer, a CVB3 IRES, a protein coding region, a 3' polyAC spacer, a 3' internal homology arm, a 5' Anabaena intron/exon fragment, and a 3' external homology arm.

[0287] Each construct described in **FIG. 2A-2C** was driven by a T7 promoter and each plasmid contained a HindIII restriction site.

[0288] The subsequent examples test the generation and functionality of aptamer tagged circRNA constructs in streptavidin sepharose bead affinity purification.

Example 2: Generation of aptamer tagged circRNA from aptamer tagged linear precursor RNA

[0289] The linear precursor RNA was synthesized by obtaining the cDNA template for IVT template via the linearization of the plasmids described in Example 1 using restriction enzyme, HindIII.

Linearized template DNA was loaded into the IVT reaction for the experimental groups, 4xS1m aptamer tagged and tRNAxS1m aptamer tagged linear precursor RNA as well as the control group was carried out using the HiScribe T7 High Yield RNA Synthesis Kit (New England Biolabs) according to manufacturer's instructions.

[0290] After IVT reactions, samples were treated with DNase I (NEB) for 15 min. After DNase treatment, circRNA was generated from the linear precursor RNA by adding 2 mM GTP to IVT product and incubating at 55°C for 15 min (i.e., circularization conditions). RNA samples were subsequently purified using LiCl precipitation and resuspended in 100 µl DEPC H2O.

[0291] After circularization conditions, three RNA species were expected to emerge from each respective sample: (1) aptamer-tagged circRNA, (2) residual aptamer-tagged linear precursor RNA that did not successfully undergo circularization, and (3) nicked aptamer-tagged circRNA. As previously reported, nicked aptamer-tagged circRNA is likely mediated by magnesium-catalyzed autohydrolysis which reduces the yield of the circRNA and is a deficiency that requires further optimization and improvement. Wesselhoeft et al., (2018), Nat Commun., 9(1):2629; Wesselhoeft et al., (2019), Mol Cell., 74(3):508-520; Li and Breaker, (1999), J. Am. Chem. Soc 121(23): 5364-5372.

Example 3: Streptavidin sepharose bead affinity purification and circRNA quantification

[0292] Samples which had been subjected to the circulation conditions in Example 2 were tested in a Sepharose bead affinity purification strategy followed by quantification of the yield of RNA recovery.

[0293] Methods for preparing the samples and binding conditions involved are disclosed in the following steps: (1) *Preparation of the streptavidin Sepharose beads.* To remove bead storage solution, 20 µL of streptavidin Sepharose beads (per sample) were spun at 0.8xg for 1 minute at 4°C. Subsequently, the beads were resuspended in 20 µL binding buffer and incubated on ice for 15 minutes. (2) *Preparation of RNA aptamer tagged circRNA containing samples and incubation conditions.* 2.5 µg of each sample was resuspended in 10 µL binding buffer. Refolding to allow aptamer to take on the expected secondary structure was performed by heating at 56°C for 5 min, 37°C for 10 min, and incubating at room temperature for 5 minutes. 2 µL of the sample was collected before binding to the sepharose beads and used as the control for input concentration. 10 µL of refolded aptamer (2.5 µg) were added to the Sepharose beads, incubated, and rotated at 4°C for 2 hours. Beads were washed 2 times with 100 µL of binding buffer. (3) *Elution of RNA aptamers from beads.* Elution was performed with 250 µL phenol-based reagent in the following steps: 50 µL cold

chloroform was added to the samples and vigorously shaken for 10 seconds. Subsequently, samples were spun at 12,000xg for 15 minutes at 4°C. Top aqueous phase (~125 µL) containing RNA was directly transferred to Monarch cleanup columns and follow manufacturer's instructions, and finally eluted from Monarch column in 40 µL DEPC H2O. (4) *Quantification of yield of RNA recovery*. RNA concentration following streptavidin affinity purification was quantified on a nanodrop. Elution, unbound, and wash fractions were run on a 2% EX Agarose Gel on an E-Gel Power Snap Electrophoresis system to visualize the RNA species present (aptamer-tagged circRNA, aptamer-tagged linear precursor RNA, and nicked RNA) in each of the fractions. Putative circRNA runs at a higher molecular weight than heavier linear precursor RNA, as indicated in **FIG. 3**.

[0294] As shown in **FIG. 3**, 4xS1m and tRNA-S1m aptamer tagged circRNA successfully underwent streptavidin Sepharose bead affinity purification relative to the no aptamer control sample (see lanes 3-5 containing eluted sample) and unbound fractions (compare lanes 3-5 with lanes 6-11). As predicted in Example 2, **FIG. 3** also shows that circularization conditions resulted in three distinct RNA species (labeled on the agarose gel as “circular”, “precursor”, and “nicked”) indicating that the aptamer did not interfere with circularization of the linear precursor RNA.

[0295] The amount of RNA recovery in each sample after streptavidin Sepharose bead affinity purification was also quantified. The results are shown in the bar graph of **FIG. 4** which also displays an additional aptamer tagged linear precursor RNA control. Affinity purified 4xS1m aptamer tagged circRNA yielded approximately a 50% RNA recovery and the tRNAxS1m tagged circRNA yielded approximately a 60% RNA recovery yield relative to the input control sample. In contrast, the affinity purified control yielded approximately less than 5% RNA recovery yield. This result indicates that introducing aptamer tag to circRNA (e.g., a 4xS1m or a tRNAxS1m aptamer tag) can potentially be used to improve affinity purification efficiency of circRNA.

Example 4: Negative selection scheme for recovery of circRNA

[0296] In Examples 1-3, aptamer-containing constructs were designed to be present in both the linear precursor RNA as well as the aptamer tagged circRNA (see **FIG. 1**). However, to optimally purify aptamer-tagged circRNA removal of the linear precursor RNA is necessary. Accordingly, linear precursor RNA were designed to create a negative selection strategy for affinity purification as diagrammed in **FIG. 6**.

[0297] Under the negative selection method, as shown in **FIG. 6**, the aptamer was localized in the linear precursor RNA at a position that would be removed upon circularization (i.e., the circRNA will

not have the aptamer). In this configuration, the linear precursor RNA binds to the affinity matrix, but the circRNA does not.

[0298] Several linear precursor RNAs were designed with the aptamer positioned at the 3' intron region. After IVT and circularization, the circularization reaction mixture was incubated with streptavidin Sepharose beads as described above. The unbound, wash, and elution fractions were all collected. Purification of a 4xS1m aptamer tagged linear precursor RNA (pML49), a tRNA-S1m (tS1m) aptamer tagged linear precursor RNA (pML50 and pML51), a no aptamer control (pML47), a 4xS1m aptamer tagged circRNA (pML26), and a tRNA-S1m aptamer tagged circRNA (pML38) was performed. The amount of recovered RNA measured is expressed as a percent of the input (i.e., the input being the total RNA in the sample). As shown in **FIG. 7**, the negative selection constructs (pML49, pML50, pML51) showed binding that was intermediate between the no aptamer control (pML47) and the circRNA with aptamer designs (pML26 & pML38), suggesting that the portion of RNA in the unbound and wash fraction for the negative selection constructs was the desired circRNA.

[0299] These results were analyzed further by taking images of agarose gels of the different samples. As shown in **FIG. 8A – FIG. 8D**, circRNA and nicked RNA species were predominantly found in the unbound and wash fraction, while linear precursor RNA was found in the eluted fraction for the negative selection constructs. A capillary electrophoresis assay was also performed to determine the various RNA species, as shown in **FIG. 9A – FIG. 9C**.

[0300] The placement of the aptamer in the linear precursor was tested. The tS1m aptamer was placed at the 3' end of the linear precursor RNA (pML123), at the 5' end of the linear precursor RNA (pML128), and at both the 5' end and 3' end of the linear precursor RNA (pML125). Each linear precursor RNA contained an ORF encoding for human erythropoietin (EPO), a gene of over 500 nucleotides. As shown in **FIG. 12A – FIG. 12B**, the placement or number of tS1m aptamers on the linear precursor did not negatively impact the purification of the circRNA. A summary of the purification is provided below in **Table 1** for the pML125 construct. The introns in **FIG. 12A** results from the homology regions of the catalytic introns co-purifying when one of them contains the aptamer.

[0301] **Table 1** - Summary of purification

Sample	Concentration	Total RNA	% Circular	Total Circular	Circular Recovery
Input	217 ng/ul	8 mg	69%	5.58 mg	N/A

Unbound	504.7 ng/ul	2.019 mg	98%	1.97 mg	35.3%
Unbound + washes	351.6 ng/ul	2.813 mg	98%	2.76 mg	49.5%

Example 5: Positive selection scheme for recovery of circRNA

[0302] In Examples 1-3, aptamer-containing constructs were designed to be present in both the linear precursor RNA as well as the aptamer tagged circRNA (see **FIG. 1**). However, to optimally purify aptamer-tagged circRNA removal of the linear precursor RNA is necessary. Accordingly, linear precursor RNA were designed to create a positive selection strategy for affinity purification as diagrammed in **FIG. 5**.

[0303] Under the positive selection method, as shown in **FIG. 5**, a linear precursor RNA will be constructed to contain a split aptamer in which the 3' and the 5' half of the aptamer will be positioned at the 5' and 3' flanking ends of the linear precursor RNA, respectively. The linear precursor RNA will not undergo affinity purification because the intact aptamer is required for binding to the affinity matrix. Upon circularization of the linear precursor RNA, the intact aptamer will form allowing for binding to the affinity matrix.

[0304] cDNA templates will be generated and IVT will be used to produce the linear precursor RNA constructs. Constructs will vary the type of aptamer and its spatial configuration within the linear precursor RNA (see **FIG. 5** for exemplary configurations). Table 2 shows the list of potential aptamer orientations for the tRNA-S1m and the 4xS1m aptamer in the linear precursor RNA. Upon completion of circularization conditions, constructs will be affinity purified using streptavidin sepharose beads and quantified as described in Example 3. Each construct will be evaluated based on RNA recovery relative to the input control sample.

Example 6: Scale-up of circRNA purification

[0305] A scale up in the total input of linear precursor was performed to determine if the aptamer purification strategy would robustly purify the circRNA. As an initial matter, the template pML50 was modified to swap out the T7 RNA polymerase promoter for the SP6 promoter. An IVT reaction was performed to produce the linear precursor and the circularization reaction was performed with an

initial 1 mg amount of RNA. As shown in **FIG. 10**, the 1 mg scale circularization followed by streptavidin purification yielded a highly pure circRNA in the unbound and wash fractions. Following the 1 mg scale purification, a larger 12 mg scale purification was attempted. In this assay, 3 rounds of the purification scheme were performed to increase purity. As shown in **FIG. 11A**, even at the higher starting amount of RNA, the circRNA was effectively purified, whether after 1, 2, or 3 rounds of purification. As shown in **FIG. 11B**, multiple rounds of purification yielded higher purities of circRNA.

Example 7: Purification of large circRNA

[0306] The circRNA purification strategies described above were attempted with circRNA encoding relatively small proteins (GFP and EPO). To test the efficacy of the aptamer purification strategy on larger circRNA, 6 different circRNA were generated with ORF sizes of 1032, 1035, 1725, 1728, 2172, and 2175 nucleotides. The full size of the 6 circRNAs were 1952, 2645, and 3092 nucleotides. As shown in **FIG. 13**, the 6 different constructs were purified through the negative selection purification scheme in which one or more aptamers are contained in the linear precursor, but lost during the circularization reaction. The data shows that the large circRNA was effectively purified.

Example 8: Activity of circRNA containing aptamers

[0307] A circRNA was next tested to ensure expression of the encoded protein occurred. The pML50 circRNA encoding GFP was used, which was purified via the negative selection scheme, where the linear precursor RNA, but not he circRNA, contains the aptamer. The circRNA encoding GFP was transfected into Hela cells at different µg of RNA / million cells. As shown in **FIG. 14**, both purified and unpurified circRNA displayed GFP expression relative to a negative control., while the purified circRNA displayed greater expression relative to the unpurified circRNA.

[0308] Other embodiments of the disclosure will be apparent to those skilled in the art from consideration of the specification and practice of the disclosure disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the disclosure being indicated by the following claims.

[0309] All patents and publications cited herein are incorporated by reference herein in their entirety.

SEQUENCES

Table 2. Linear precursor RNA-encoding nucleotide sequences

SEQ ID NO / Description	SEQUENCE
SEQ ID NO. 1: pML23_CVB 3-EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG AGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGC GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCAT CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAACAAAAAACAAAA CGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTC GGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAA TCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGTAGCTGTG AACCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGG GTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCA GAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGGGCTA TTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTC TTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCC TGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 2:	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA

pML24_CVB3-EGFP-	AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG AGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGC GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCAT CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAACAAAAAACAAAA CGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTC GGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAA TCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGTAGCTGTG AACCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGG GTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCA GAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAGCTC GCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAA CTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTAT TTTCATTGCAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAA GTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTA ATAAAAAACATTTATTTTCATTGCGGCTATTATGCGTTACCGGCGAGACGCTACGGACT TAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAA CCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGA CCAGTGGACAATCGACG
SEQ ID NO. 3: pML25_UTR-CVB3-E	TAATACGACTCACTATAGGGGATCCAGAGCGGCCGCTTTTTCAGCAAGATTAAGCCCA GGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCT CAAACAGACACCGGGAGACCCTCGACCGTCGATTGTCCACTGGTCAACAATAGATGAC TTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTAACGTCAAGACGAG GGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAAAGCTGCAAGAGAA TGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTCCACCCCCACGCCG GAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAAAACCAAAAAAACAA AACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCAC TCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTA GAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGC ACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGC

	GTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGT GTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACG GGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACG CTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCC TGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGT CGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTAT TCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGGATT GGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTTAG CTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACAGCAAAATGGTGAG CAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCG ACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGCGATGCCACCTACG GCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCA CCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACA TGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCA CCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG GCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCA ACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGC CGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCACAACATCGAGGA CGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCC CCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACC CCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCA CTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAACAAAAAACAAAACGTAGAAAATG CGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCAT CTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTG CGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGTAGCTGTGAACCGTAGAAA ATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCC GCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAATCATGCA AGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAGCTCGCTTTCTTGC TGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATA TTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAG CTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACT AAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATT TATTTTCATTGCGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAG CCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGT TATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAA TCGACG
SEQ ID NO. 4: pML26_CVB 3-EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG

	TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG AGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGC GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCAT CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAGTAGAAAATGCGGCCGC CGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGG GAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGAT AGTCGCGGGTCGGCGGCCGCATCTGCTGGGTAGCTGTGAACCGTAGAAAATGCGGCC GCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCT GGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAA GATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAAAAAACAAAAAACAAAACGGCTA TTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTC TTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCC TGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 5: pML27_CVB 3-EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG AGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGC GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCAT CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC

	CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAGTAGAAAATGCGGCCGC CGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGG GAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGAT AGTCGCGGGTCGGCGGCCGCATCTGCTGGGTAGCTGTGAACCGTAGAAAATGCGGCC GCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCT GGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAA GATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAGCTCGCTTTCTTGCTGTCCAATTT CTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGG GCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAGCTCGCTTTC TTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGG GATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATT GCAAAAAACAAAAAACAAAACGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTA AATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACC TAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACC AGTGGACAATCGACG
SEQ ID NO. 6: pML28_CVB 3-EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG AGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGC GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCAT CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCTCGCTTTCTTGCTGT CCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTA TGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAGCT CGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAA ACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTA TTTTCATTGCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGT CGCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGC CGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGG

	GTAGCTGTGAACCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGAT AGTCGCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCC GCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCT GGGAAAAAACAAAAAACAAAACGGCTATTATGCGTTACCGGCGAGACGCTACGGACTT AAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAAC CTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGAC CAGTGGACAATCGACG
SEQ ID NO. 7: pML29_CVB 3-GLuc-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGGAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAA GCCCACCGAGAACAACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGAC CACGGATCTCGATGCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGT GCTCAAAGAGATGGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGAT CTGCCTGTCCCACATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGC CACACCTACGAAGGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGT CGACATTCCTGAGATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCA CAGGTCGATCTGTGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTG CAGTGTTCTGACCTGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCA AGATCCAGGGCCAGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAAAAAAAACAAA AAACAAAACGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTC GCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCC GACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGG TAGCTGTGAACCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATA GTCGCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCC GCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCT GGGGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAG AAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACA AGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 8: pML30_CVB 3-GLuc-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC

	ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGGAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAA GCCCACCGAGAACAACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGAC CACGGATCTCGATGCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGT GCTCAAAGAGATGGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGAT CTGCCTGTCCCACATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGC CACACCTACGAAGGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGT CGACATTCCTGAGATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCA CAGGTCGATCTGTGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTG CAGTGTTCTGACCTGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCA AGATCCAGGGCCAGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAAAAAAAACAAA AAACAAAACGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTC GCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCC GACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGG TAGCTGTGAACCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATA GTCGCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCC GCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCT GGGAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCA ACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAA AACATTTATTTTCATTGCAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTT GTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGA TTCTGCCTAATAAAAAACATTTATTTTCATTGCGGCTATTATGCGTTACCGGCGAGACG CTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACT CAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAA TTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 9: pML31_CVB 3-GLuc-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGGAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAA

	GCCCACCGAGAACAACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGAC CACGGATCTCGATGCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGT GCTCAAAGAGATGGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGAT CTGCCTGTCCCACATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGC CACACCTACGAAGGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGT CGACATTCCTGAGATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCA CAGGTCGATCTGTGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTG CAGTGTTCTGACCTGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCA AGATCCAGGGCCAGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAAGTAGAAAATG CGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCAT CTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTG CGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGTAGCTGTGAACCGTAGAAA ATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCC GCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAATCATGCA AGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAAAAAACAAAAAACA AAACGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAA GAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGAC AAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 10: pML32_CVB 3-GLuc-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGGAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAA GCCCACCGAGAACAACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGAC CACGGATCTCGATGCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGT GCTCAAAGAGATGGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGAT CTGCCTGTCCCACATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGC CACACCTACGAAGGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGT CGACATTCCTGAGATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCA CAGGTCGATCTGTGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTG CAGTGTTCTGACCTGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCA AGATCCAGGGCCAGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAAAGCTCGCTTT CTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGG GGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCAT TGCAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAA CTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAA ACATTTATTTTCATTGCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAA GATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCG GCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCT

	GCTGGGTAGCTGTGAACCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCG TAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAAT GCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGC ATCTGCTGGGAAAAAACAAAAAACAAAACGGCTATTATGCGTTACCGGCGAGACGCTA CGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCA GGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATT AGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 11: pML33_CVB 3-GLuc-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGGAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAA GCCCACCGAGAACAACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGAC CACGGATCTCGATGCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGT GCTCAAAGAGATGGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGAT CTGCCTGTCCCACATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGC CACACCTACGAAGGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGT CGACATTCCTGAGATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCA CAGGTCGATCTGTGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTG CAGTGTTCTGACCTGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCA AGATCCAGGGCCAGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAAGTAGAAAATG CGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCAT CTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTG CGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGTAGCTGTGAACCGTAGAAA ATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCC GCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAATCATGCA AGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAGCTCGCTTTCTTGC TGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATA TTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAG CTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACT AAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATT TATTTTCATTGCAAAAAACAAAAAACAAAACGGCTATTATGCGTTACCGGCGAGACGCT ACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTC AGGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAAT TAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 12:	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA

pML34_4xS1m-CVB3	AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGGTAGAAAATGCGGCCGCCGACCAGAATCA TGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACGATC CGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTC GGCGGCCGCATCTGCTGGGTAGCTGTGAACCGTAGAAAATGCGGCCGCCGACCAGAA TCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACG ATCCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGG GTCGGCGGCCGCATCTGCTGGGAAAAACAAAAAACAAAAAAAACAAAAAAAAAACCAA AAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGC GCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACT GTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTT GATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAG GAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGT TGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCAT TCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCC ATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCT CCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGG CAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTT CATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTA TTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATAC CACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACAGCAAAAT GGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGG ACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGCGATGCC ACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCC TGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCC GACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAG GAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAG TTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG GACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATA TCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCACAACAT CGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCG ACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCA AAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCG GGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAACAAAAAACAAAACGGCT ATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATT CTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATC CTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 13: pML35_CVB3-EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT

	ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG AGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGC GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCAT CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAACAAAAAACAAAA CAAAAAAAAAAAAAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGC CGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGC CGCGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCACTGCAGAAAAAAAAAAAAGGCTAT TATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCT TTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCCT GAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 14: pML36_CVB 3-EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG AGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGC GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCAT CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAACAAAAAACAAAA CAAAAAAAAAAAAAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGC

	CGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGC CGCGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCACTGCAGAAAAAAAAAAAAAGCTCG CTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAAC TGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATT TTCATTGCAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAG TCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAAT AAAAAACATTTATTTTCATTGCGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTA AATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACC TAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACC AGTGGACAATCGACG
SEQ ID NO. 15: pML37_UTR- CVB3-E	TAATACGACTCACTATAGGGGATCCAGAGCGGCCGCTTTTTCAGCAAGATTAAGCCCA GGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCT CAAACAGACACCGGGAGACCCTCGACCGTCGATTGTCCACTGGTCAACAATAGATGAC TTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTAACGTCAAGACGAG GGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAAAGCTGCAAGAGAA TGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTCCACCCCCACGCCG GAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAAAACCAAAAAAACAA AACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCAC TCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTA GAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGC ACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGC GTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGT GTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACG GGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACG CTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCC TGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGT CGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTAT TCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGGATT GGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTTAG CTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACAGCAAAATGGTGAG CAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCG ACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGCGATGCCACCTACG GCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCA CCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACA TGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCA CCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG GCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCA ACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGC CGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCACAACATCGAGGA CGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCC CCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACC CCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCA CTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAACAAAAAACAAAACAAAAAAAAAA AAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCA TGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGG TTCAAGTCCCTGTTCGGGCGCCACTGCAGAAAAAAAAAAAAAGCTCGCTTTCTTGCTGT CCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTA TGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAGCT CGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAA ACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTA TTTTCATTGCGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCC TTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTAT

	AGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 16: pML38_CVB3-EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTCAACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTAACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAAAGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTCCACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAAAACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACAGCAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAAAAAAAAAAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCACTGCAGAAAAAAAAAAAAAAAAAACAAAAAACAAAACGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 17: pML39_CVB3-EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTCAACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTAACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAAAGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTCCACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAAAACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG

	TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG AGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGC GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCAT CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCTCGCTTTCTTGCTGT CCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTA TGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAGCT CGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAA ACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTA TTTTCATTGCAAAAAAAAAAAAAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTAT GCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGC ATTCGAGGCCGCGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCACTGCAGAAAAAAAA AAAAAAAAAACAAAAAACAAAACGGCTATTATGCGTTACCGGCGAGACGCTACGGACT TAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAA CCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGA CCAGTGGACAATCGACG
SEQ ID NO. 18: pML40_CVB 3-EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG AGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGC GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG

	TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCAT CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAAAAAAAAAGCCCG GATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCATGCAAGT GCGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGGTTCAAGT CCCTGTTCGGGCGCCACTGCAGAAAAAAAAAAAAAGCTCGCTTTCTTGCTGTCCAATTT CTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGG GCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAGCTCGCTTTC TTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGG GATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATT GCAAAAAACAAAAAACAAAACGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTA AATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACC TAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACC AGTGGACAATCGACG
SEQ ID NO. 19: pML41_CVB 3-GLuc-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGGAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAA GCCCACCGAGAACAACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGAC CACGGATCTCGATGCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGT GCTCAAAGAGATGGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGAT CTGCCTGTCCCACATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGC CACACCTACGAAGGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGT CGACATTCCTGAGATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCA CAGGTCGATCTGTGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTG CAGTGTTCTGACCTGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCA AGATCCAGGGCCAGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAAAAAAAACAAA AAACAAAACAAAAAAAAAAAAAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATG CGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCAT TCGAGGCCGCGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCACTGCAGAAAAAAAAAA AAGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGA AGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAA GGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG

SEQ ID NO. 20: pML42_CVB 3-GLuc-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGGAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAA GCCCACCGAGAACAACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGAC CACGGATCTCGATGCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGT GCTCAAAGAGATGGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGAT CTGCCTGTCCCACATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGC CACACCTACGAAGGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGT CGACATTCCTGAGATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCA CAGGTCGATCTGTGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTG CAGTGTTCTGACCTGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCA AGATCCAGGGCCAGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAAAAAAAACAAA AAACAAAACAAAAAAAAAAAAAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATG CGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCAT TCGAGGCCGCGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCACTGCAGAAAAAAAAAA AAAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAAC TACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAA CATTTATTTTCATTGCAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTT CCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTC TGCCTAATAAAAAACATTTATTTTCATTGCGGCTATTATGCGTTACCGGCGAGACGCTA CGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCA GGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATT AGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 21: pML43_CVB 3-GLuc-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC

	AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGGAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAA GCCCACCGAGAACAACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGAC CACGGATCTCGATGCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGT GCTCAAAGAGATGGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGAT CTGCCTGTCCCACATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGC CACACCTACGAAGGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGT CGACATTCCTGAGATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCA CAGGTCGATCTGTGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTG CAGTGTTCTGACCTGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCA AGATCCAGGGCCAGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAAAAAAAAAAAA AAAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATC ATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGG GTTCAAGTCCCTGTTCGGGCGCCACTGCAGAAAAAAAAAAAAAAAAAACAAAAAACAAA ACGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGA AGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAA GGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 22: pML44_CVB 3-GLuc-	TAATACGACTCACTATAGGGGATCCAGAGCGGCCGCTTTTTCAGCAAGATTAAGCCCA GGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCT CAAACAGACACCGGGAGACCCTCGACCGTCGATTGTCCACTGGTCAACAATAGATGAC TTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTAACGTCAAGACGAG GGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAAAGCTGCAAGAGAA TGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTCCACCCCCACGCCG GAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAAAACCAAAAAAACAA AACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCAC TCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTA GAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGC ACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGC GTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGT GTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACG GGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACG CTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCC TGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGT CGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTAT TCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGGATT GGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTTAG CTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACAGCAAAATGGGAGT CAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAAGCCCACCGAGAAC AACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGACCACGGATCTCGAT GCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGTGCTCAAAGAGAT GGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGATCTGCCTGTCCCA CATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGCCACACCTACGAA GGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGTCGACATTCCTGA GATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCACAGGTCGATCTG TGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTGCAGTGTTCTGACC TGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCAAGATCCAGGGCC AGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAAAGCTCGCTTTCTTGCTGTCCAAT TTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAG GGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAGCTCGCTTT CTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGG

	GGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCAT TGCAAAAAAAAAAAAAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCC GCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAG GCCGCGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCACTGCAGAAAAAAAAAAAAAAAAA AACAAAAAACAAAACGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATT GAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCT AGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGA CAATCGACG
SEQ ID NO. 23: pML45_tS1m -CVB3-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAAAAAAAAAAAGCCCGGATAGCTCAGTC GGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGT CGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGGTTCAAGTCCCTGTTCGGGC GCCACTGCAGAAAAAAAAAAAAAAAAACAAAAAACAAAAAAAACAAAAAAAAAACCAAA AAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCG CTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTG TAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTG ATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGG AGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTT GCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATT CCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCA TGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTC CGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGGC AGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTC ATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTAT TGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACC ACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACAGCAAAATG GTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGA CGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGCGATGCCA CCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCT GGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCG ACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGA GCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTT CGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGA CGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATC ATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCACAACATC GAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGA CGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAA AGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGG GATCACTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAACAAAAAACAAAACGGCTA TTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTC TTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCC TGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 24:	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC

pML46_CVB 3-GLuc-	CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGGAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAA GCCCACCGAGAACAACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGAC CACGGATCTCGATGCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGT GCTCAAAGAGATGGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGAT CTGCCTGTCCCACATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGC CACACCTACGAAGGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGT CGACATTCCTGAGATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCA CAGGTCGATCTGTGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTG CAGTGTTCTGACCTGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCA AGATCCAGGGCCAGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAAAAAAAACAAA AAACAAAACGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCC TTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTAT AGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCG ACG
SEQ ID NO. 25: pML47_CVB 3-EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG AGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGC GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCAT

	CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAACAAAAAACAAAA CGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAA GAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAG GCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 26: pML48_CVB 3-EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG AGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGC GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCAT CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAACAAAAAACAAAA CGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAA GAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAG GCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACGGATA ACAGCATATCTAGTAAGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAA GATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCG GCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCT GCTGGGTAGCTGTGAACCGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCG TAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAAT GCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGC AT
SEQ ID NO. 27:	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA

pML49_CVB 3-GLuc-	AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGGAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAA GCCCACCGAGAACAACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGAC CACGGATCTCGATGCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGT GCTCAAAGAGATGGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGAT CTGCCTGTCCCACATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGC CACACCTACGAAGGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGT CGACATTCCTGAGATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCA CAGGTCGATCTGTGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTG CAGTGTTCTGACCTGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCA AGATCCAGGGCCAGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAAAAAAAACAAA AAACAAAACGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCC TTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTAT AGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCG ACGGATAACAGCATATCTAGTAAGTAGAAAATGCGGCCGCCGACCAGAATCATGCAAG TGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGTAGA AAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGC CGCATCTGCTGGGTAGCTGTGAACCGTAGAAAATGCGGCCGCCGACCAGAATCATGC AAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGAAGCTACGATCCGT AGAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGC GGCCGCAT
SEQ ID NO. 28: pML50_CVB 3-EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG AGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGC

	GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCAT CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAACAAAAAACAAAA CGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAA GAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAG GCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACGGATA ACAGCATATCTAAAAAAAAAAAAAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTA TGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCG CATTCGAGGCCGCGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCA
SEQ ID NO. 29: pML51_CVB 3-Gluc-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAA AACCAAAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCA TTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCC CCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCC ACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCG TGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTC ACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGG GAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGG TAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCC AGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTC CGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCAT ATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGG TTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACA GCAAAATGGGAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAA GCCCACCGAGAACAACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGAC CACGGATCTCGATGCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGT GCTCAAAGAGATGGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGAT CTGCCTGTCCCACATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGC CACACCTACGAAGGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGT CGACATTCCTGAGATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCA CAGGTCGATCTGTGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTG CAGTGTTCTGACCTGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCA AGATCCAGGGCCAGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAAAAAAAACAAA AAACAAAACGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCC TTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTAT AGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCG ACGGATAACAGCATATCTAAAAAAAAAAAAAGCCCGGATAGCTCAGTCGGTAGAGCAG CGGCCTATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGG CGGCCGCATTCGAGGCCGCGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCA

SEQ ID NO. 30: pML75_EGFP-CVB3-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGAAACAAAAAACAAAAAAAACAAAAAAAAAA CCAAAAAAACAAAACACAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTG CCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGC GAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACC GGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAG TGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGC CCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGA CCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAG GGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTAC AACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACT TCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGC AGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCA CCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGG AGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATTAAA ACAGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGTATCA CGGTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTAACAC ACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGCACTTCTGTTA CCCCGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGTTATC CGGCCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCA GCACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGT GGCGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACA GACATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGAATGCGG CTAATCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGTCGTAACGGG CAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACTG GCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCATCCG GTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAAGAG GTTAAAACATTACAATTCATTGTTAAGTTGAATACAGCAAAAAAAAACAAAAAACAAAAC GGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAG AAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGG CAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 31: pML76_EGFP-CVB3-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGACAAAAAAAACAAAAAAAAAACCAAAAAAA CAAAACACAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTG GTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGA GGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCT GCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAG CCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGG CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGC CGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGA CTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCA CAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATC CGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACC CCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCC GCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTG ACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATTAAAACAGCCTG TGGGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGTATCACGGTACCTT

	TGTGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTAACACACACCGATC AACAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGCACTTCTGTTACCCCGGACT GAGTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACT ACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCC CAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGGCGGTGGC TGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGACATGGTG CGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAA CTGCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGTCGTAACGGGCAACTCTGCA GCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACTGGCTGCTTATG GTGACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCATCCGGTGACTAATAG AGCTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAAGAGGTTAAAACATT ACAATTCATTGTTAAGTTGAATACAGCAAAAAAAAACAAAAAACAAAACGGCTATTATGC GTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAG TGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGC CAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 32: pML77_EGF P-CVB3-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGACAAAAAAAAAACCAAAAAAACAAAACACA ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCT GGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGCGATG CCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGC CCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACC CCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCA GGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAA GTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGA GGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTAT ATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCACAACA TCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGC GACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGC AAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCC GGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATTAAAACAGCCTGTGGGTTGAT CCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCT GTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTAACACACACCGATCAACAGTCAG CGTGGCACACCAGCCACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAA TAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAA AACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGA TCAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGC GGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTC TATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGC ACACACCCTCAAGCCAGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCG ACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTG AGAGATCGTTACCATATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATA TATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATT GTTAAGTTGAATACAGCAAAAAAAAACAAAAAACAAAACGGCTATTATGCGTTACCGGC GAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTC TCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAA GTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 33:	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA

pML78_EGFP-CVB3-	AGCTGCAAGAGAATGAAAATCCGTCGCCGGAAACGCAATAGCCGACAAAAAACAAAAA AAACAAAAAAAAAACCAAAAAAACAAAACACAATGGTGAGCAAGGGCGAGGAGCTGTT CACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTT CAGCGTGTCTGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTT CATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGAC CTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTC AAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGAC GGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCG CATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCT GGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGC ATCAAGGCGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCC GACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAAC CACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCAC ATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTG TACAAGTAATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCGCTAG CACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTGTAAC TTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGATCA AGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGGAGAA AGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAG AGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCC ACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGG ACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGC CCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGGCAGTG TGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTT TATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGG ATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTT AGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACAGCAAAAAAAAAC AAAAAACAAAACGGCTATTATGCGTTACCGGCGACGGACTTAAATAATTGAGCCTTAAA GAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGAC AAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 34: pML79_EGFP-CVB3-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGACAAAAAACAAAAAAAACAAAAAAAAAACC AAAAAAACAAAACACAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCC CATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGA GGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGG CAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTG CTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCC GAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACC CGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGG CATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAA CAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTC AAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAG AACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACC CAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAG TTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATTAAAAC AGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGTATCACG GTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTAACACAC ACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGCACTTCTGTTACC CCGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGTTATCCG GCCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGC

	ACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGG CGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGA CATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGAATGCGGCTA ATCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGTCGTAACGGGCAA CTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACTGGCT GCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCATCCGGTGA CTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAAGAGGTTA AAACATTACAATTCATTGTTAAGTTGAATACAGCAAAAAAAAAACAAAAAACAAAACAAAA AAAAAAAAAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACC AGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGT CCAGGGTTCAAGTCCCTGTTCGGGCGCCACTGCAGAAAAAAAAAAAAAGGCTATTATGC GTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAG TGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGC CAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 35: pML80_EGF P-CVB3-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGACAAAAAACAAAAAAAACAAAAAAAAAACC AAAAAAACAAAACACAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCC CATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGA GGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGG CAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTG CTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCC GAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACC CGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGG CATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAA CAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTC AAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAG AACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACC CAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAG TTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATTAAAAC AGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGTATCACG GTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTAACACAC ACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGCACTTCTGTTACC CCGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGTTATCCG GCCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGC ACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGG CGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGA CATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGAATGCGGCTA ATCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGTCGTAACGGGCAA CTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACTGGCT GCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCATCCGGTGA CTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAAGAGGTTA AAACATTACAATTCATTGTTAAGTTGAATACAGCAAAAAAAAAAAAAAAAAGCCCGGATAG CTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCATGCAAGTGCGTA AGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGGTTCAAGTCCCTGT TCGGGCGCCACTGCAGAAAAAAAAAAAAAAAAAACAAAAAACAAAACGGCTATTATGC GTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAG TGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGC CAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG

SEQ ID NO. 36: pML81_EGFP-CVB3-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGCAAAAAACAAAAAAAACAAAAAAAAAACCA AAAAAACAAAACACAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCC ATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAG GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGC AAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGC TTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCG AAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCC GCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGC ATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACA GCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAA GATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAA CACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCA GTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTT CGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATTAAAACA GCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGTATCACGG TACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTAACACACA CCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGCACTTCTGTTACCC CGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGTTATCCGG CCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCA CTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGGC GGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGAC ATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGAATGCGGCTAA TCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGTCGTAACGGGCAAC TCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACTGGCTG CTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCATCCGGTGAC TAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAAGAGGTTAA AACATTACAATTCATTGTTAAGTTGAATACAGCAAAAAAAAACAAAAAACAAAACGGCTA TTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTC TTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCC TGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACGAAAAAAAAAAA AAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCA TGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGG TTCAAGTCCCTGTTCGGGCGCCA
SEQ ID NO. 37: pML82_EGFP-CVB3-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGCAAAAAACAAAAAAAACAAAAAAAAAACCA AAAAAACAAAACACAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCC ATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAG GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGC AAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGC TTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCG AAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCC GCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGC ATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACA GCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAA GATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAA CACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCA

	GTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTT CGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATTAAAACA GCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGTATCACGG TACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTAACACACA CCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGCACTTCTGTTACCC CGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGTTATCCGG CCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCA CTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGGC GGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGAC ATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGAATGCGGCTAA TCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGTCGTAACGGGCAAC TCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACTGGCTG CTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCATCCGGTGAC TAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAAGAGGTTAA AACATTACAATTCATTGTTAAGTTGAATACAGCAAAAAAAAACAAAAAACAAAACGGCTA TTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTC TTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCC TGAGCCAAGCCGAAGTAGTAATTAGTAAAAAAAAAAAAAAAGCCCGGATAGCTCAGTC GGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGT CGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGGTTCAAGTCCCTGTTCGGGC GCCACTGCAGAAAAAAAAAAAAGACCAGTGGACAATCGACG
SEQ ID NO. 38: pML83_tS1m -EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACAAAAAAAAAAAAAAGCCCGG ATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCATGCAAGTG CGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGGTTCAAGTCC CTGTTCGGGCGCCACTGCAGAAAAAAAAAAAAACGTCGATTGTCCACTGGTCAACAATA GATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTAACGTCAA GACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAAAGCTGCA AGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTCCACCCCC ACGCCGGAAACGCAATAGCCGCAAAAAACAAAAAAAACAAAAAAAAAAACCAAAAAAACA AAACACAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGT CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGG GCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGC CCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCC GCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTA CGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGA GGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTT CAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAA CGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGC CACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCC ATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCC CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACC GCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATTAAAACAGCCTGTG GGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGTATCACGGTACCTTTG TGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTAACACACACCGATCAA CAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGA GTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTAC TTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCA GTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTG CGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGACATGGTGCG AAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACT GCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGC GGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGT GACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCATCCGGTGACTAATAGAG

	CTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAAGAGGTTAAAACATTAC AATTCATTGTTAAGTTGAATACAGCAAAAAAAAACAAAAAACAAAACGGCTATTATGCGT TACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTG GATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAA GCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 39: pML84_tS1m -EGFP-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AAAAAAAAAAAAAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCC GACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCC GCGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCACTGCAGAAAAAAAAAAAAAAACAATA GATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTAACGTCAA GACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAAAGCTGCA AGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTCCACCCCC ACGCCGGAAACGCAATAGCCGCAAAAAACAAAAAAAACAAAAAAAAAACCAAAAAAACA AAACACAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGT CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGG GCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGC CCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCC GCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTA CGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGA GGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTT CAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAA CGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGC CACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCC ATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCC CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACC GCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATTAAAACAGCCTGTG GGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGTATCACGGTACCTTTG TGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTAACACACACCGATCAA CAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGA GTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTAC TTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCA GTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTG CGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGACATGGTGCG AAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACT GCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGC GGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGT GACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCATCCGGTGACTAATAGAG CTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAAGAGGTTAAAACATTAC AATTCATTGTTAAGTTGAATACAGCAAAAAAAAACAAAAAACAAAACGGCTATTATGCGT TACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTG GATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAA GCCGAAGTAGTAATTAGTAAAAAAAAAAAAAAAGCCCGGATAGCTCAGTCGGTAGAGC AGCGGCCTATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTC GGCGGCCGCATTCGAGGCCGCGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCACTGC AGAAAAAAAAAAAAGACCAGTGGACAATCGACG
SEQ ID NO. 40:	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACAAAAAAAAAAAAAGCCCGG ATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCATGCAAGTG CGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGGTTCAAGTCC CTGTTCGGGCGCCACTGCAGAAAAAAAAAAAAACGTCGATTGTCCACTGGTCAACAATA GATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTAACGTCAA GACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAAAGCTGCA AGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTCCACCCCC

pML85_tS1m-EGFP-	ACGCCGGAAACGCAATAGCCGCAAAAAACAAAAAAAACAAAAAAAAAAACCAAAAAAACA AAACACAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGT CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGG GCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGC CCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCC GCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTA CGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGA GGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTT CAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAA CGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGC CACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCC ATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCC CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACC GCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATTAAAACAGCCTGTG GGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGTATCACGGTACCTTTG TGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTAACACACACCGATCAA CAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGA GTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTAC TTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCA GTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTG CGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGACATGGTGCG AAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACT GCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGC GGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGT GACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCATCCGGTGACTAATAGAG CTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAAGAGGTTAAAACATTAC AATTCATTGTTAAGTTGAATACAGCAAAAAAAAACAAAAAACAAAACGGCTATTATGCGT TACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTG GATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAA GCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACGAAAAAAAAAAAAAGCCCGG ATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCATGCAAGTG CGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGGTTCAAGTCC CTGTTCGGGCGCCA
SEQ ID NO. 41: pML86_EGFP-tS1m-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGCAAAAAACAAAAAAAACAAAAAAAAAACCA AAAAAACAAAACACAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCC ATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAG GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGC AAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGC TTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCG AAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCC GCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGC ATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACA GCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAA GATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAA CACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCA GTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTT CGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAAAAAAAAA AAAAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAAT CATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAG

	GGTTCAAGTCCCTGTTCGGGCGCCACTGCAGAAAAAAAAAAAATTAAAACAGCCTGTG GGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGTATCACGGTACCTTTG TGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTAACACACACCGATCAA CAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGCACTTCTGTTACCCCGGACTGA GTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTAC TTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCCA GTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTG CGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGACATGGTGCG AAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACT GCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGTCGTAACGGGCAACTCTGCAGC GGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGT GACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCATCCGGTGACTAATAGAG CTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAAGAGGTTAAAACATTAC AATTCATTGTTAAGTTGAATACAGCAAAAAAAAACAAAAAACAAAACGGCTATTATGCGT TACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTG GATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAA GCCGAAGTAGTAATTAGTAAGACCAGTGGACAATCGACG
SEQ ID NO. 42: pML87_EGFP-tS1m-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCACGCCGGAAACGCAATAGCCGCAAAAAACAAAAAAAACAAAAAAAAAACCA AAAAAACAAAACACAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCC ATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAG GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGC AAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGC TTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCG AAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCC GCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGC ATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACA GCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAA GATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAA CACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCA GTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTT CGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAACAAAAAAC AAAAAAAACAAAAAAAAAACCAAAAAAACAAAACACAAAAAAAAAAAAAAGCCCGGATA GCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCATGCAAGTGCGT AAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGGTTCAAGTCCCTG TTCGGGCGCCACTGCAGAAAAAAAAAAAACAAAAAACAAAAAAAACAAAAAAAAACCA AAAAAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGG CGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAA CTGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTT TTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCGGTTGA AGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCGTGGAA GTTGCAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGC ATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAAC CCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTC CTCCGGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCCAGAG GGCAGTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTG TTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAG CTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTAT ACCACTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACAGCAAA AAAAAACAAAAAACAAAACGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAAT

	AATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAA ATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAGACCAGT GGACAATCGACG
SEQ ID NO. 43: pML89_tS1m -CVB3-	TAATACGACTCACTATAGGGGATCCGGGAGACCCTCGACCGTCGATTGTCCACTGGTC AAAAAAAAAAAAAGCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCC GACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCC GCGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCACTGCAGAAAAAAAAAAAAAAACAATA GATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTAACGTCAA GACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAAAGCTGCA AGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTCCACCCCC ACGCCGGAAACGCAATAGCCGAAAAACAAAAAACAAAAAAAACAAAAAAAAAACCAAAA AAACAAAACACATTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCGC TAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTGT AACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGA TCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGGA GAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTTG CAGAGTGTTTCGCTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATTC CCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCAT GGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCC GGCCCCTGAATGCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGGCA GTGTGTCGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCA TTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATT GGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCA CTTAGCTTGAAAGAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACAGCAAAATGG GAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAAGCCCACCGA GAACAACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGACCACGGATCT CGATGCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGTGCTCAAAGA GATGGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGATCTGCCTGTC CCACATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGCCACACCTAC GAAGGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGTCGACATTCCT GAGATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCACAGGTCGATC TGTGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTGCAGTGTTCTGA CCTGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCAAGATCCAGGG CCAGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAAAAAAAACAAAAAACAAAACG GCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGA AATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGC AATCCTGAGCCAAGCCGAAGTAGTAATTAGTAAAAAAAAAAAAAAAGCCCGGATAGCTC AGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGA TAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGGTTCAAGTCCCTGTTCG GGCGCCACTGCAGAAAAAAAAAAAAGACCAGTGGACAATCGACG

Table 3. DNA sequences encoding linear RNA precursor and circular RNA elements

SEQ ID NO / Description	**SEQUENCE**

SEQ ID NO. 44: T7 promoter	TAATACGACTCACTATAGG
SEQ ID NO. 45: 5' external homology arm	CGTCGATTGTCCACTGGTC
SEQ ID NO. 46: 5' internal homology arm	CGCCGGAAACGCAATAGCCG
SEQ ID NO. 47: 3' internal homology arm	GGCTATTATGCGTTACCGGCG
SEQ ID NO. 48: 3' external homology arm	GACCAGTGGACAATCGACG

SEQ ID NO. 49: Ana2.0 PIE 3'	AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTC CACCCCCA
SEQ ID NO. 50: Ana2.0 PIE 5'	AGACGCTACGGACTTAAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCT CAAACTCAGGGAAACCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAG TAGTAATTAGTAA
SEQ ID NO. 51: CVB3 IRES	TTAAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCATTGGGCGCTAGCACTCTGGT ATCACGGTACCTTTGTGCGCCTGTTTTATACCCCCTCCCCCAACTGTAACTTAGAAGTA ACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGATCAAGCACTTCT GTTACCCCGGACTGAGTATCAATAGACTGCTCACGCGGTTGAAGGAGAAAGCGTTCGT TATCCGGCCAACTACTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCG CTCAGCACTACCCCAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGGCGA CCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTA ATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGAAT GCGGCTAATCCTAACTGCGGAGCACACACCCTCAAGCCAGAGGGCAGTGTGTCGTAA CGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTAT ACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCAT CCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAA GAGGTTAAAACATTACAATTCATTGTTAAGTTGAATACAGCAAA
SEQ ID NO. 52: S1m aptamer	ATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCC GCAT
SEQ ID NO. 53: 4xS1m aptamer	ATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCC GCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAATCATGCA AGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATCTGCTGGGTAGCTGTGAACCGTA GAAAATGCGGCCGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGTCGGCG GCCGCATCTGCTGGGAAGCTACGATCCGTAGAAAATGCGGCCGCCGACCAGAATCAT GCAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCAT

SEQ ID NO. 54: tRNA-S1m aptamer	GCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCTATGCGGCCGCCGACCAGAATCATG CAAGTGCGTAAGATAGTCGCGGGTCGGCGGCCGCATTCGAGGCCGCGTCCAGGGTT CAAGTCCCTGTTCGGGCGCCA
SEQ ID NO. 55: EGFP	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCT GGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCTGGCGAGGGCGAGGGCGATG CCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGC CCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACC CCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCA GGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAA GTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGA GGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTAT ATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCACAACA TCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGC GACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGC AAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCC GGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA
SEQ ID NO. 56: hGLuc	ATGGGAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCCAAGCCCA CCGAGAACAACGAAGACTTCAACATCGTGGCCGTGGCCAGCAACTTCGCGACCACGG ATCTCGATGCTGACCGCGGGAAGTTGCCCGGCAAGAAGCTGCCGCTGGAGGTGCTCA AAGAGATGGAAGCCAATGCCCGGAAAGCTGGCTGCACCAGGGGCTGTCTGATCTGCC TGTCCCACATCAAGTGCACGCCCAAGATGAAGAAGTTCATCCCAGGACGCTGCCACAC CTACGAAGGCGACAAAGAGTCCGCACAGGGCGGCATAGGCGAGGCGATCGTCGACAT TCCTGAGATTCCTGGGTTCAAGGACTTGGAGCCCATGGAGCAGTTCATCGCACAGGTC GATCTGTGTGTGGACTGCACAACTGGCTGCCTCAAAGGGCTTGCCAACGTGCAGTGTT CTGACCTGCTCAAGAAGTGGCTGCCGCAACGCTGTGCGACCTTTGCCAGCAAGATCC AGGGCCAGGTGGACAAGATCAAGGGGGCCGGTGGTGACTAA
SEQ ID NO. 57: 5' UTR	AGAGCGGCCGCTTTTTCAGCAAGATTAAGCCCAGGGCAGAGCCATCTATTGCTTACAT TTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACC
SEQ ID NO. 58: 3' UTR	AGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTA CTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACA TTTATTTTCATTGCAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCC CTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTG CCTAATAAAAAACATTTATTTTCATTGC
SEQ ID NO. 59: 5' spacer	AAAAACAAAAAACAAAAAAAAACAAAAAAAAAACCAAAAAAACAAAACACA

poly(A) site 1 50 bp	
SEQ ID NO. 60: 3'spacer poly(A) site 2 19 bp	AAAAAACAAAAAACAAAAC
SEQ ID NO. 61: Ana2.0 3' intron	AACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTA ACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAA AGCTGCAAGAGAATG
SEQ ID NO. 62: Ana2.0 3' exon	AAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTCCACCCCCA
SEQ ID NO. 63: Ana2.0 5' intron	AAATAATTGAGCCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGAAAC CTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGAAGTAGTAATTAGTAA
SEQ ID NO. 64: Ana2.0 5' exon	AGACGCTACGGACTT

Table 4. RNA sequences of linear RNA precursor and circular RNA elements

SEQ ID NO / Description	**SEQUENCE**
SEQ ID NO. 68: T7 promoter	UAAUACGACUCACUAUAGG
SEQ ID NO. 69: 5' external homology arm	CGUCGAUUGUCCACUGGUC
SEQ ID NO. 70: 5' internal homology arm	CGCCGGAAACGCAAUAGCCG
SEQ ID NO. 71: 3' internal homology arm	GGCUAUUAUGCGUUACCGGCG
SEQ ID NO. 72:	GACCAGUGGACAAUCGACG

3' external homology arm	
SEQ ID NO. 73: Ana2.0 PIE 3'	AACAAUAGAUGACUUACAACUAAUCGGAAGGUGCAGAGACUCGACGGGAGCUACCC UAACGUCAAGACGAGGGUAAAGAGAGAGUCCAAUUCUCAAAGCCAAUAGGCAGUAG CGAAAGCUGCAAGAGAAUGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAG UCCCUCCACCCCCA
SEQ ID NO. 74: Ana2.0 PIE 5'	AGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCU CUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCG AAGUAGUAAUUAGUAA
SEQ ID NO. 75: CVB3 IRES	UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUG GUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCCAACUGUAACUUAG AAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGC ACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAA GCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA GAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUC CCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCA UGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCC UCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAG GGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCG UGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACC AUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUG UUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGU UGAAUACAGCAAA
SEQ ID NO. 76:	AGAGCGGCCGCUUUUUCAGCAAGAUUAAGCCCAGGGCAGAGCCAUCUAUUGCUUAC AUUUGCUUCUGACACAACUGUGUUCACUAGCAACCUCAAACAGACACC

5' UTR	
SEQ ID NO. 77: 3' UTR	AGCUCGCUUUCUUGCUGUCCAAUUUCUAUUAAAGGUUCCUUUGUUCCCUAAGUCCA ACUACUAAACUGGGGGGAUAUUAUGAAGGGCCUUGAGCAUCUGGAUUCUGCCUAAUA AAAAACAUUUAUUUUCAUUGCAGCUCGCUUUCUUGCUGUCCAAUUUCUAUUAAAGGU UCCUUUGUUCCCUAAGUCCAACUACUAAACUGGGGGGAUAUUAUGAAGGGCCUUGAG CAUCUGGAUUCUGCCUAAUAAAAAACAUUUAUUUUCAUUGC
SEQ ID NO. 78: 5' spacer poly(A) site 1 50 bp	AAAAACAAAAAACAAAAAAAACAAAAAAAAAACCAAAAAAACAAAACACA
SEQ ID NO. 79: 3'spacer poly(A) site 2 19 bp	AAAAAACAAAAAACAAAAC
SEQ ID NO. 80: Ana2.0 3' intron	AACAAUAGAUGACUUACAACUAAUCGGAAGGUGCAGAGACUCGACGGGAGCUACCC UAACGUCAAGACGAGGGUAAAGAGAGAGUCCAAUUCUCAAAGCCAAUAGGCAGUAG CGAAAGCUGCAAGAGAAUG
SEQ ID NO. 81: Ana2.0 3' exon	AAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCA

SEQ ID NO. 82: Ana2.0 5' intron	AAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAA
SEQ ID NO. 83: Ana2.0 5' exon	AGACGCUACGGACUU

CLAIMS

What is claimed is:

1. A circular RNA comprising a protein coding region and at least one RNA aptamer.

2. The circular RNA of claim 1, wherein the at least one RNA aptamer binds to an affinity ligand.

3. The circular RNA of claim 2, wherein the affinity ligand comprises protein A, protein G, streptavidin, glutathione, dextran, a fluorescent molecule, or 6xHis.

4. The circular RNA of claim 2 or 3, wherein the affinity ligand comprises streptavidin.

5. The circular RNA of any one of claims 2-4, wherein the affinity ligand is immobilized on a chromatography resin.

6. The circular RNA of any one of claims 1-5, wherein the RNA aptamer is S1m, Sm, or a derivative or fragment thereof.

7. The circular RNA of any one of claims 1-6, wherein the circular RNA comprises between one to four RNA aptamers.

8. The circular RNA of any one of claims 1-7, wherein the RNA aptamers are identical.

9. The circular RNA of any one of claims 1-7, wherein at least one of the RNA aptamers is distinct.

10. The circular RNA of any one of claims 1-9, wherein the RNA aptamer is synthetically derived.

11. The circular RNA of any one of claims 1-9, wherein the RNA aptamer is a split aptamer or an X-aptamer.

12. The circular RNA of any one of claims 1-9, wherein RNA aptamer is naturally-derived.

13. The circular RNA of any one of claims 1-12, wherein the RNA aptamer is derived from a hairpin RNA, a tRNA, or a riboswitch.

14. The circular RNA of any one of claims 1-12, wherein the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 65 or 66.

15. The circular RNA of any one of claims 1-14, wherein the RNA aptamer is about 30-200 nucleotides in length.

16. The circular RNA of any one of claims 1-15, wherein the RNA aptamer is about 50-200 nucleotides in length.

17. The circular RNA of any one of claims 1-16, wherein the RNA aptamer is not a histone stem-loop.

18. The circular RNA of any one of claims 1-17, wherein the RNA aptamer does not bind eIF4G.

19. The circular RNA of any one of claims 1-18, wherein the RNA aptamer is embedded in an RNA scaffold.

20. The circular RNA of claim 19, wherein the RNA scaffold comprises at least one secondary structure motif.

21. The circular RNA of claim 20, wherein the secondary structure motif is a tetraloop, a pseudoknot, or a stem-loop.

22. The circular RNA of any one of claims 19-21, wherein the RNA scaffold comprises at least one tertiary structure.

23. The circular RNA of claim 22, wherein the secondary structure motif and/or tertiary structure are nuclease resistant.

24. The circular RNA of any one of claims 19-23, wherein the RNA scaffold comprises a transfer RNA (tRNA).

25. The circular RNA of claim 24, wherein the RNA aptamer is embedded in a tRNA hairpin loop of the tRNA.

26. The circular RNA of claim 24, wherein the RNA aptamer is embedded in a tRNA anticodon loop of the tRNA.

27. The circular RNA of claim 24, wherein the RNA aptamer is embedded in a tRNA D loop of the tRNA.

28. The circular RNA of claim 24, wherein RNA aptamer embedded tRNA comprises the nucleotide sequence of SEQ ID NO: 67.

29. The circular RNA of any one of claims 1-28, wherein an internal ribosome entry site (IRES) is positioned at the 5' end of the protein coding region.

30. The circular RNA of any one of claims 1-28, wherein an IRES is positioned at the 3' end of the protein coding region.

31. The circular RNA of any one of claims 1-30, wherein the IRES is derived from Coxsackievirus B3 (CVB3), Encephalomyocarditis virus (EMCV), Dicistroviruses, hepatitis C virus (HCV), poliovirus (PV), enterovirus 71 (EV71), human rhinovirus (HRV), foot-and-mouth disease virus (FMDV), or synthetic IRES.

32. The circular RNA of any one of claims 1-31, wherein the IRES comprises a polynucleotide sequence of SEQ ID NO: 75.

33. The circular RNA of any one of claims 1-32, wherein the protein coding region encodes at least one polypeptide or peptide.

34. The circular RNA of claim 33, wherein the polypeptide is a biologically active polypeptide, a therapeutic polypeptide, or an antigenic polypeptide.

35. The circular RNA of any one of claims 1-34, wherein the circular RNA comprises at least one 5' internal homology arm and at least one 3' internal homology arm.

36. The circular RNA of claim 35, wherein the 5' internal homology arm is about 5 to about 50 nucleotides in length.

37. The circular RNA of claim 35 or 36, wherein the 5' internal homology arm comprises the nucleotide sequence of SEQ ID NO: 70.

38. The circular RNA of any one of claims 35-37, wherein the 3' internal homology arm is about 5 to about 50 nucleotides in length.

39. The circular RNA of any one of claims 35-38, wherein the 3' internal homology arm comprises the nucleotide sequence of SEQ ID NO: 71.

40. The circular RNA of any one of claims 1-39, wherein the circular RNA comprises at least one 3' exon element.

41. The circular RNA of claim 40, wherein the 3' exon element comprises the nucleotide sequence of SEQ ID NO: 81.

42. The circular RNA of any one of claims 1-41, wherein the circular RNA comprises at least one 5' exon element.

43. The circular RNA of claim 42, wherein the 5' exon element comprises the nucleotide sequence of SEQ ID NO: 83.

44. The circular RNA of any one of claims 1-43, wherein the circular RNA comprises at least one spacer sequence.

45. The circular RNA of claim 44, wherein the spacer sequence is about 5 to about 75 nucleotides in length.

46. The circular RNA of claim 44 or 45, wherein the spacer sequence comprises the nucleotide sequence of SEQ ID NO: 78 or 79.

47. The circular RNA of any one of claims 44-46, wherein the spacer sequence is positioned at one or both of a 5' end and 3' end of any one of the following elements: the protein coding region, the IRES, the 5' internal homology arm, the 3' internal homology arm, the 5' exon element, and the 3' exon element.

48. The circular RNA of any one of claims 44-47, wherein the circular RNA comprises the following elements, from 5' to 3': a) the 3' exon element, b) the 5' internal homology arm, c) the spacer sequence, d) the IRES, e) the protein coding region, f) the spacer sequence, g) the 3' internal homology arm, and h) the 5' exon element.

49. The circular RNA of any one of claims 44-47, wherein the circular RNA comprises the following elements, from 5' to 3': a) the 3' exon element, b) the 5' internal homology arm, c) the spacer sequence, d) the protein coding region, e) the IRES, f) the spacer sequence, g) the 3' internal homology arm, and h) the 5' exon element.

50. The circular RNA of claim 48 or 49, wherein the at least one RNA aptamer is positioned at a 5' end or a 3' end of any one of elements a)-h).

51. The circular RNA of any one of claims 1-50, wherein the circular RNA contains at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or at least one polyadenylation (polyA) sequence.

52. The circular RNA of claim 51, wherein the 5' UTR, the 3' UTR, and/or the polyA sequence are spacer sequences.

53. The circular RNA of any one of claims 44-52, wherein the at least one RNA aptamer is positioned: a) before the 3' exon element, b) between the 3' exon element and the 5' internal

homology arm, c) between the 5' internal homology arm and the 5' spacer sequence, d) between the 5' spacer sequence and the IRES, e) between the protein coding region and the 3' spacer sequence, f) between the 3' spacer sequence and the 3' internal homology arm, g) between the 3' internal homology arm and the 5' exon element, h) after the 5' exon element, i) between the 3' exon and the IRES, and/or j) between the IRES and the 5' exon element.

54. The circular RNA of any one of claims 44-52, wherein the at least one RNA aptamer is positioned: a) before the 3' exon element, b) between the 3' exon element and the 5' internal homology arm, c) between the 5' internal homology arm and the 5' spacer sequence, d) between the 5' spacer sequence and the protein coding region, e) between the IRES and the 3' spacer sequence, f) between the 3' spacer sequence and the 3' internal homology arm, g) between the 3' internal homology arm and the 5' exon element, h) after the 5' exon element, i) between the 3' exon and the protein coding region, and/or j) between the protein coding region and the 5' exon element.

55. The circular RNA of any one of claims 1-54, wherein the circular RNA comprises at least one chemical modification.

56. The circular RNA of claim 55, wherein the chemical modification is pseudouridine, N1-methylpseudouridine, 2-thiouridine, 4'-thiouridine, 5- methylcytosine, 2-thio-l-methyl-1-deaza-pseudouridine, 2-thio-l-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-l-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methyluridine, 5-methyluridine, 5-methoxyuridine, 2'-O-methyl uridine, or N6-methyladenosine.

57. The circular RNA of claim 55, wherein the chemical modification is pseudouridine, N1-methylpseudouridine, 5-methylcytosine, 5- methoxyuridine, N6-methyladenosine or a combination thereof.

58. The circular RNA of claim 55, wherein the chemical modification is N1-methylpseudouridine.

59. A linear precursor RNA comprising at least a self-splicing ribozyme and a protein coding region, wherein the linear precursor RNA comprises at least one RNA aptamer.

60. The linear precursor RNA of claim 59, wherein the at least one RNA aptamer binds to an affinity ligand.

61. The linear precursor RNA of claim 60, wherein the affinity ligand comprises protein A, protein G, streptavidin, glutathione, dextran, a fluorescent molecule, or 6xHis.

62. The linear precursor RNA of claim 60 or 61, wherein the affinity ligand comprises streptavidin.

63. The linear precursor RNA of any one of claims 60-62, wherein the affinity ligand is immobilized on a chromatography resin.

64. The linear precursor RNA of any one of claims 59-63, wherein the RNA aptamer is S1m, Sm, or a derivative or fragment thereof.

65. The linear precursor RNA of any one of claims 59-64, wherein the circular RNA comprises between one to four RNA aptamers.

66. The linear precursor RNA of any one of claims 59-65, wherein the RNA aptamers are identical.

67. The linear precursor RNA of any one of claims 59-65, wherein at least one of the RNA aptamers is distinct.

68. The linear precursor RNA of any one of claims 59-67, wherein the RNA aptamer is synthetically derived.

69. The linear precursor RNA of any one of claims 59-67, wherein the RNA aptamer is a split aptamer or an X-aptamer.

70. The linear precursor RNA of any one of claims 59-67, wherein RNA aptamer is naturally-derived.

71. The linear precursor RNA of any one of claims 59-70, wherein the RNA aptamer is derived from a hairpin RNA, a tRNA, or a riboswitch.

72. The linear precursor RNA of any one of claims 59-71, wherein the RNA aptamer comprises the nucleotide sequence of SEQ ID NO: 65 or 66.

73. The linear precursor RNA of any one of claims 59-72, wherein the RNA aptamer is about 30-200 nucleotides in length.

74. The linear precursor RNA of any one of claims 59-73, wherein the RNA aptamer is about 50-200 nucleotides in length.

75. The linear precursor RNA of any one of claims 59-74, wherein the RNA aptamer is not a histone stem-loop.

76. The linear precursor RNA of any one of claims 59-75, wherein the RNA aptamer does not bind eIF4G.

77. The linear precursor RNA of any one of claims 59-76, wherein the RNA aptamer is embedded in an RNA scaffold.

78. The linear precursor RNA of claim 77, wherein the RNA scaffold comprises at least one secondary structure motif.

79. The linear precursor RNA of claim 78, wherein the secondary structure motif is a tetraloop, a pseudoknot, or a stem-loop.

80. The linear precursor RNA of any one of claims 77-79, wherein the RNA scaffold comprises at least one tertiary structure.

81. The linear precursor RNA of claim 80, wherein the secondary structure motif and/or tertiary structure are nuclease resistant.

82. The linear precursor RNA of any one of claims 77-81, wherein the RNA scaffold comprises a transfer RNA (tRNA).

83. The linear precursor RNA of claim 82, wherein the RNA aptamer is embedded in a tRNA hairpin loop of the tRNA.

84. The linear precursor RNA of claim 82, wherein the RNA aptamer is embedded in a tRNA anticodon loop of the tRNA.

85. The linear precursor RNA of claim 82, wherein the RNA aptamer is embedded in a tRNA D loop of the tRNA.

86. The linear precursor RNA of claim 82, wherein RNA aptamer embedded tRNA comprises the nucleotide sequence of SEQ ID NO: 67.

87. The linear precursor RNA of any one of claims 59-86, wherein the self-splicing ribozyme comprises at least two catalytic subunits.

88. The linear precursor RNA of claim 87, wherein the self-splicing ribozyme catalytic subunits derive from either a group I intron or a group II intron RNA transcript or a fragment thereof.

89. The linear precursor RNA of claim 87 or 88, wherein the self-splicing ribozyme catalytic subunits derive from a permuted intron-exon (PIE) sequence from *Cyanobacterium Anabaena* pre-tRNA-Leu gene, T4 phage Td gene, or *Tetrahymena* pre-rRNA.

90. The linear precursor RNA of any one of claims 87-89, wherein the catalytic activity of the two subunits results in a circularized RNA.

91. The linear precursor RNA of any one of claims 59-90, wherein the linear precursor RNA comprises the following elements, from 5' to 3': a) a 5' external homology arm, b) a 3' self-splicing PIE fragment, c) a 5' internal homology arm, d) a 5' spacer sequence, e) an internal ribosome entry site (IRES) f) a protein coding region, g) a 3' spacer sequence, h) a 3' internal homology arm, i) a 5'

self-splicing PIE fragment, and j) a 3' external homology arm, wherein the RNA aptamer is present at one or both of the 5' end or 3' end of any one of elements a)-j).

92. The linear precursor RNA of any one of claims 59-90, wherein the linear precursor RNA comprises the following elements, from 5' to 3': a) a 5' external homology arm, b) a 3' self-splicing PIE fragment, c) a 5' internal homology arm, d) a 5' spacer sequence, e) a protein coding region, f) an IRES, g) a 3' spacer sequence, h) a 3' internal homology arm, i) a 5' self-splicing PIE fragment, and j) a 3' external homology arm, wherein the RNA aptamer is present at one or both of the 5' end or 3' end of any one of elements a)-j).

93. The linear precursor RNA of claims 91 or 92, wherein the 5' external homology arm and the 3' external homology arm are each independently about 5 to about 50 nucleotides in length.

94. The linear precursor RNA of any one of claims 91-93, wherein the 5' external homology arm and the 3' external homology arm comprises the nucleotide sequence of SEQ ID NO: 69 or SEQ ID NO: 72.

95. The linear precursor RNA of any one of claims 91-94, wherein the 5' self-splicing PIE fragment comprises the nucleotide sequence of SEQ ID NO: 74.

96. The linear precursor RNA of any one of claims 91-95, wherein the 5' internal homology arm is about 5 to about 50 nucleotides in length.

97. The linear precursor RNA of any one of claims 91-96, wherein the 5' internal homology arm comprises the nucleotide sequence of SEQ ID NO: 70.

98. The linear precursor RNA of any one of claims 91-97, wherein the 5' spacer and the 3' spacer are each independently about 5 to 75 nucleotides in length.

99. The linear precursor RNA of any one of claims 91-98, wherein the 5' spacer and the 3' spacer comprises the nucleotide sequence of SEQ ID NO: 78 or SEQ ID NO: 79.

100. The linear precursor RNA of any one of claims 91-99, wherein the 3' self-splicing PIE fragment comprises the nucleotide sequence of SEQ ID NO: 73.

101. The linear precursor RNA of any one of claims 91-100, wherein the IRES is derived from Coxsackievirus B3 (CVB3), Encephalomyocarditis virus (EMCV), Dicistroviruses, hepatitis C virus (HCV), poliovirus (PV), enterovirus 71 (EV71), human rhinovirus (HRV), foot-and-mouth disease virus (FMDV), or synthetic IRES.

102. The linear precursor RNA of any one of claims 91-101, wherein the IRES comprises the nucleotide sequence of SEQ ID NO: 75.

103. The linear precursor RNA of any one of claims 59-101, wherein the linear precursor RNA comprises at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or a polyadenylation (polyA) sequence.

104. The linear precursor RNA of any one of claims 59-103, wherein the protein coding region encodes at least one polypeptide.

105. The linear precursor RNA of claim 104, wherein the polypeptide is a biologically active polypeptide, a therapeutic polypeptide, or an antigenic polypeptide.

106. The linear precursor RNA of any one of claims 59-103, wherein the RNA aptamer is a split aptamer comprising a 5' portion and a 3' portion.

107. The linear precursor RNA of claim 106, wherein the 5' portion of the split aptamer is positioned 3' of the 5' exon element and the 3' portion of the split aptamer is positioned 5' of the 3' exon element.

108. The linear precursor RNA of claim 106 or 107, wherein the 5' portion of the split aptamer is positioned 3' of the 3' internal homology arm and the 3' portion of the split aptamer is positioned 5' of the 5' internal homology arm.

109. The linear precursor RNA of any one of claims 106-108, wherein the split aptamer is reformed to a functional aptamer upon circularization of the linear precursor RNA.

110. The linear precursor RNA of any one of claims 91-109, wherein the at least one RNA aptamer is positioned: a) before the 5' external homology arm, b) between the 5' external homology arm and the 3' self-splicing PIE fragment, c) between the 3' self-splicing PIE fragment and the 5' internal homology arm, d) between the 5' internal homology arm and the 5' spacer sequence, e) between the 5' space sequence and the IRES, f) after the protein coding region but before the 3' spacer sequence, g) between the 3' spacer sequence and the 3' internal homology arm, h) between the 3' internal homology arm and the 5' self-splicing PIE fragment, i) between the 5' self-splicing PIE fragment and the 3' external homology arm, and/or j) after the 3' external homology arm.

111. The linear precursor RNA of any one of claims 91-109, wherein the at least one RNA aptamer is positioned: a) before the 5' external homology arm, b) between the 5' external homology arm and the 3' self-splicing PIE fragment, c) between the 3' self-splicing PIE fragment and the 5' internal homology arm, d) between the 5' internal homology arm and the 5' spacer sequence, e) between the 5' space sequence and the protein coding region, f) after the IRES but before the 3' spacer sequence, g) between the 3' spacer sequence and the 3' internal homology arm, h) between the 3' internal homology arm and the 5' self-splicing PIE fragment, i) between the 5' self-splicing PIE fragment and the 3' external homology arm, and/or j) after the 3' external homology arm.

112. The linear precursor RNA of any one of claims 91-109, wherein the linear precursor RNA comprises at least one chemical modification.

113. The linear precursor RNA of claim 112, wherein the chemical modification is pseudouridine, N1-methylpseudouridine, 2-thiouridine, 4'-thiouridine, 5- methylcytosine, 2-thio-l-methyl-1-deaza-pseudouridine, 2-thio-l-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-l-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methyluridine, 5-methyluridine, 5-methoxyuridine, 2'-O-methyl uridine, or N6-methyladenosine..

114. The linear precursor RNA of claim 112, wherein the chemical modification is pseudouridine, N1-methylpseudouridine, 5-methylcytosine, 5- methoxyuridine, N6-methyladenosine, or a combination thereof.

115. The linear precursor RNA of claim 112, wherein the chemical modification is N1-methylpseudouridine.

116. The linear precursor RNA of any one of claims 59-115, wherein the linear precursor RNA is synthesized using *in vitro* transcription (IVT).

117. A circular RNA comprising a protein coding region and at least one RNA aptamer, wherein the circular RNA is formed from the linear precursor RNA of any one of claims 59-116.

118. A circular RNA comprising a protein coding region, wherein the circular RNA is formed from the linear precursor RNA of any one of claims 59-116, and wherein the circular RNA lacks an RNA aptamer.

119. A nucleic acid that encodes the linear precursor RNA of any one of claims 59-116.

120. A vector comprising the nucleic acid of claim 119.

121. A pharmaceutical composition comprising the circular RNA of any one of claims 1-58, 117, or 118, or the linear precursor RNA of any one of claims 59-116.

122. A method of producing a circular RNA, comprising incubating the linear precursor RNA of any one of claims 59-116 under conditions that result in the circularization of the linear precursor RNA.

123. The method of claim 122, wherein the linear precursor RNA is incubated with GTP and Mg2+.

124. The method of claim 122 or 123, wherein the linear precursor RNA is incubated with GTP and Mg2+ for a time sufficient to circularize the linear precursor RNA.

125. The method of claim 123 or 124, wherein the GTP is present at a concentration of about 1 mM to about 15 mM.

126. The method of any one of claims 123-125, wherein the GTP is present at a concentration of about 2 mM.

127. The method of any one of claims 123-126, wherein the Mg2+ is present at a concentration of about 1 mM to about 50 mM.

128. The method of any one of claims 123-127, wherein the Mg2+ is present at a concentration of about 10 mM.

129. A method of producing a plurality of circular RNA molecules, comprising incubating a plurality of linear precursor RNA molecules under conditions that result in the circularization of at least a portion of the linear precursor RNA molecules, wherein each linear precursor RNA molecule comprises the linear precursor RNA of any one of claims 59-116.

130. The method of claim 129, wherein at least about 30% of the linear precursor RNA molecules in the plurality are circularized.

131. A method for purifying a circular RNA, comprising the steps of: (a) contacting a sample comprising the circular RNA of any one of claims 1-58 with an affinity ligand that is immobilized on a chromatography resin, wherein the RNA aptamer comprises binding affinity for the affinity ligand; (b) eluting the circular RNA from the chromatography resin; and (c) purifying the circular RNA from the sample.

132. A method for purifying a linear precursor RNA, comprising the steps of: (a) contacting a sample comprising the linear precursor RNA of any one of claims 59-116 with an affinity ligand that is immobilized on a chromatography resin, wherein the RNA aptamer comprises binding affinity for the affinity ligand; (b) eluting the linear precursor RNA from the chromatography resin; and (c) purifying the linear precursor RNA from the sample.

133. The method of claim 131 or 132, comprising one or more washing steps between the contacting step (a) and the eluting step (b).

134. A method of purifying a circular RNA, comprising the steps of: (a) contacting a sample comprising the circular RNA with an affinity ligand that is immobilized on a chromatography resin; (b) eluting the circular RNA from the chromatography resin; and (c) isolating the circular RNA from the sample, wherein the circular RNA comprises a protein coding region and at least one RNA aptamer, wherein the RNA aptamer comprises binding affinity for the affinity ligand.

135. A method of purifying a linear precursor RNA, comprising the steps of: (a) contacting a sample comprising the linear precursor RNA with an affinity ligand that is immobilized on a chromatography resin; (b) eluting the linear precursor RNA from the chromatography resin; and (c) isolating the linear precursor RNA from the sample, wherein the linear precursor RNA comprises a protein coding region and at least one RNA aptamer, wherein the RNA aptamer comprises binding affinity for the affinity ligand.

136. A method of purifying a circular RNA, comprising the steps of: (a) contacting a sample comprising a plurality of linear precursor RNA molecules and a plurality of circular RNA molecules with an affinity ligand that is immobilized on a chromatography resin; and (b) isolating the circular RNA molecules from the sample, wherein the linear precursor RNA molecules comprise a protein coding region and at least one RNA aptamer and wherein the RNA aptamer comprises binding affinity for the affinity ligand, and wherein the circular RNA molecules lack an RNA aptamer.

137. The method of claim 136, wherein the circular RNA molecules do not bind the affinity ligand.

138. The method of any one of claims 131-137, wherein the circular RNA or linear precursor RNA is greater than or equal to 90% pure.

139. A method of treating or preventing a disease or disorder, comprising administering to a subject in need thereof the pharmaceutical composition of claim 121.

140. A pharmaceutical composition comprising a plurality of circular RNA molecules, wherein at least about 90% of the circular RNA comprise a protein coding region and at least one RNA aptamer.

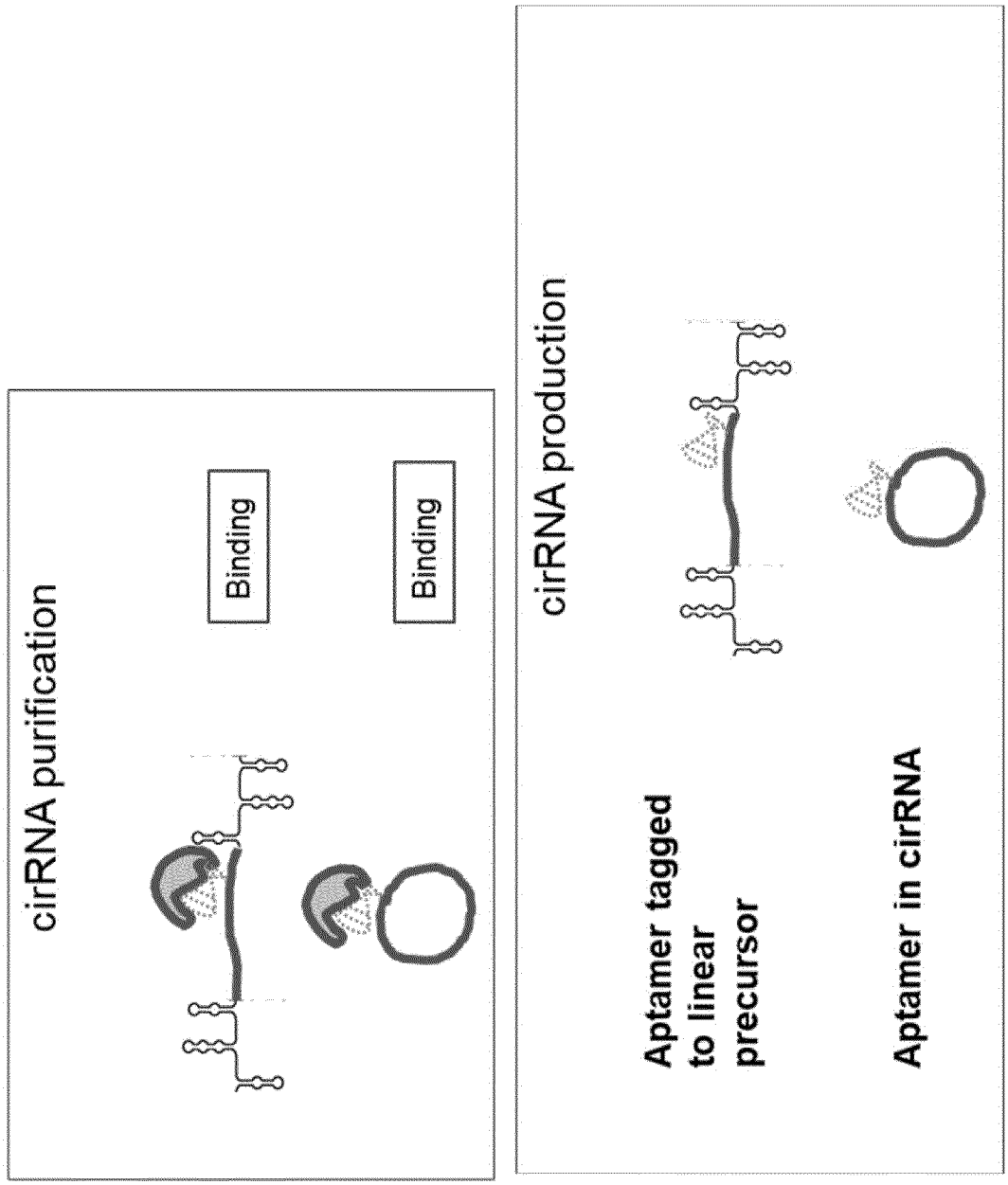
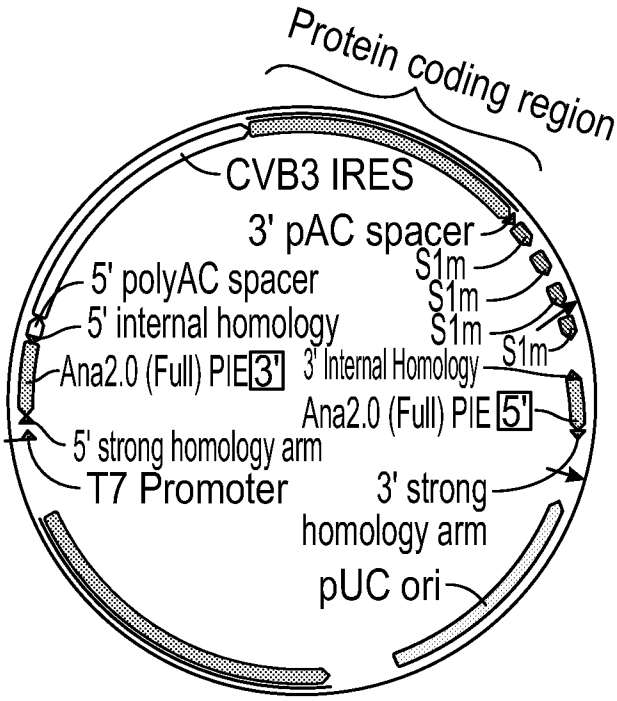
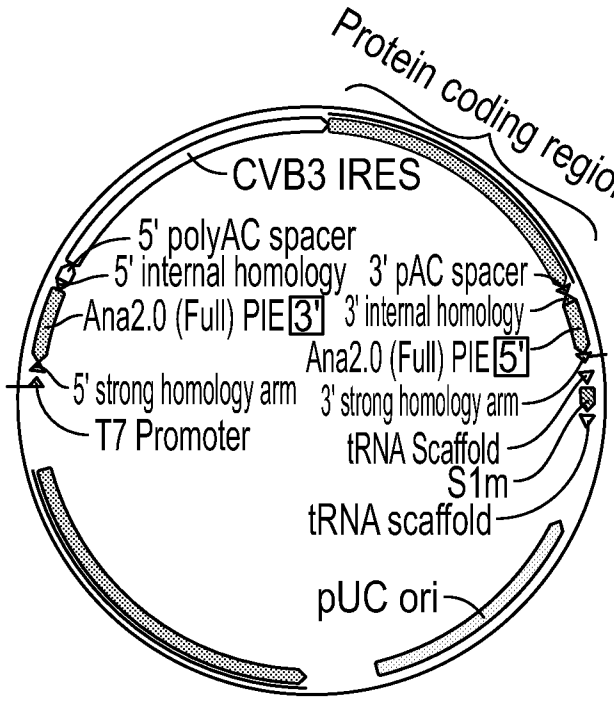

cirRNA purification
Binding
Binding
cirRNA production
Aptamer tagged to linear precursor
Aptamer in cirRNA

FIGURE 1



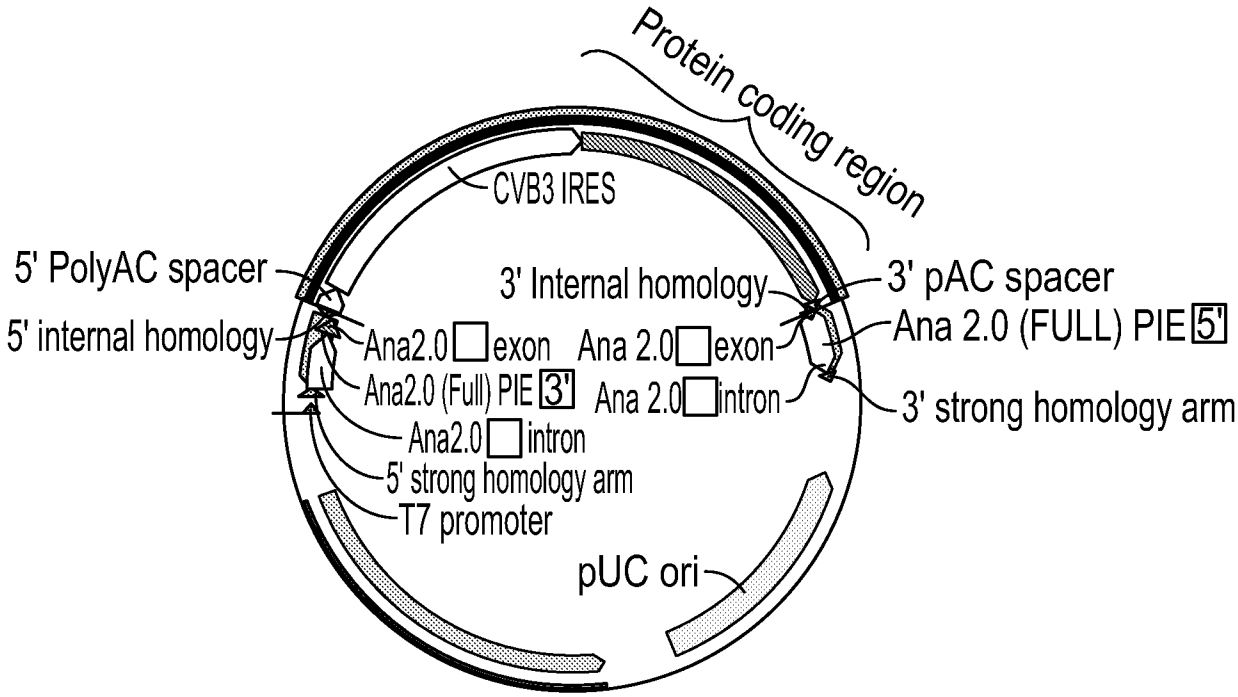
4xS1m

FIG. 2A



tRNA-S1m

FIG. 2B



No aptamer

FIG. 2C

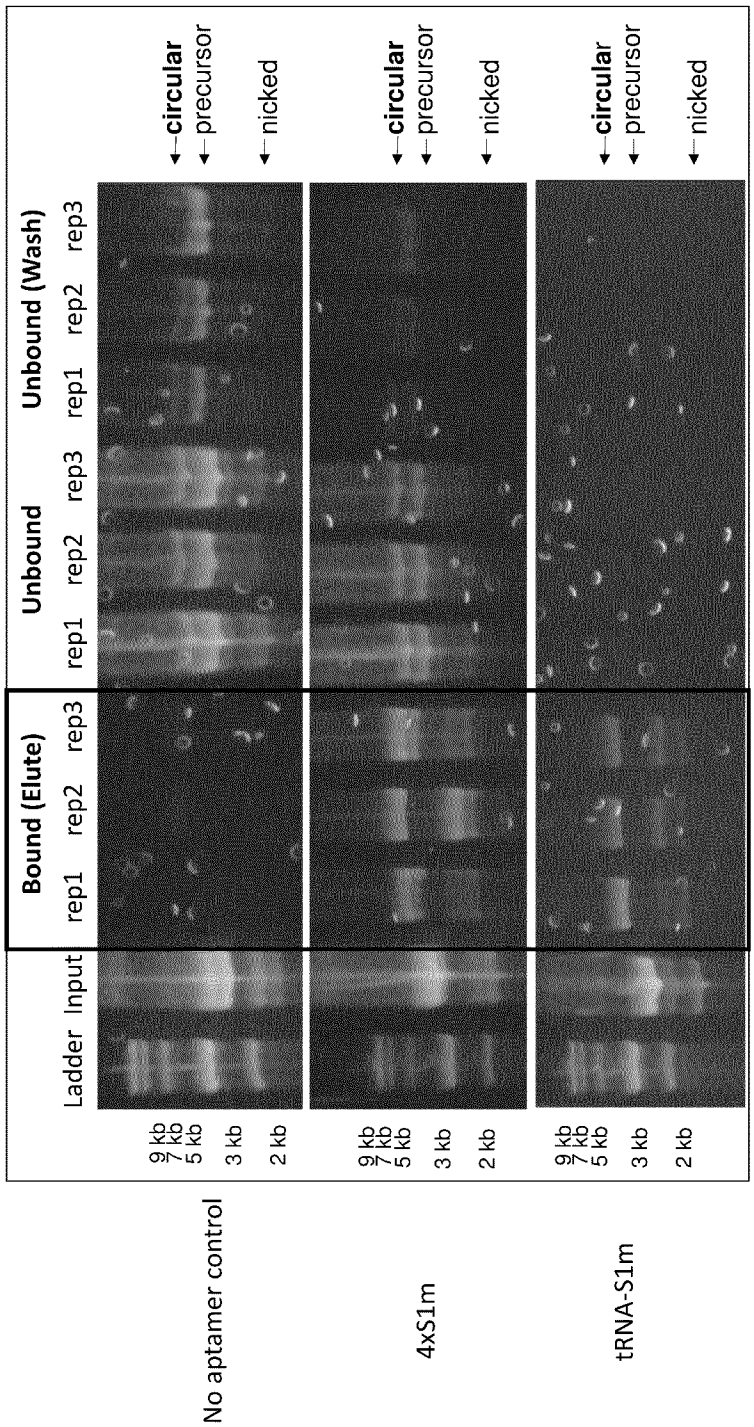

Ladder
Input
Bound (Elute)
rep1
rep2
rep3
Unbound
rep1
rep2
rep3
Unbound (Wash)
rep1
rep2
rep3
9 kb
7 kb
5 kb
3 kb
2 kb
circular
precursor
nicked
No aptamer control
4xS1m
tRNA-S1m

FIGURE 3

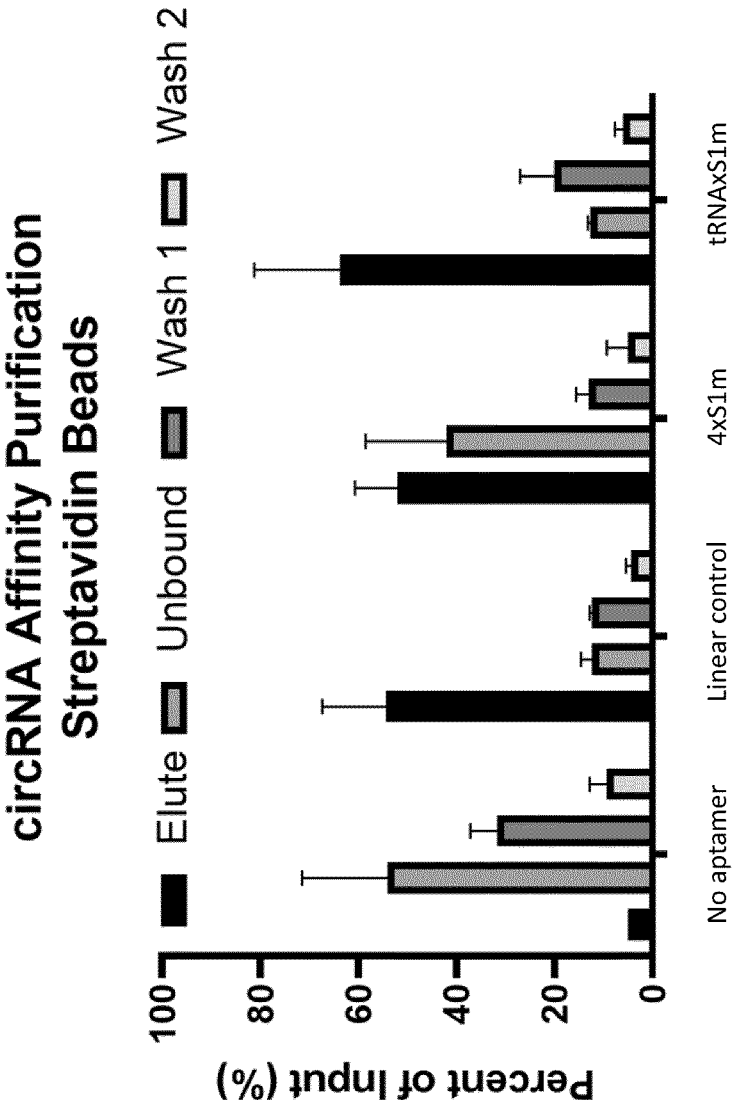

circRNA Affinity Purification
Streptavidin Beads
Elute
Unbound
Wash 1
Wash 2
Percent of Input (%)
100
80
60
40
20
0
No aptamer
Linear control
4xS1m
tRNAxS1m

FIGURE 4

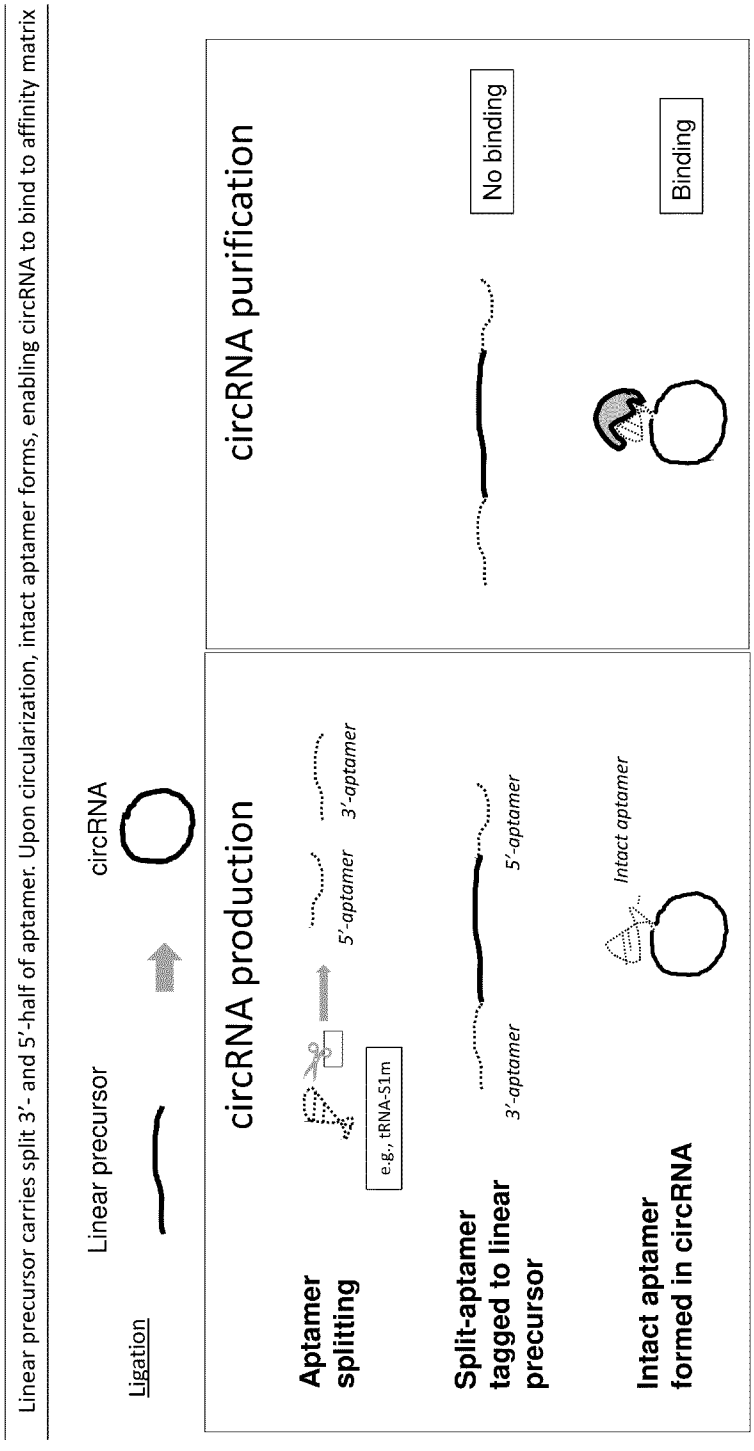

Linear precursor carries split 3'- and 5'-half of aptamer. Upon circularization, intact aptamer forms, enabling circRNA to bind to affinity matrix
Ligation
Linear precursor
circRNA
circRNA production
Aptamer splitting
5'-aptamer
3'-aptamer
e.g., tRNA-S1m
Split-aptamer tagged to linear precursor
3'-aptamer
5'-aptamer
Intact aptamer formed in circRNA
Intact aptamer
circRNA purification
No binding
Binding

FIGURE 5

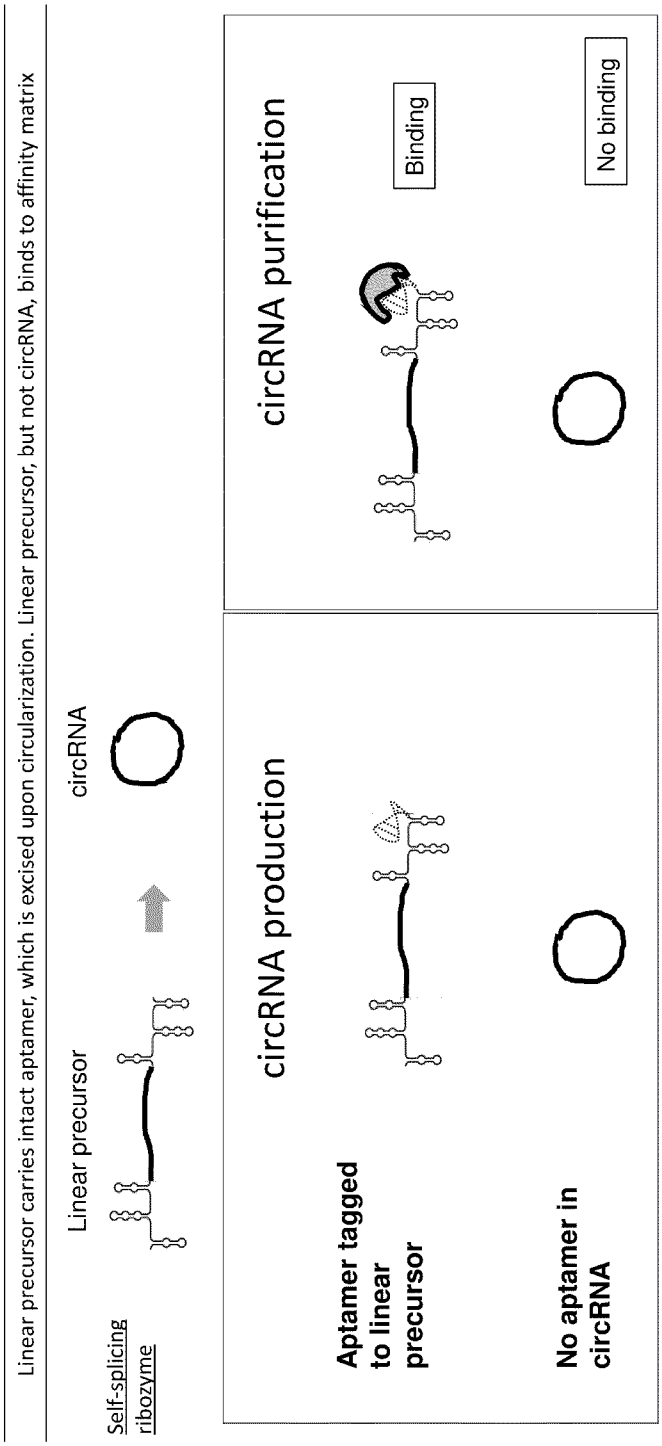

Linear precursor carries intact aptamer, which is excised upon circularization. Linear precursor, but not circRNA, binds to affinity matrix
Linear precursor
circRNA
Self-splicing ribozyme
circRNA production
Aptamer tagged to linear precursor
No aptamer in circRNA
circRNA purification
Binding
No binding

FIGURE 6

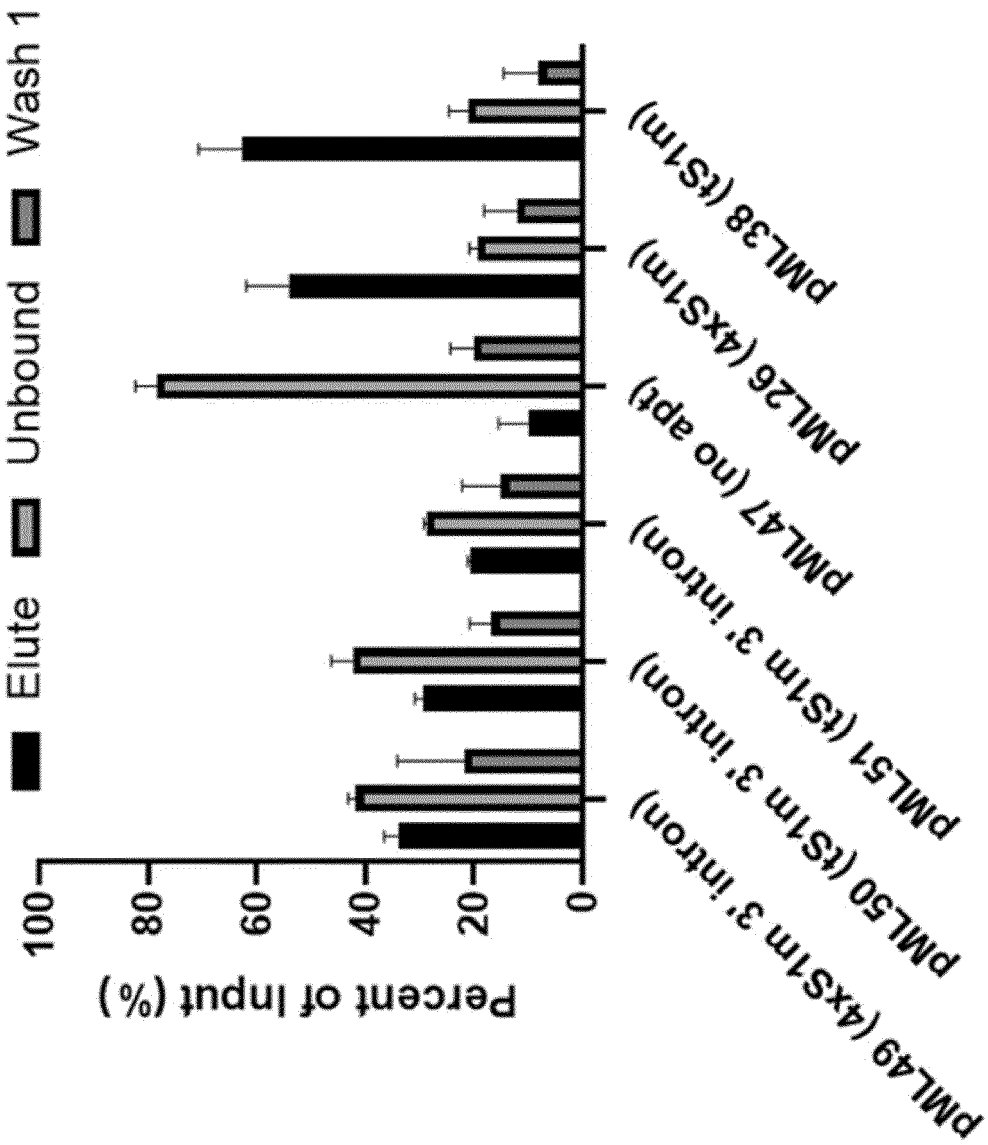

Elute
Unbound
Wash 1
Percent of Input (%)
100
80
60
40
20
0
pML49 (4xS1m 3' intron)
pML50 (tS1m 3' intron)
pML51 (tS1m 3' intron)
pML47 (no apt)
pML26 (4xS1m)
pML38 (tS1m)

FIGURE 7

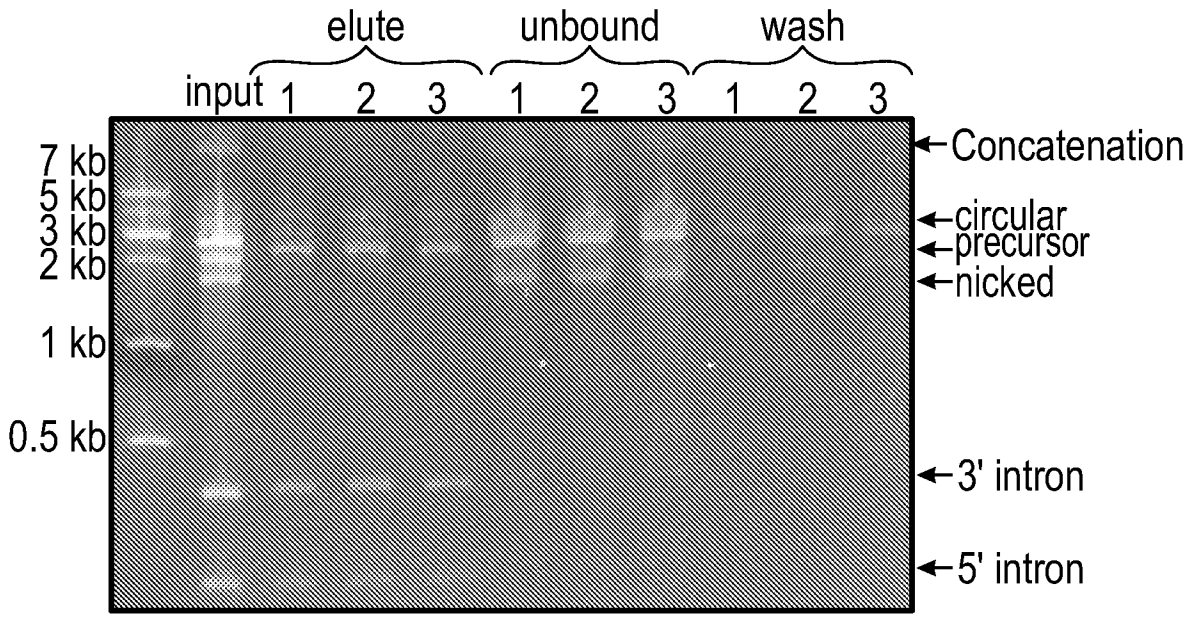

elute
unbound
wash
input
1
2
3
1
2
3
1
2
3
7 kb
5 kb
3 kb
2 kb
1 kb
0.5 kb
Concatenation
circular
precursor
nicked
3' intron
5' intron

FIG. 8A

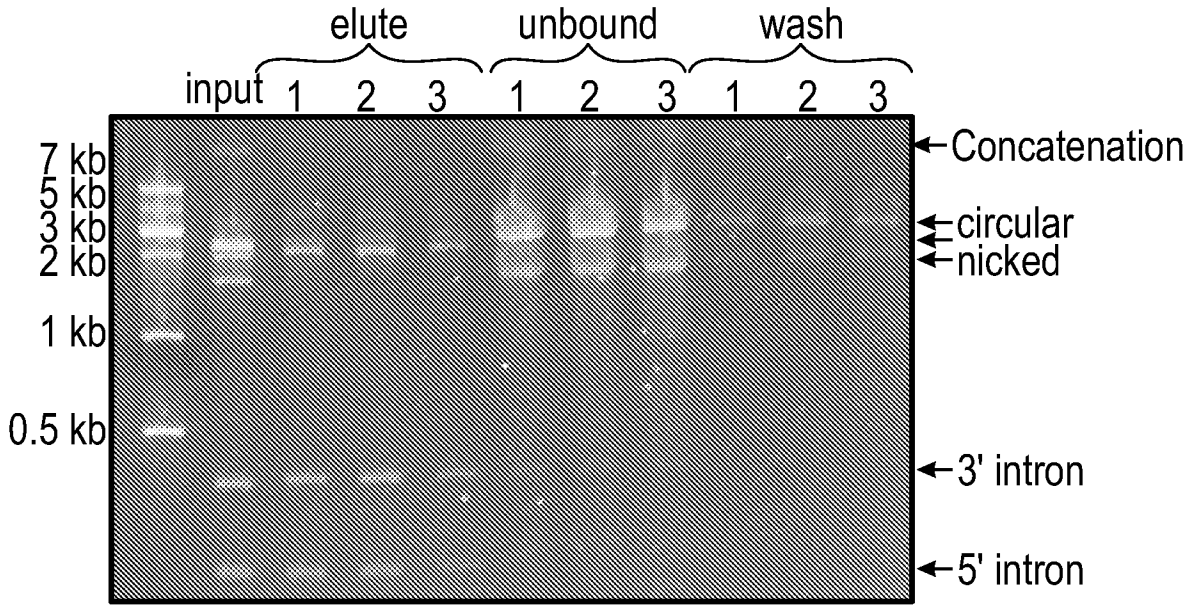

elute
unbound
wash
input
1
2
3
1
2
3
1
2
3
7 kb
5 kb
3 kb
2 kb
1 kb
0.5 kb
Concatenation
circular
nicked
3' intron
5' intron

FIG. 8B

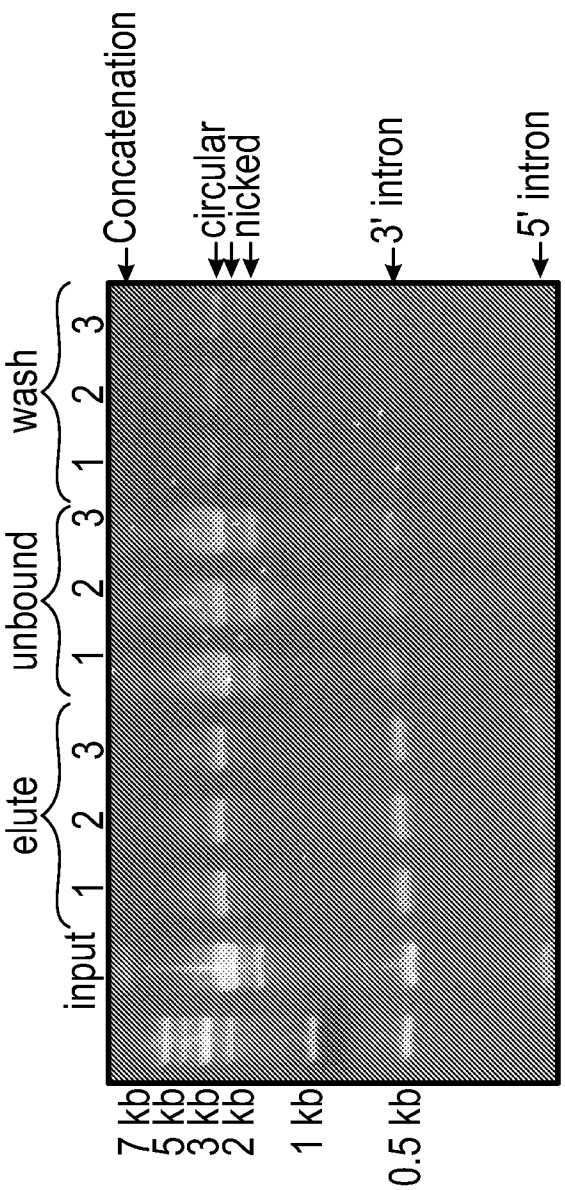

input
elute
1
2
3
unbound
1
2
3
wash
1
2
3
Concatenation
circular
nicked
3' intron
5' intron
7 kb
5 kb
3 kb
2 kb
1 kb
0.5 kb

FIG. 8C

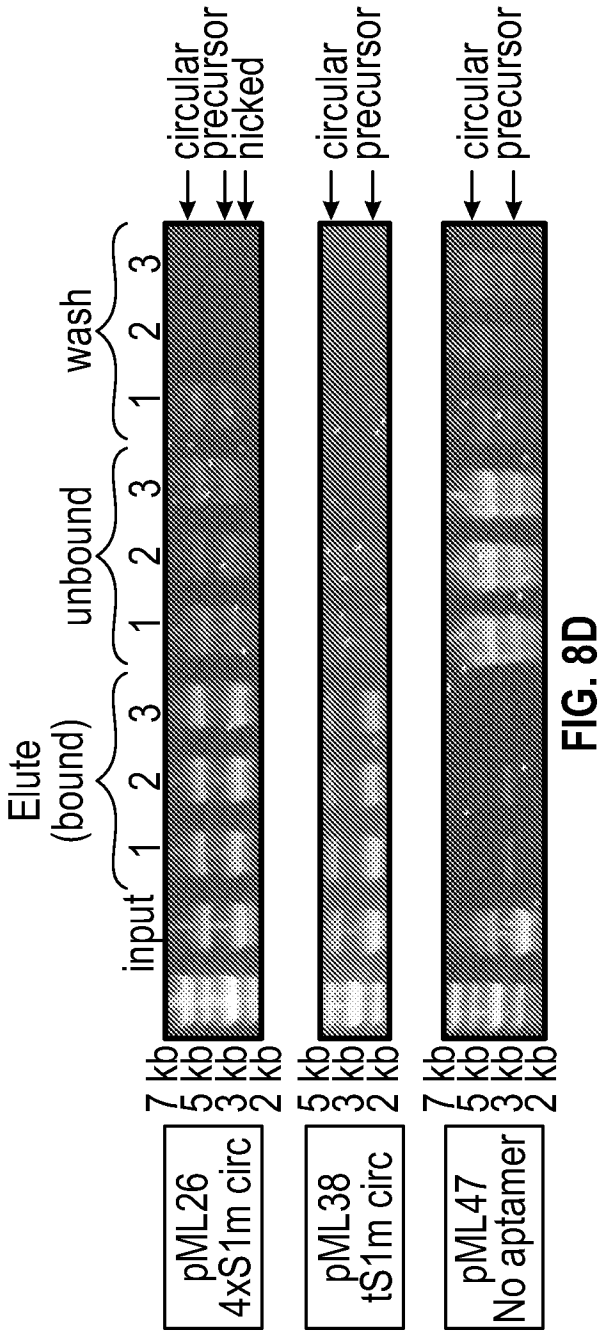

input
Elute
(bound)
1
2
3
unbound
1
2
3
wash
1
2
3
circular
precursor
nicked
circular
precursor
circular
precursor
7 kb
5 kb
3 kb
2 kb
5 kb
3 kb
2 kb
7 kb
5 kb
3 kb
2 kb
pML26
4xS1m circ
pML38
tS1m circ
pML47
No aptamer

FIG. 8D

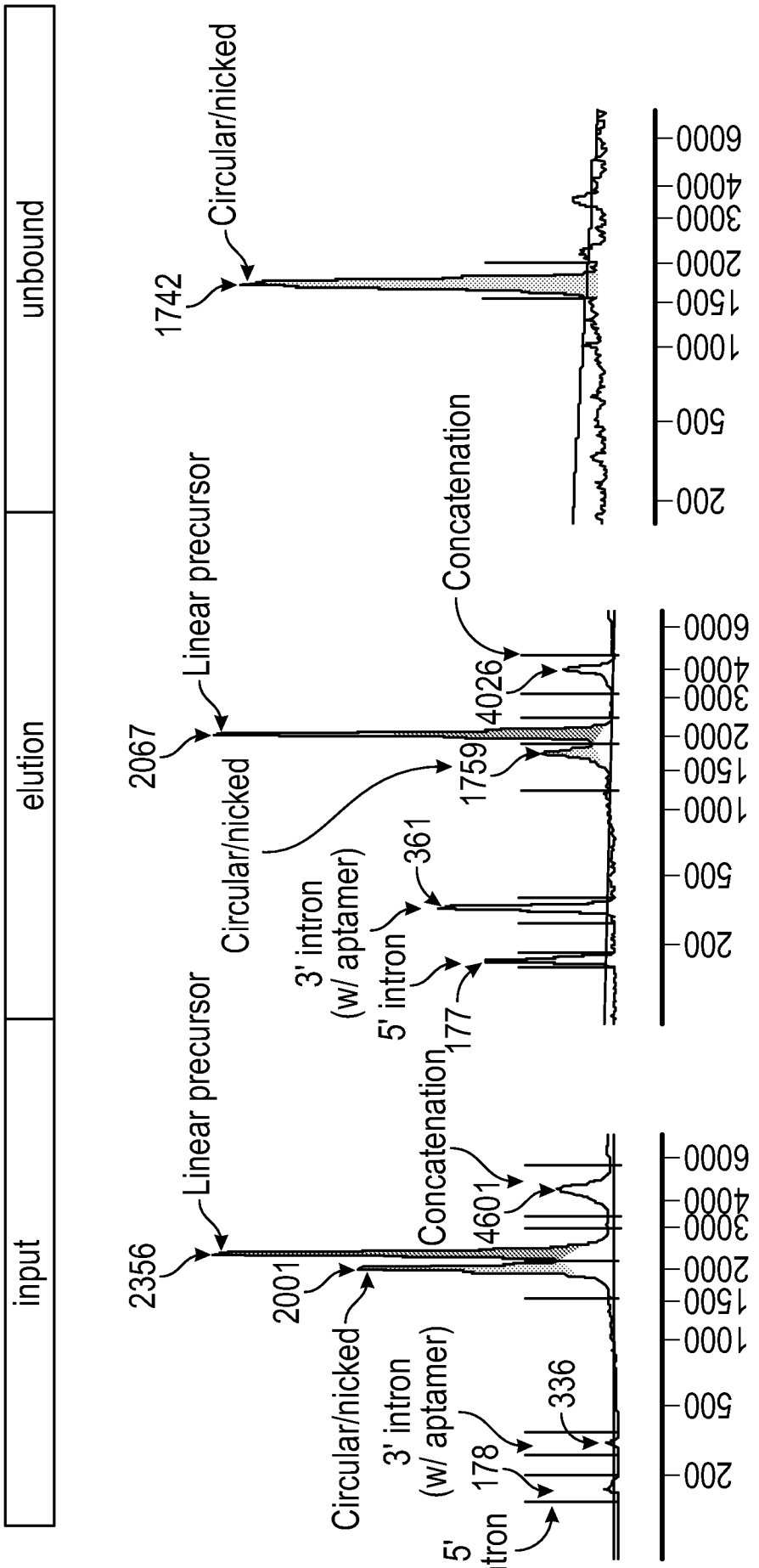

input
elution
unbound
2356
Linear precursor
2001
Circular/nicked
Concatenation
4601
3' intron
(w/ aptamer)
336
178
5'
ntron
2067
Linear precursor
Circular/nicked
Concatenation
4026
1759
3' intron
(w/ aptamer)
361
5' intron
177
1742
Circular/nicked
6000
4000
3000
2000
1500
1000
500
200

FIG. 9A

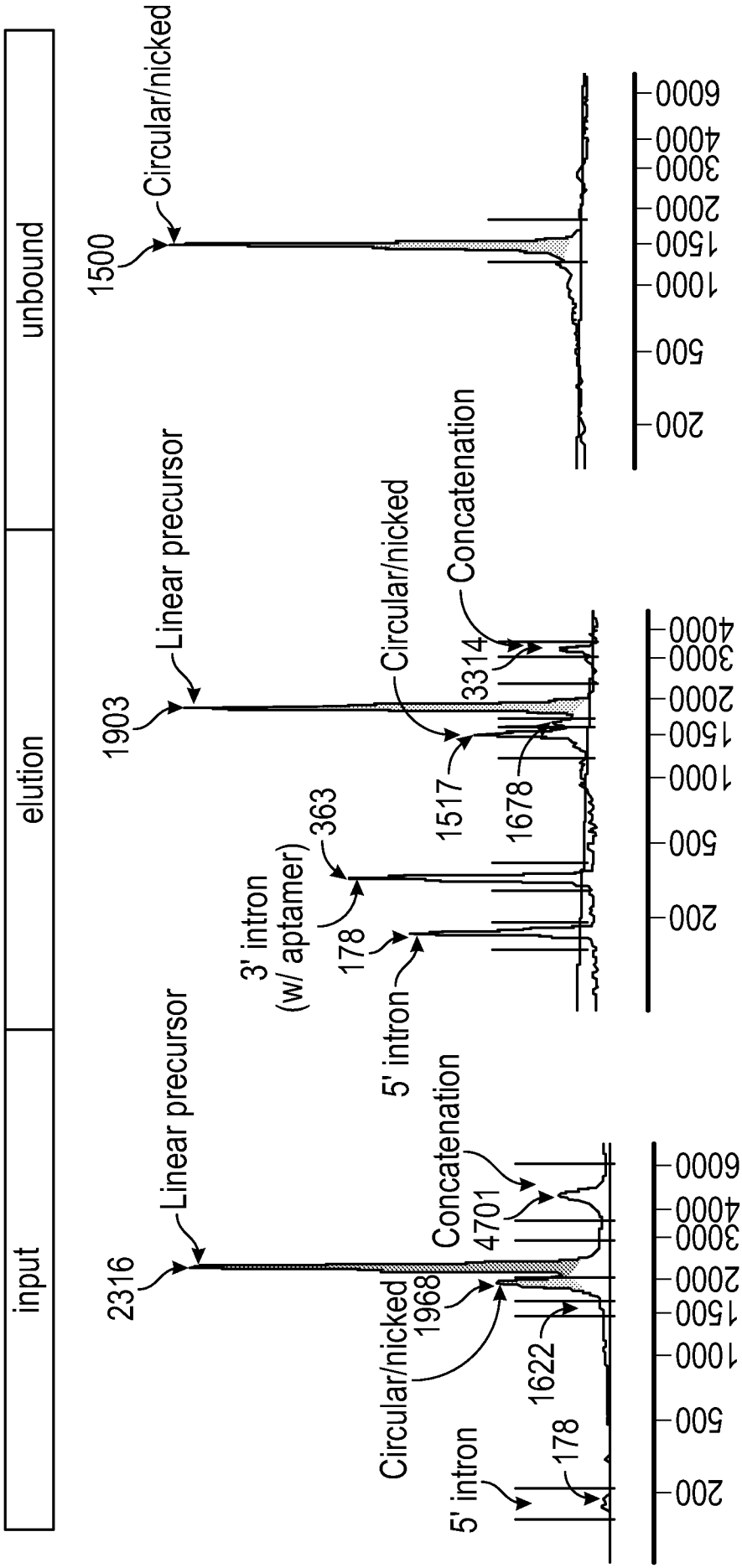

input
elution
unbound
Circular/nicked
1500
6000
4000
3000
2000
1500
1000
500
200
Linear precursor
1903
Circular/nicked
Concatenation
3314
1517
1678
3' intron
(w/ aptamer)
363
178
5' intron
Linear precursor
2316
Concatenation
4701
Circular/nicked
1968
1622
178
5' intron

FIG. 9B

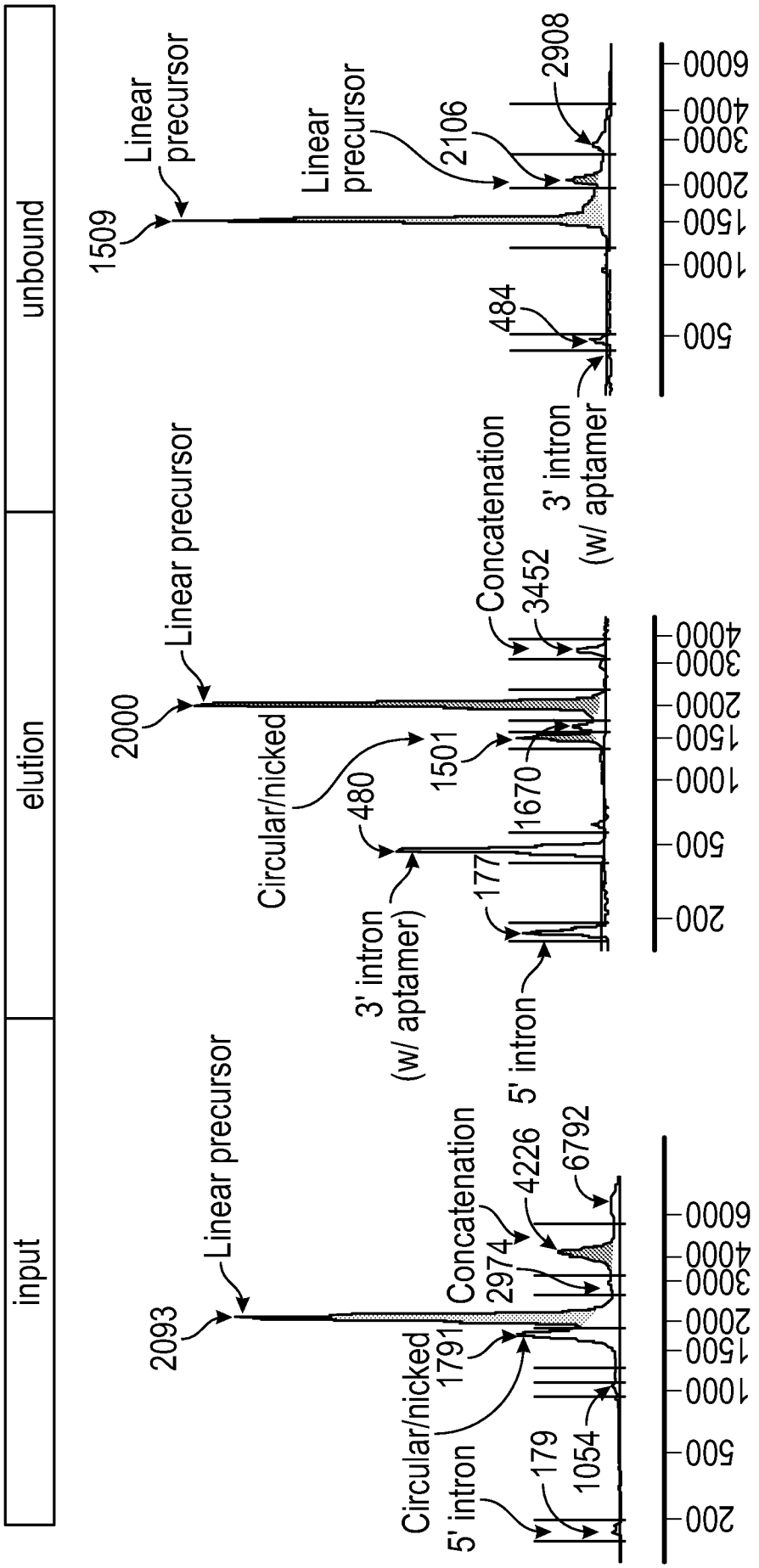

input
elution
unbound
Linear precursor
1509
Linear precursor
2106
2908
484
3' intron (w/ aptamer
Linear precursor
2000
Concatenation
3452
Circular/nicked
480
1501
1670
3' intron (w/ aptamer)
177
5' intron
Linear precursor
2093
Concatenation
2974
4226
6792
Circular/nicked
1791
5' intron
179
1054
6000
4000
3000
2000
1500
1000
500
200

FIG. 9C

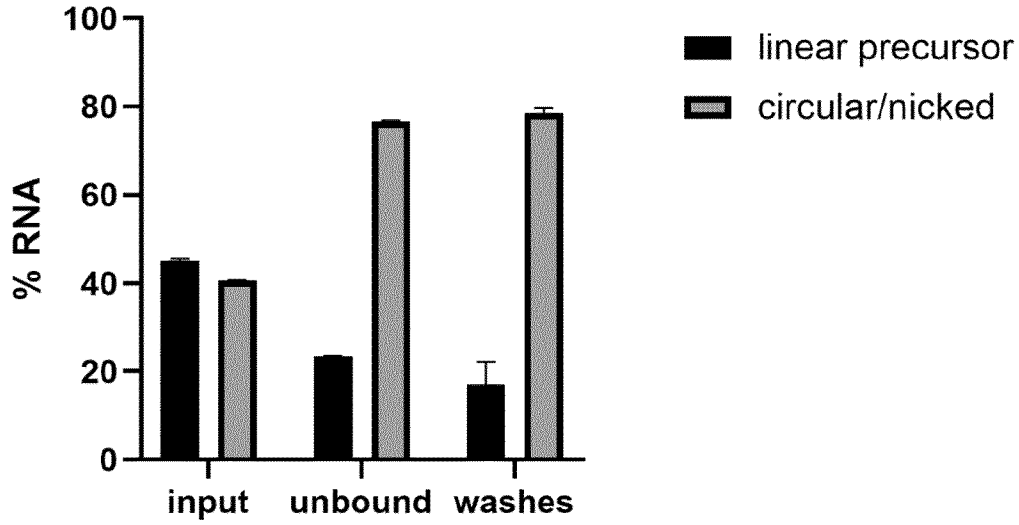

100
80
60
40
20
0
% RNA
input
unbound
washes
linear precursor
circular/nicked

FIGURE 10

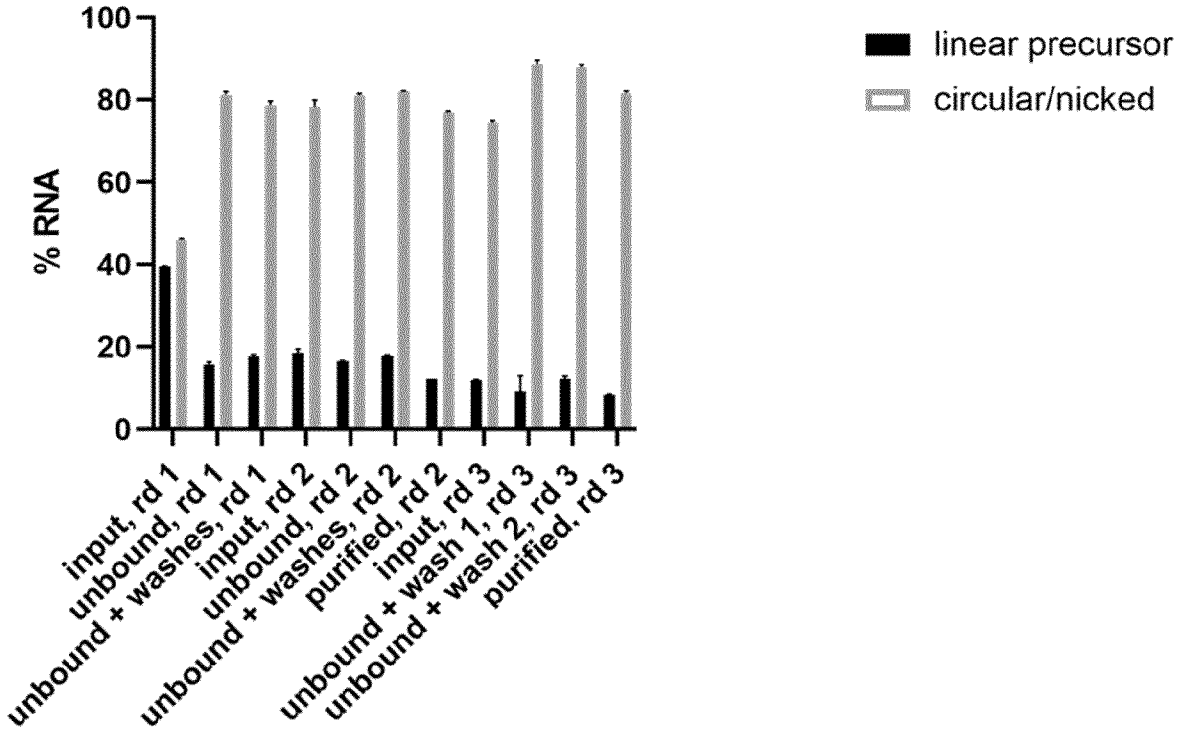

100
80
60
40
20
0
% RNA
linear precursor
circular/nicked
input, rd 1
unbound, rd 1
unbound + washes, rd 1
input, rd 2
unbound, rd 2
unbound + washes, rd 2
purified, rd 2
input, rd 3
unbound + wash 1, rd 3
unbound + wash 2, rd 3
purified, rd 3

FIGURE 11A

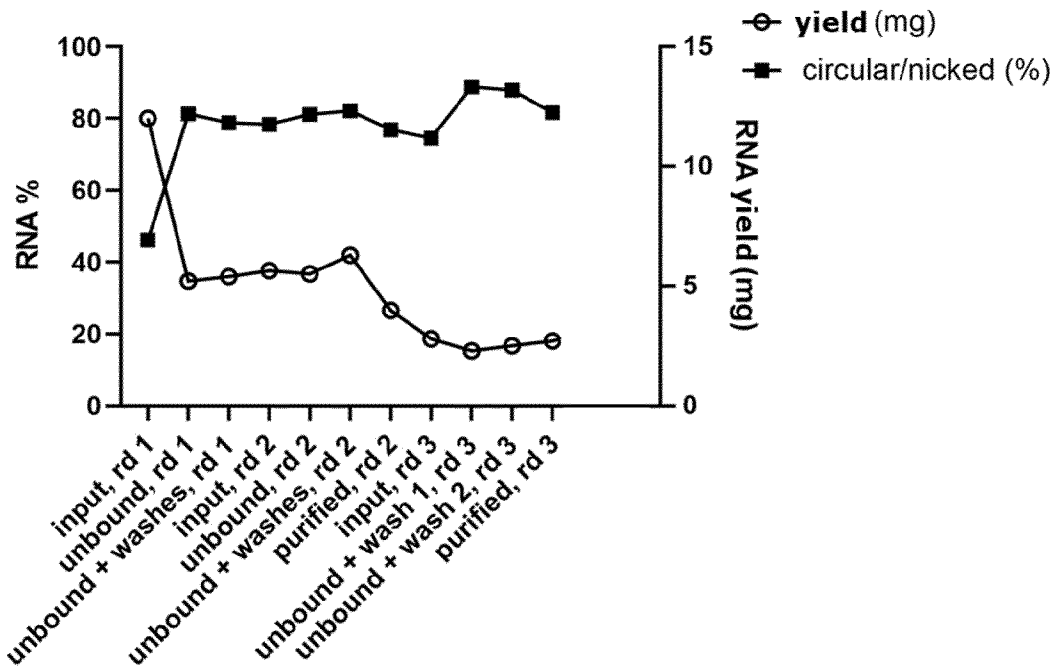

yield (mg)
circular/nicked (%)
RNA %
RNA yield (mg)
100
80
60
40
20
0
15
10
5
0
input, rd 1
unbound, rd 1
unbound + washes, rd 1
input, rd 2
unbound, rd 2
unbound + washes, rd 2
purified, rd 2
input, rd 3
unbound + wash 1, rd 3
unbound + wash 2, rd 3
purified, rd 3

FIGURE 11B

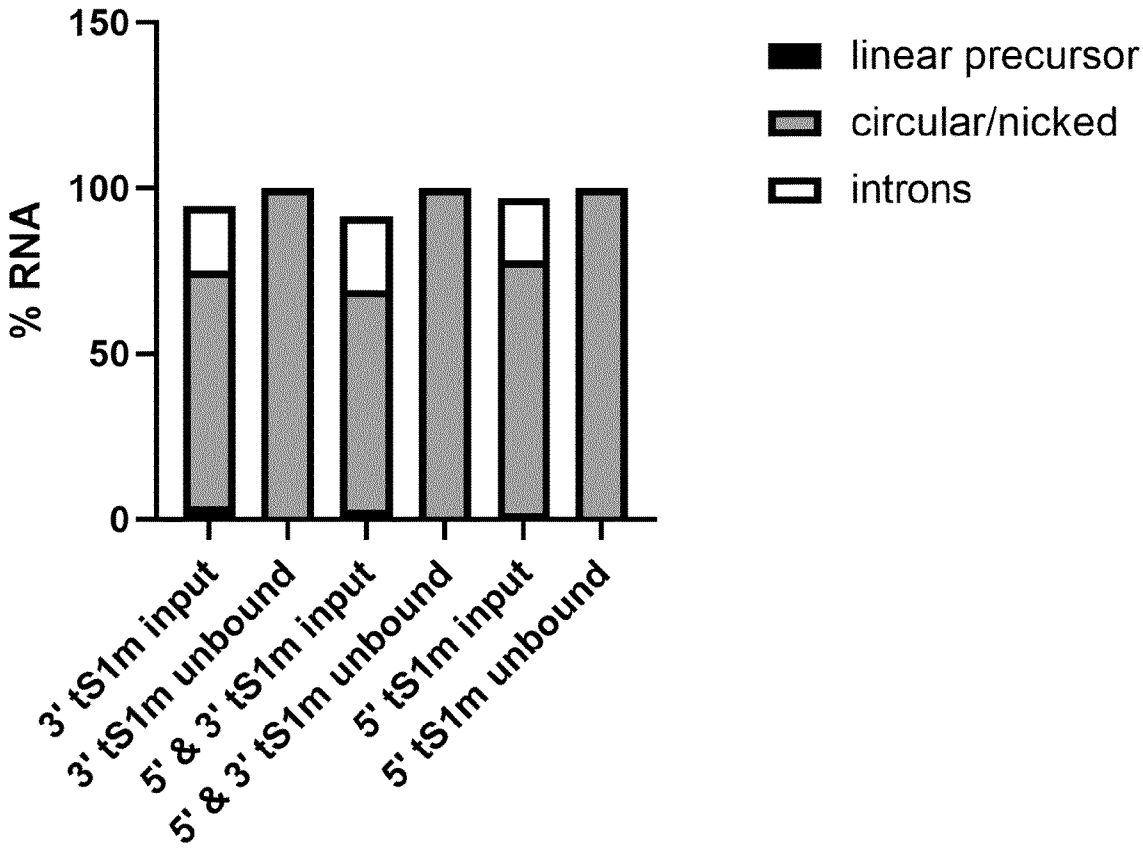

150
100
50
0
% RNA
linear precursor
circular/nicked
introns
3' tS1m input
3' tS1m unbound
5' & 3' tS1m input
5' & 3' tS1m unbound
5' tS1m input
5' tS1m unbound

FIGURE 12A

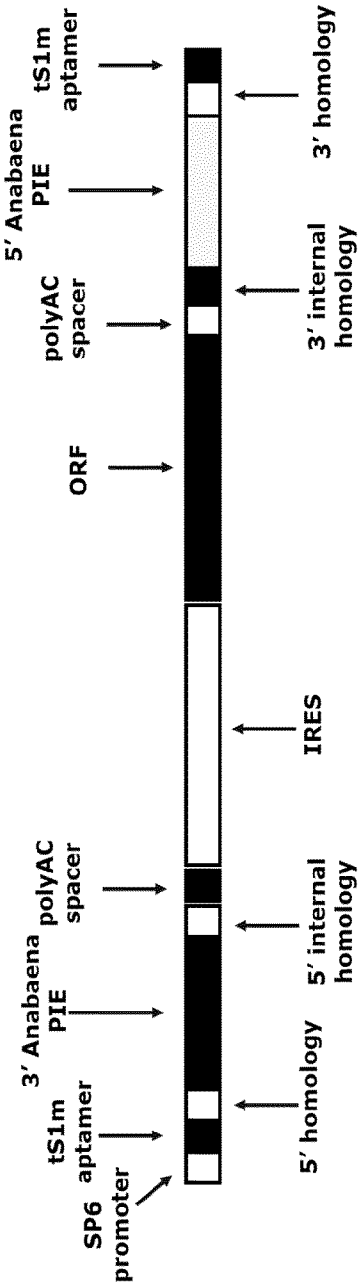

SP6 promoter
tS1m aptamer
3′ Anabaena PIE
polyAC spacer
5′ homology
5′ internal homology
IRES
ORF
3′ internal homology
5′ Anabaena PIE
tS1m aptamer
3′ homology

FIGURE 12B

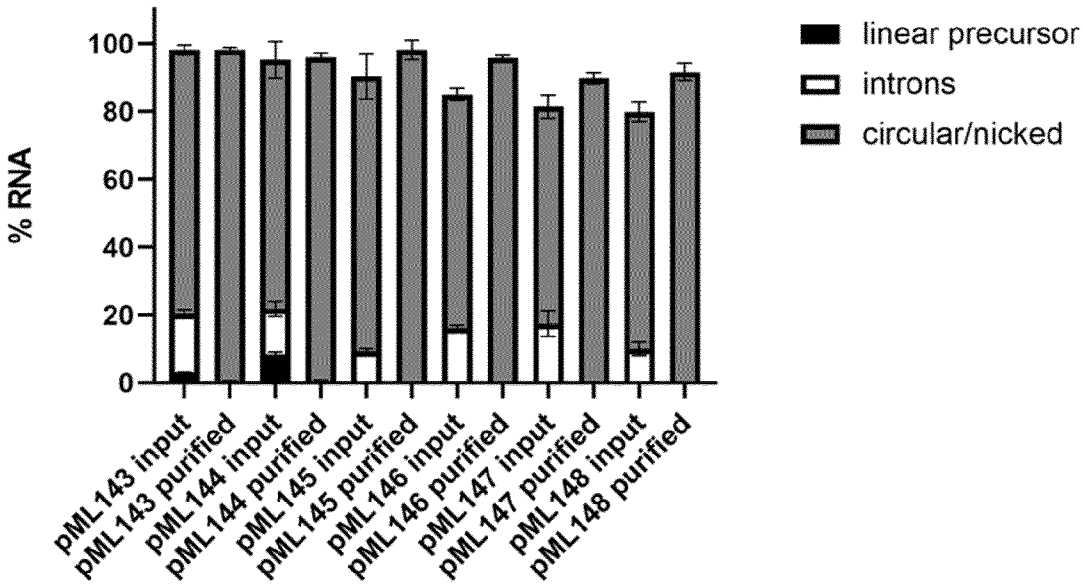

% RNA
100
80
60
40
20
0
linear precursor
introns
circular/nicked
pML143 input
pML143 purified
pML144 input
pML144 purified
pML145 input
pML145 purified
pML146 input
pML146 purified
pML147 input
pML147 purified
pML148 input
pML148 purified

FIGURE 13

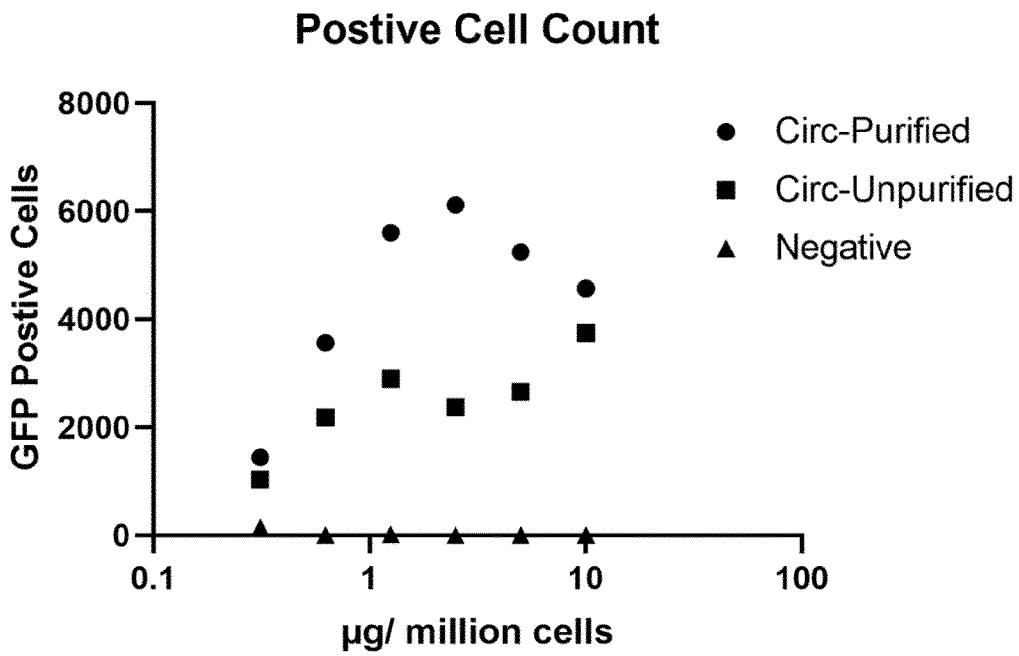

Postive Cell Count
GFP Postive Cells
8000
6000
4000
2000
0
0.1
1
10
100
µg/ million cells
Circ-Purified
Circ-Unpurified
Negative

FIGURE 14

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2023/066315

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/7088 A61K31/7105 A61K48/00 C07K14/25 C12N15/11
C12N15/115 C12N15/64
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
A61K C12N C12R C40B C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2019/236673 A1 (MASSACHUSETTS INST TECHNOLOGY [US]) 12 December 2019 (2019-12-12) [0006], [0017]-[0018], [00133], [00137], [00139], [0006]-[0014], [0019]-[0022], [0027]-[0028], [0030], [0048], [00108], [00119], [00122], [00170]-[00171], [0015], [0048], [00138], [0025], [00174], [0036], [00170], [00192], [00141], [00191], [00154], [0038], [00148], [00149]; claims 1, 2-17, 30, 37-42, 46-53, 56-62	1-65, 67-140

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 2023	29/09/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Landré, Julien

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2023/066315

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/237227 A1 (MASSACHUSETTS INST TECHNOLOGY [US]; ORNA THERAPEUTICS INC [US] ET AL.) 26 November 2020 (2020-11-26) [2], [6], [9], [15], [205], [272] [318], [6]-[7], [19], [21], [31], [108], [112]-[113], [198]-[199], [201], [213], [377], [481], [10], [205], [43], [103], [359], [288]-[291], [292], [384]; examples 1, 2B, 4A, 14-15	1-22, 24-65, 67-80, 82-140
Y	CN 114 438 127 A (SUZHOU KOMAIDE BIOMEDICAL SCIENCE AND TECH CO LTD) 6 May 2022 (2022-05-06) p. 1, 1st -2nd , p. 7, point 14, p. 31, 7th -last , p. 1, last , p. 43, point 3, p. 7, 7th , p. 24, 6th , p. 25, 3rd , p. 51, 3rd , p. 51, SEQ. ID. No. 37, p. 25, 4th , p. 15, 3rd , p. 45, point (4) 1)-2), p. 1, last , p. 22, 3rd , p. 5, 10th , p. 7, 7th , p. 22, 2nd , p. 2, penultimate -last , p. 5, 5th , p. 20, 1st , p. 22, penultimate , p. 3, 1st , p. 4, 2nd -p. 5, 4th , p. 5, last -p. 6, 7th , p. 43, 6th , p. 47, 8th , p. 7, 4th , p. 51, 5th , p. 8, point 22, p.8, point 24; example 19; sequences 1, 37	1-140
Y	SRISAWAT C ET AL: "STREPTAVIDIN APTAMERS: AFFINITY TAGS FOR THE STUDY OF RNAS AND RIBONUCLEOPROTEINS", RNA, COLD SPRING HARBOR LABORATORY PRESS, US, vol. 7, no. 4, 1 April 2001 (2001-04-01), pages 632-641, XP001120463, ISSN: 1355-8382, DOI: 10.1017/S135583820100245X abstract p. 633 col. 1, 1st , p. 634 col. 1, 1st , p. 664 col. 2, penultimate , p. 635 col. 1, last , p. 635 col. 2, penultimate , p. 636 col. 1, penultimate , p. 637 col. 1, 2nd -p. 637 col. 2, last	1-140

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2023/066315

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2023/066315

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2019236673 A1	12-12-2019	AU 2019280583 A1	03-12-2020
		AU 2023201630 A1	13-04-2023
		BR 112020024292 A2	02-03-2021
		CA 3100276 A1	12-12-2019
		CN 112399860 A	23-02-2021
		EP 3801639 A1	14-04-2021
		IL 279157 A	31-01-2021
		JP 2021526792 A	11-10-2021
		KR 20210018323 A	17-02-2021
		SG 11202011162R A	30-12-2020
		US 2020080106 A1	12-03-2020
		US 2021198688 A1	01-07-2021
		US 2021363540 A1	25-11-2021
		US 2021403944 A1	30-12-2021
		US 2022025395 A1	27-01-2022
		US 2023050306 A1	16-02-2023
		WO 2019236673 A1	12-12-2019
WO 2020237227 A1	26-11-2020	AU 2020280105 A1	20-01-2022
		BR 112021023411 A2	01-02-2022
		CA 3139032 A1	26-11-2020
		CN 115867291 A	28-03-2023
		EP 3972653 A1	30-03-2022
		IL 288284 A	01-01-2022
		JP 2022533796 A	25-07-2022
		KR 20220027855 A	08-03-2022
		SG 11202112922W A	30-12-2021
		US 2021371494 A1	02-12-2021
		US 2022177540 A1	09-06-2022
		WO 2020237227 A1	26-11-2020
CN 114438127 A	06-05-2022	CN 114438127 A	06-05-2022
		EP 4239073 A1	06-09-2023
		US 2023279389 A1	07-09-2023