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(54) **SYSTEMS AND METHODS FOR
PRODUCING RNA CONSTRUCTS WITH
INCREASED TRANSLATION AND
STABILITY**

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(57) **ABSTRACT**

(21) Appl. No.: **18/053,324**

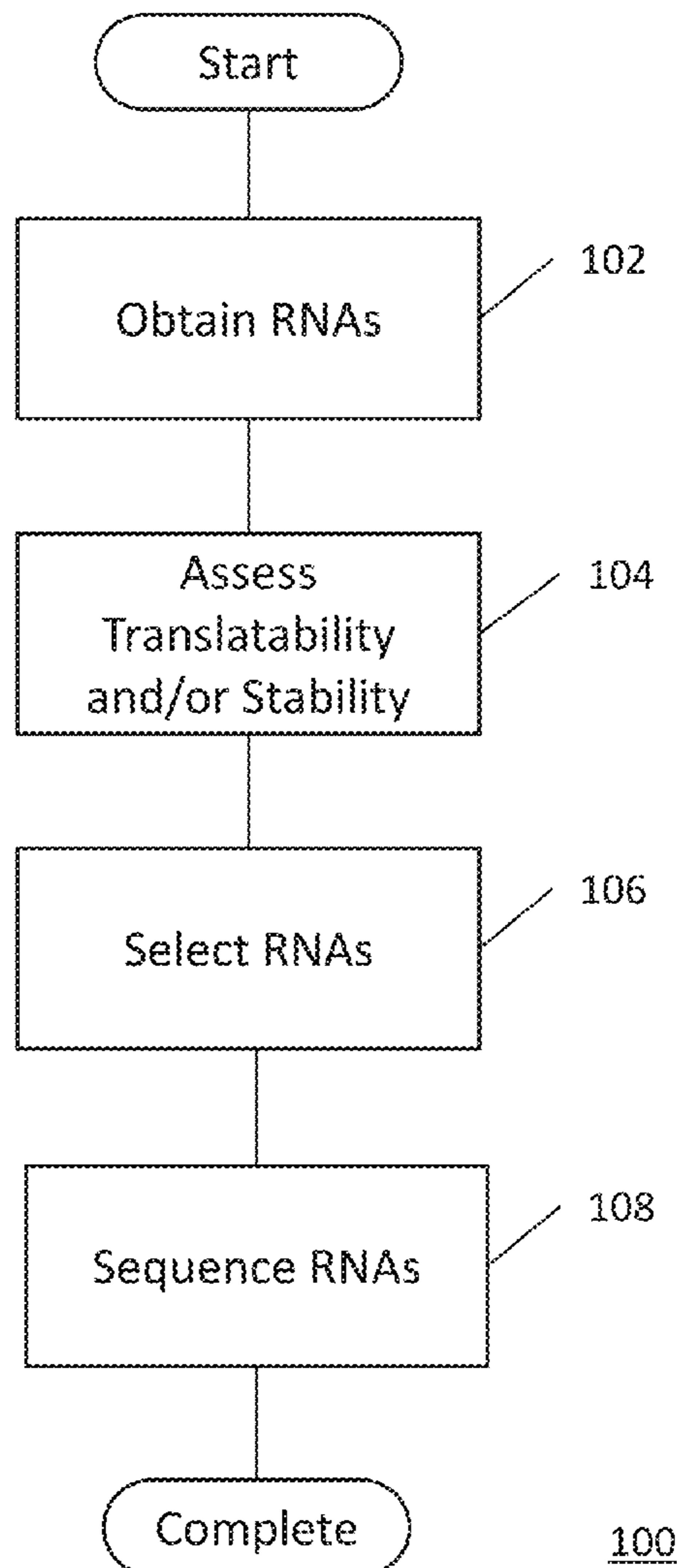
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Systems and methods for enhancing RNA translatability and stability are disclosed. Some embodiments describe RNA molecules exhibiting increased translatability and/or stability. Additional embodiments describe methods for screening RNA molecules for increased translatability and/or stability. Various embodiments utilize screening methods, including degenerative sequences to identify sequences or regions that increase the translatability and/or stability of RNA molecules.

Related U.S. Application Data

(62) Division of application No. 17/463,466, filed on Aug. 31, 2021, now Pat. No. 11,492,611.

Specification includes a Sequence Listing.



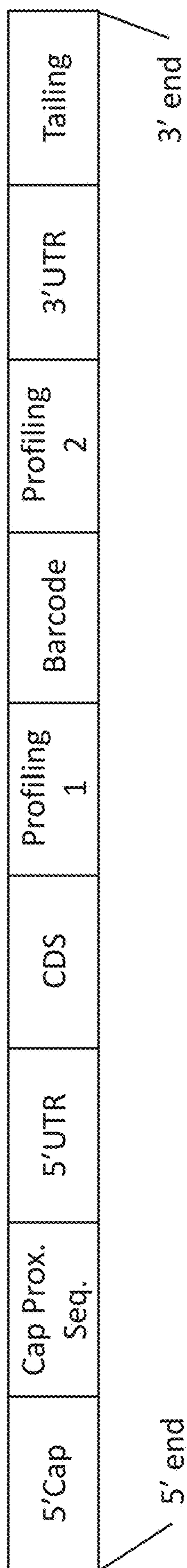


Fig. 1A

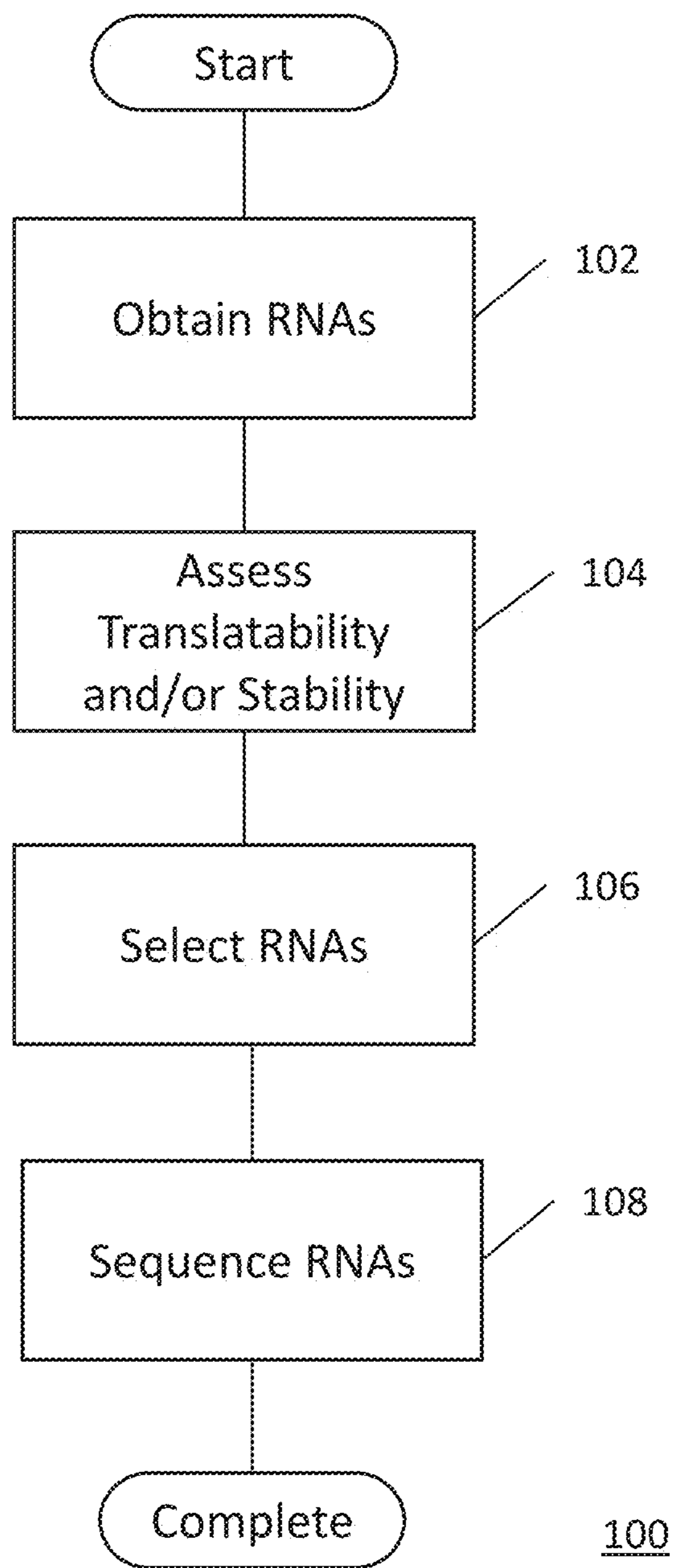


Fig. 1B

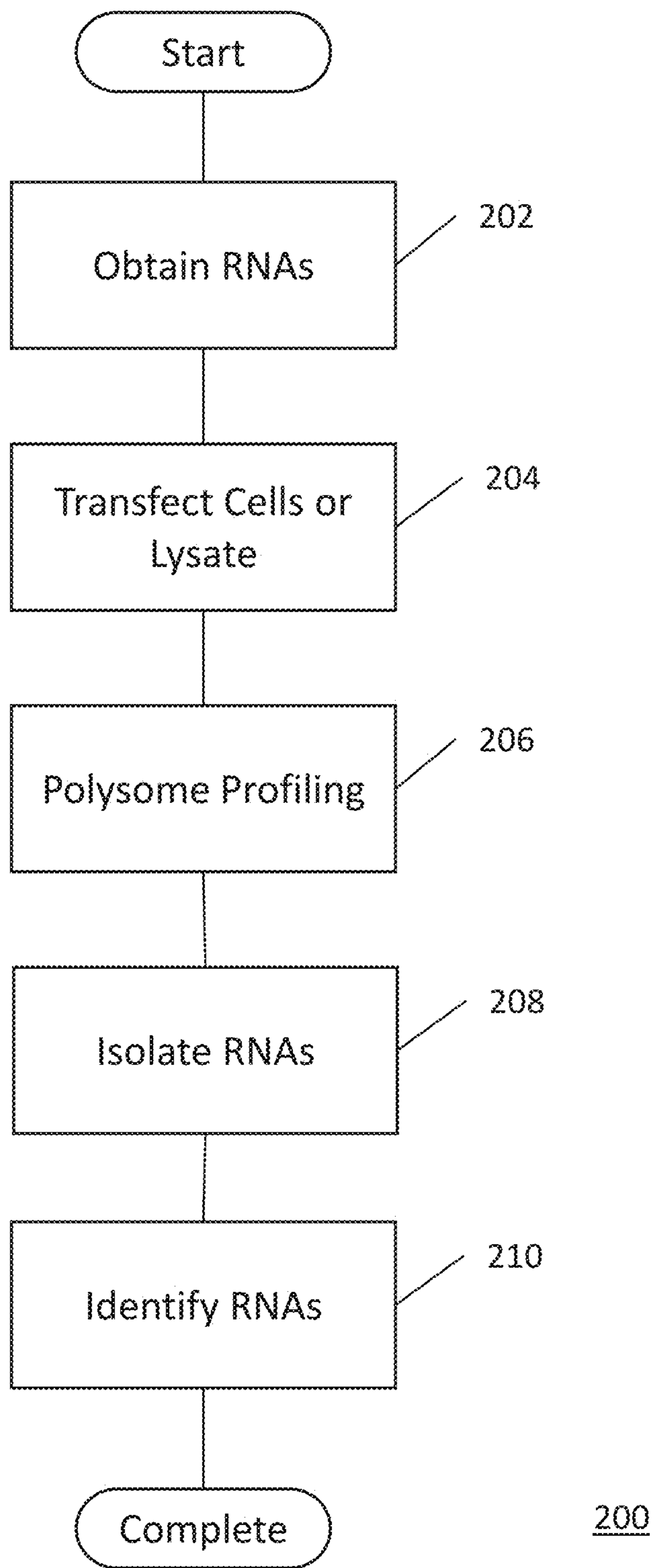


Fig. 2

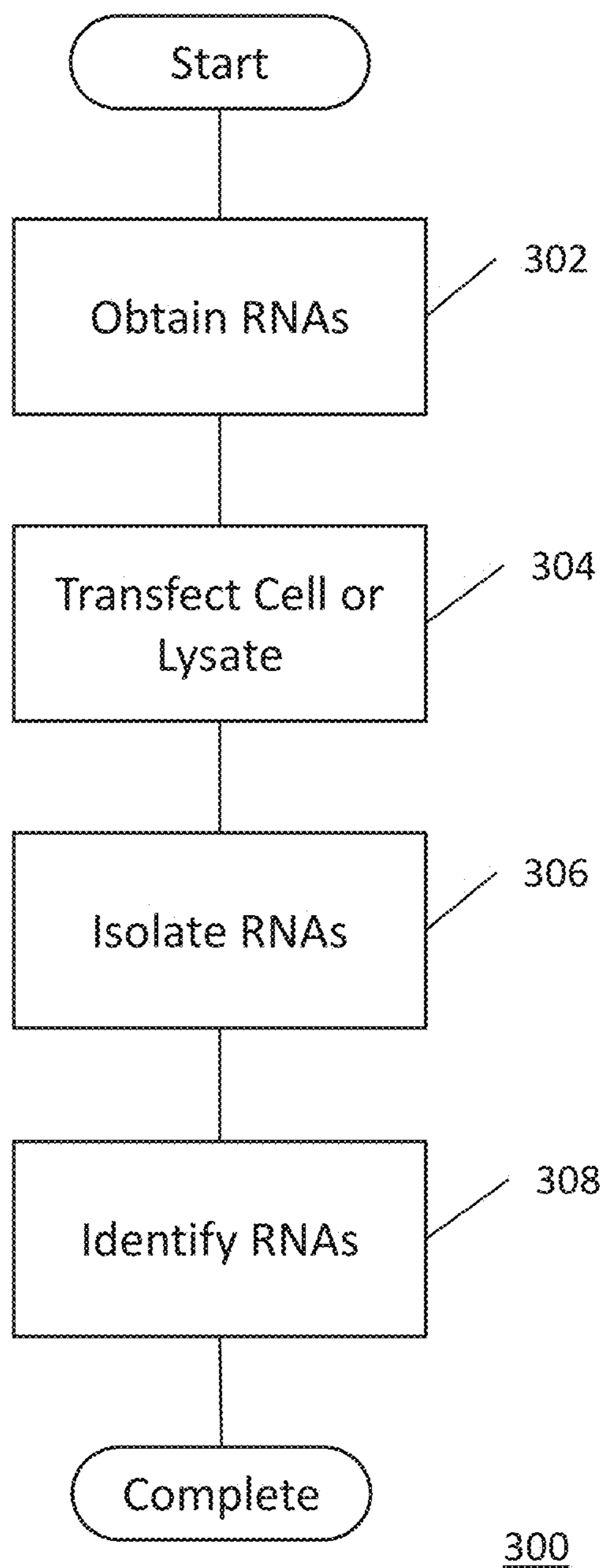


Fig. 3

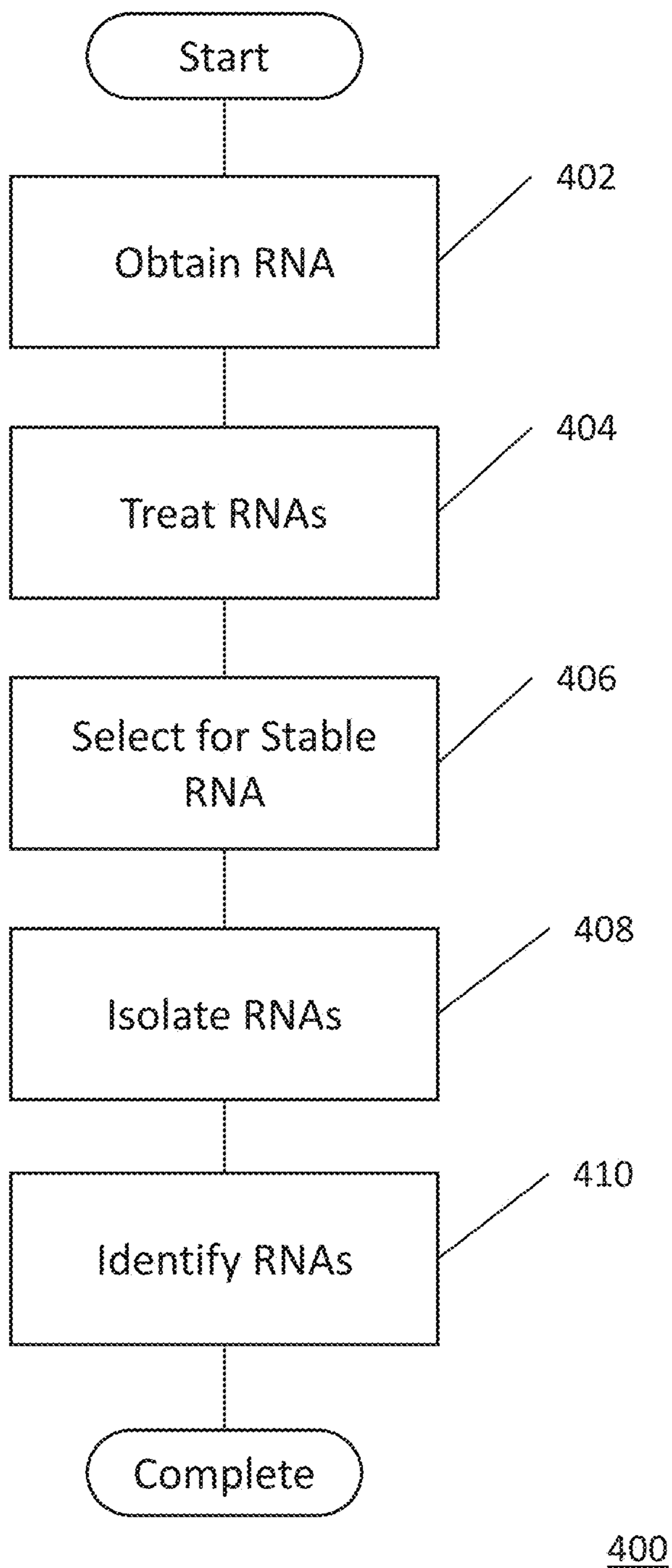


Fig. 4

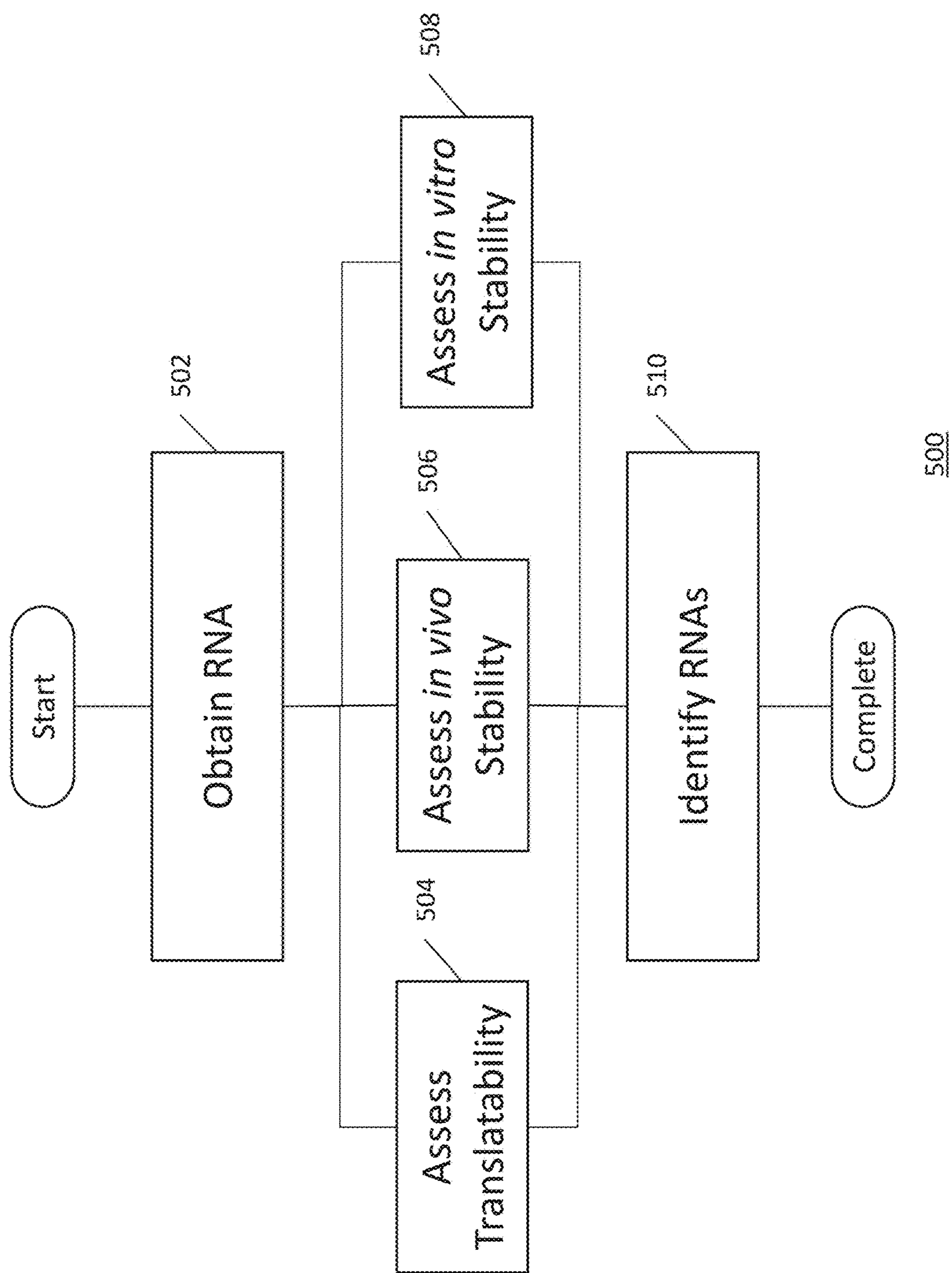


Fig. 5A

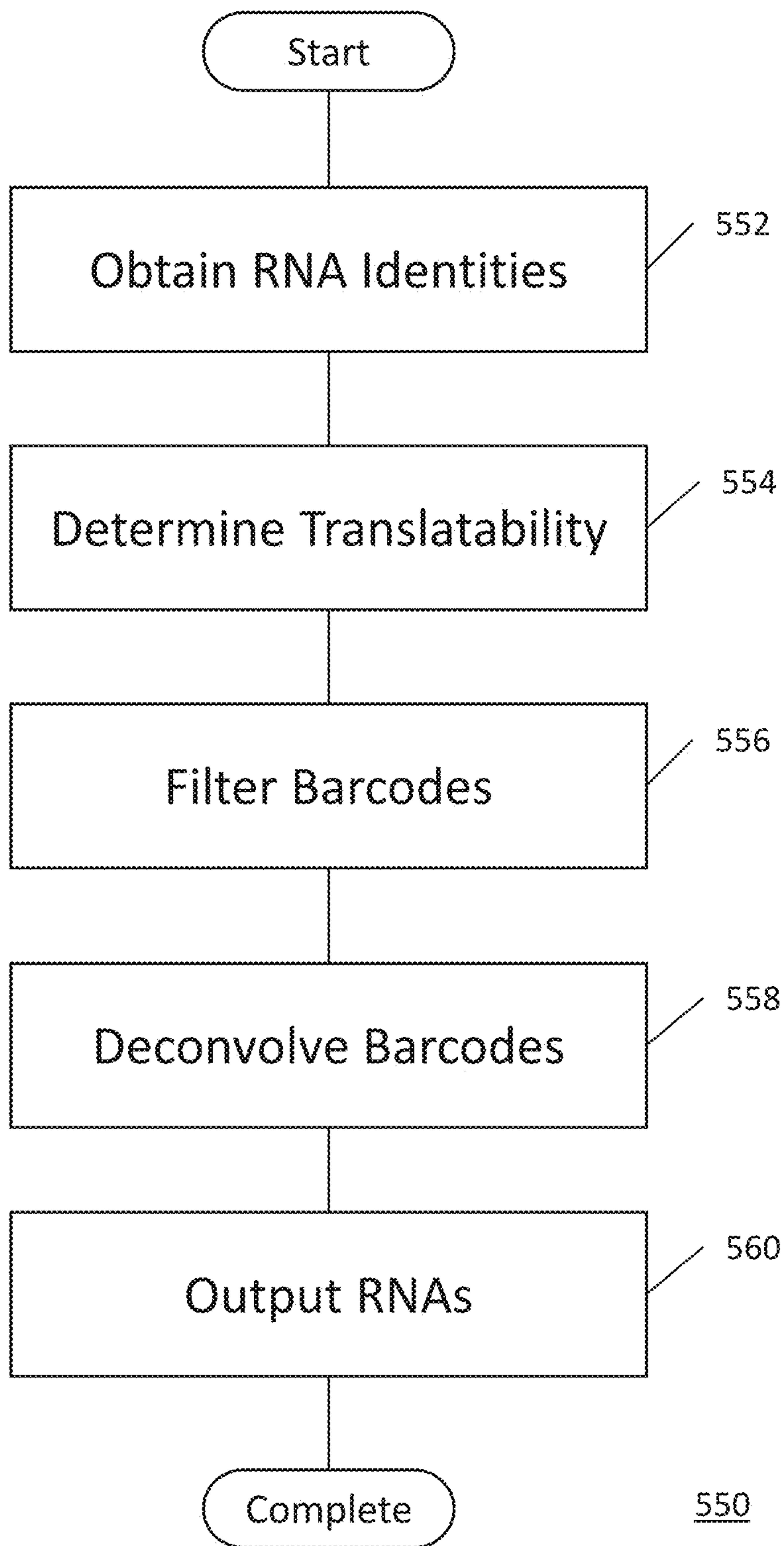


Fig. 5B

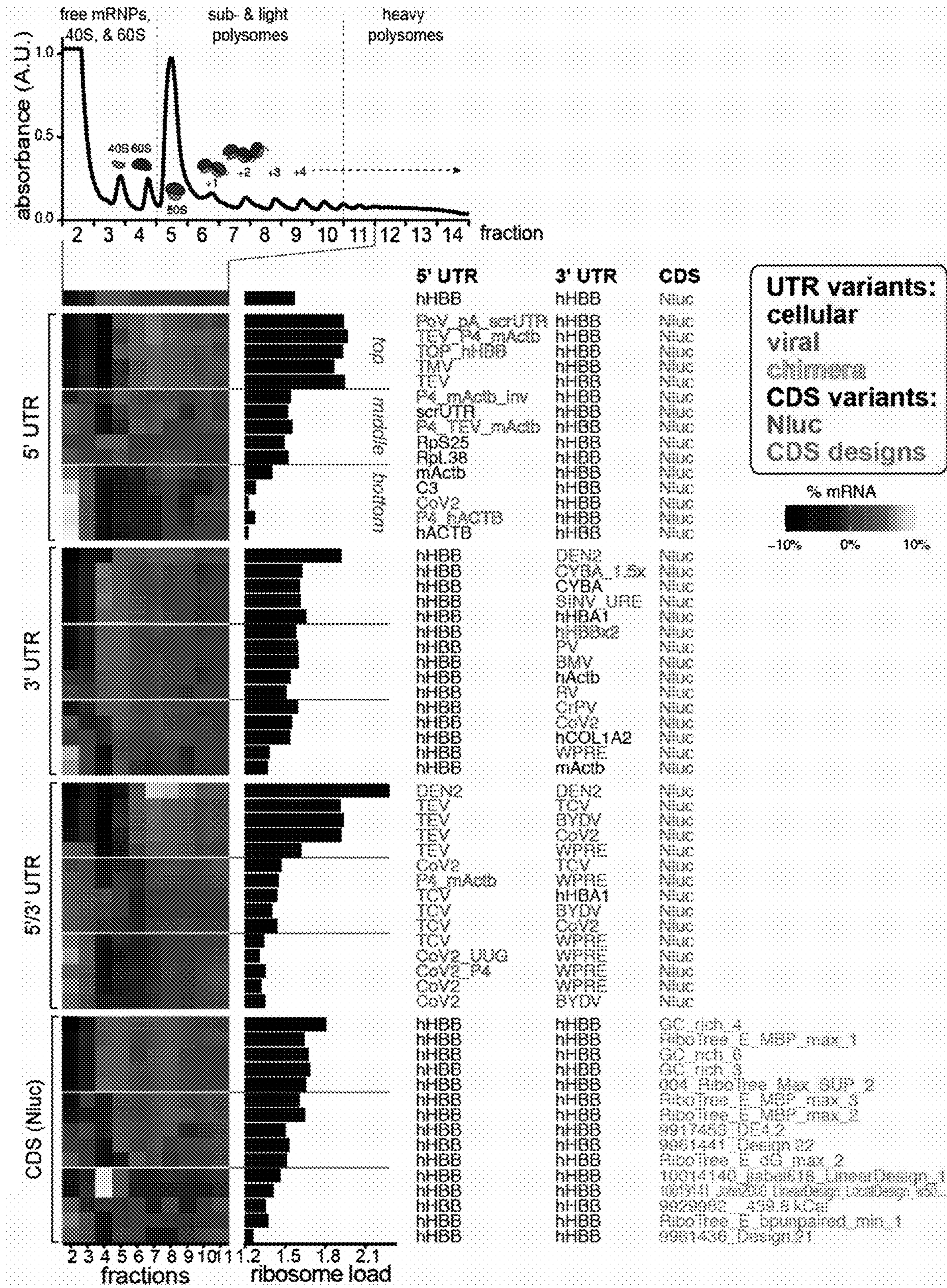


Fig. 6A

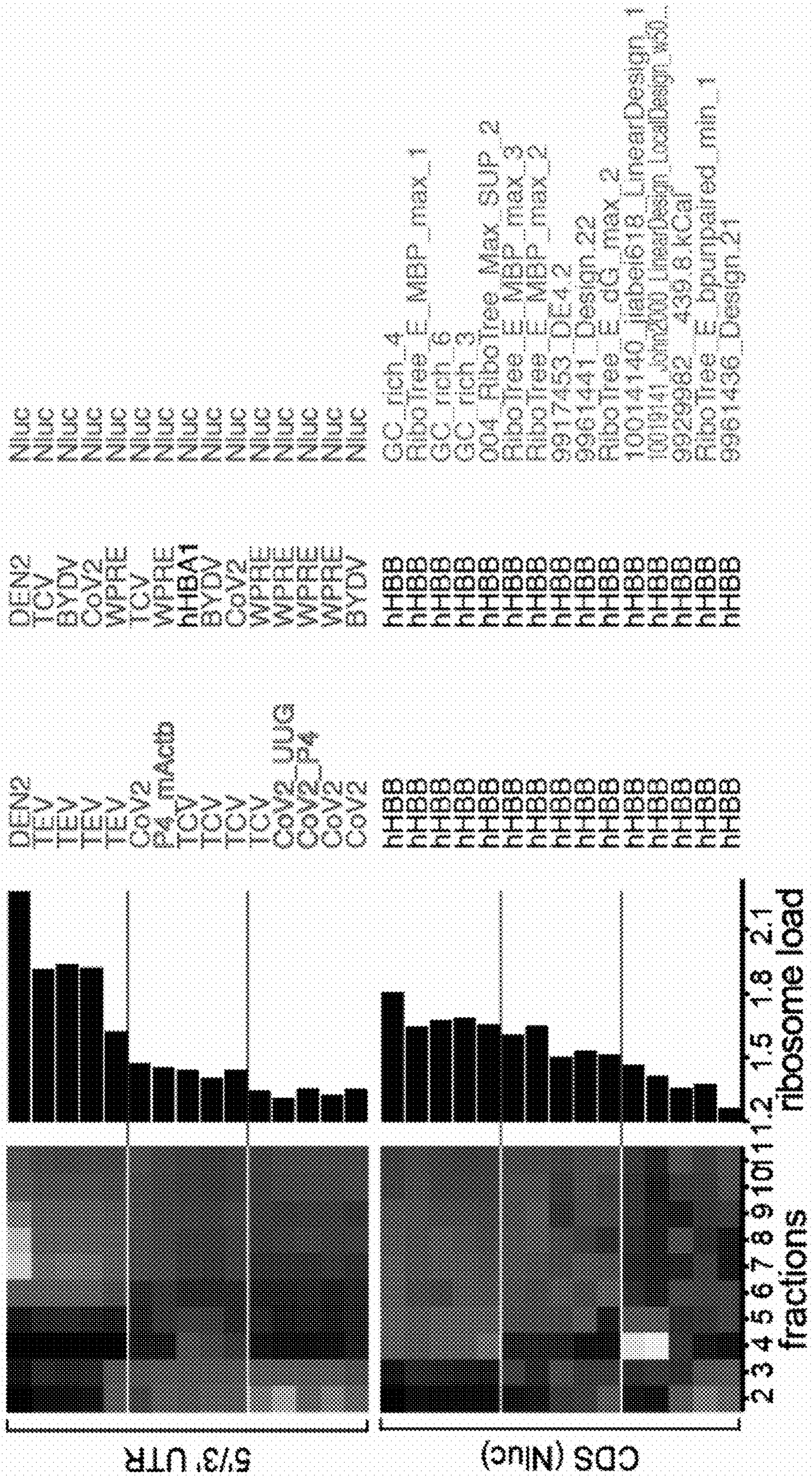


Fig. 6C

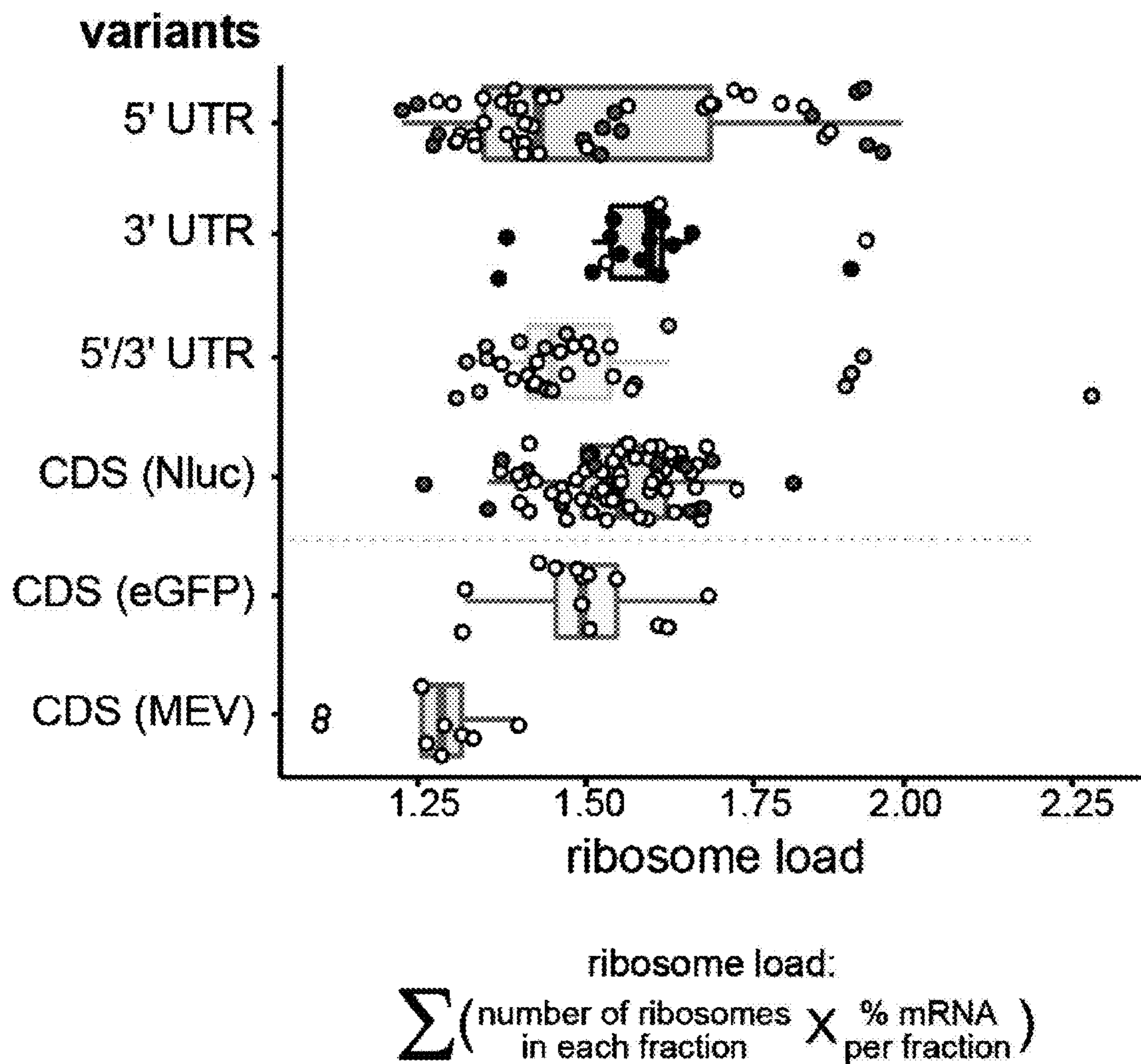


Fig. 7A

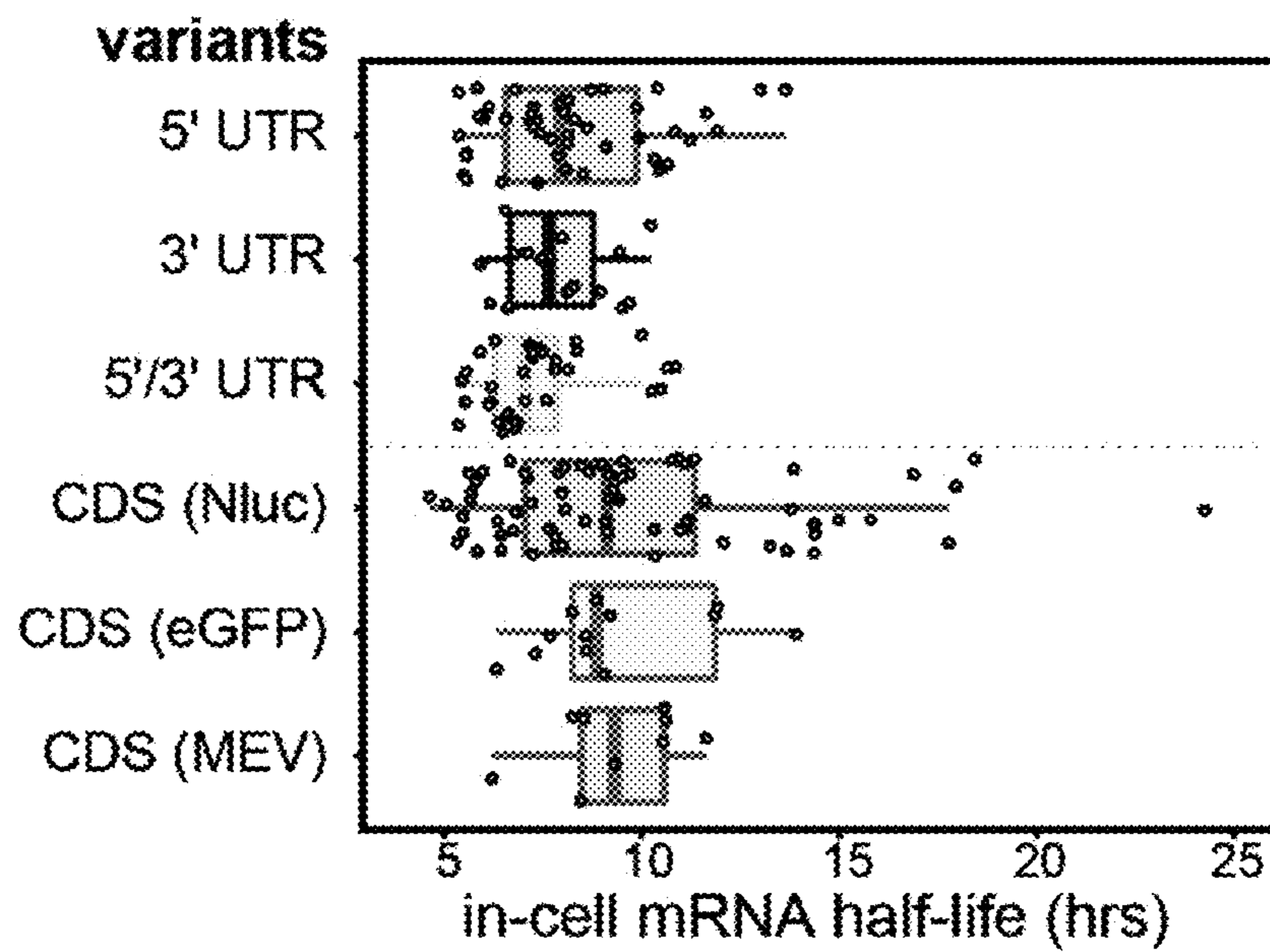


Fig. 7B

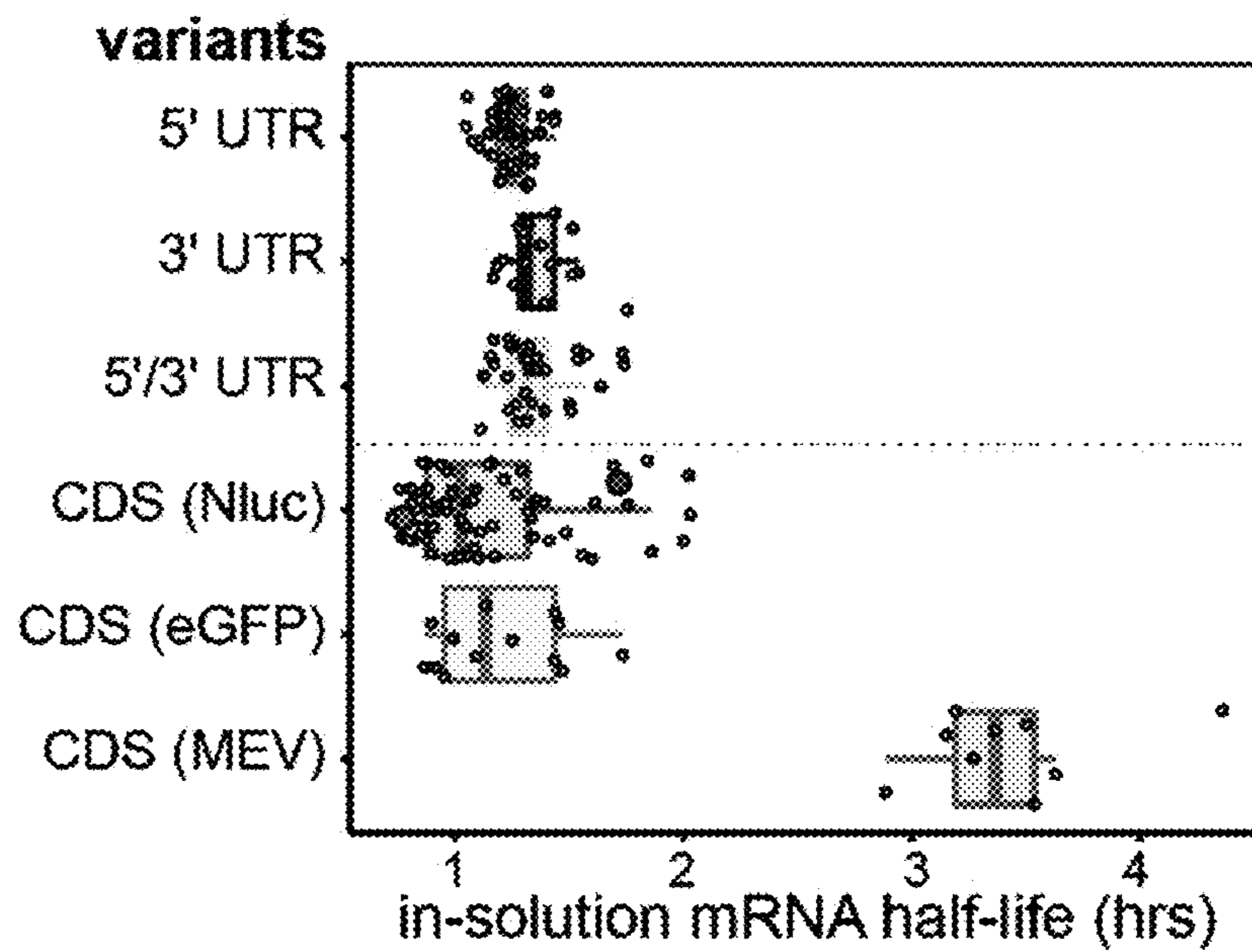


Fig. 7C

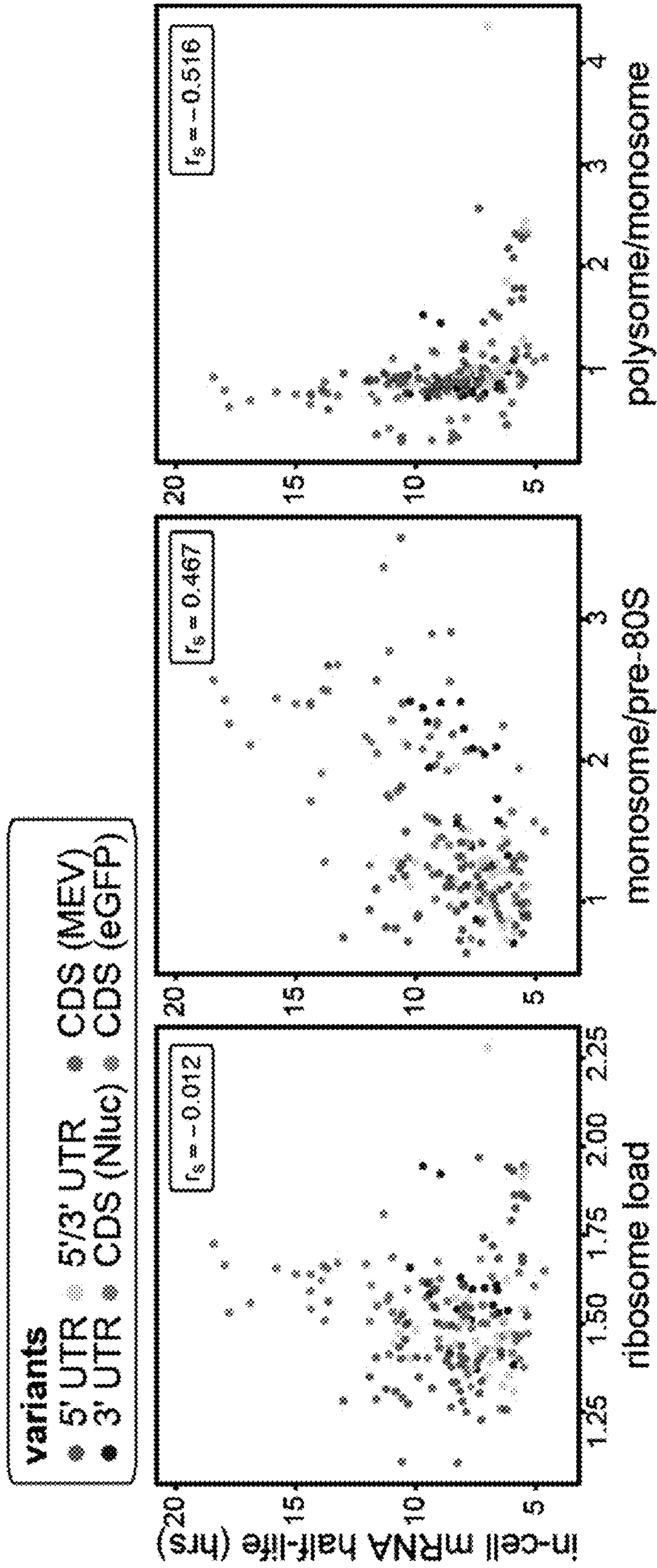


Fig. 8A

Fig. 8B

Fig. 8C

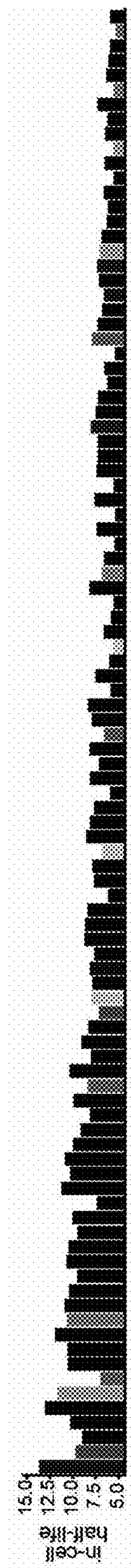


Fig. 9A

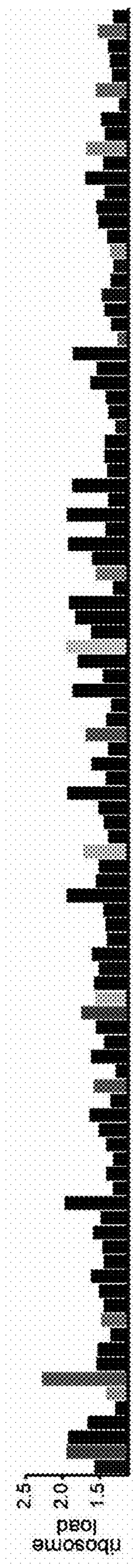


Fig. 9B

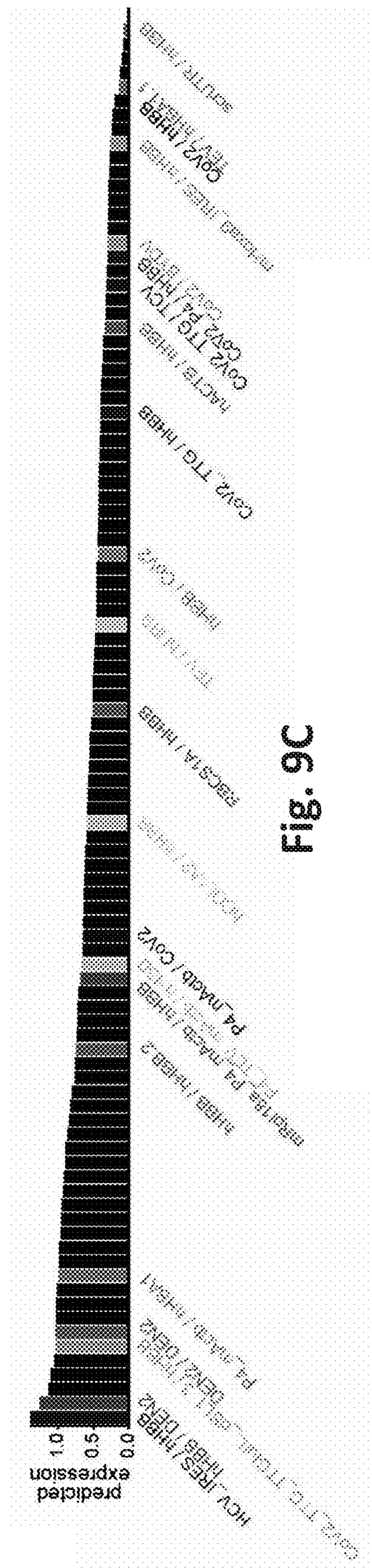


Fig. 9C

**SYSTEMS AND METHODS FOR
PRODUCING RNA CONSTRUCTS WITH
INCREASED TRANSLATION AND
STABILITY**

CROSS-REFERENCE TO RELATED
APPLICATIONS

[0001] The current application is a divisional of U.S. patent application Ser. No. 17/463,466, filed Aug. 31, 2021, which claims priority to U.S. Provisional Patent Application No. 63/072,669, filed Aug. 31, 2020; the disclosures of which is hereby incorporated by reference in its entirety.

FIELD OF THE DISCLOSURE

[0002] The present invention relates to ribonucleic acid (RNA). More specifically, the present invention relates to systems and methods to enhance RNA translatability and assessment thereof.

INCORPORATION OF SEQUENCE LISTING

[0003] This application hereby incorporates by reference the material of the electronic Sequence Listing filed concurrently herewith. The material in the electronic Sequence Listing is submitted as an XML file entitled "06753 DIV_Seq_List.xml" created on Nov. 7, 2022, which has a file size of approximately 412 KB, and is herein incorporated by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

[0004] There are multiple problems with prior methodologies of effecting protein expression. For example, introduced DNA can integrate into host cell genomic DNA at some frequency, resulting in alterations and/or damage to the host cell genomic DNA. Alternatively, the heterologous deoxy-ribonucleic acid (DNA) introduced into a cell can be inherited by daughter cells (whether or not the heterologous DNA has integrated into the chromosome) or by offspring.

[0005] In addition, assuming proper delivery and no damage or integration into the host genome, there are multiple steps which must occur before the encoded protein is made. Once inside the cell, DNA must be transported into the nucleus where it is transcribed into RNA. The RNA transcribed from DNA must then enter the cytoplasm where it is translated into protein. Not only do the multiple processing steps from administered DNA to protein create lag times before the generation of the functional protein, each step represents an opportunity for error and damage to the cell. Further, it is known to be difficult to obtain DNA expression in cells as DNA frequently enters a cell but is not expressed or not expressed at reasonable rates or concentrations. This can be a particular problem when DNA is introduced into primary cells or modified cell lines.

SUMMARY OF THE DISCLOSURE

[0006] This summary is meant to provide examples and is not intended to be limiting of the scope of the invention in any way. For example, any feature included in an example of this summary is not required by the claims, unless the claims explicitly recite the feature. Also, the features described can be combined in a variety of ways. Various features and steps as described elsewhere in this disclosure can be included in the examples summarized here.

[0007] In one embodiment, a method to determine RNA translatability includes obtaining a pool of RNA molecules, where each RNA molecule is uniquely encoded with a barcoding sequence and each barcoding sequence is flanked by at least one profiling sequence, transfecting a cell or cell lysate with the pool of RNA molecules, performing polysome profiling on the pool of RNA molecules to segregate RNA molecules based on the number of ribosomes bound to the RNA molecule, and isolating a first fraction from the polysome profile to generate a first set of RNA molecules showing a first level of ribosomes bound to the RNA molecules in the set of RNA molecules.

[0008] In a further embodiment, the method further includes sequencing the barcode sequence of each RNA molecule in the first set of RNA molecules to identify the presence of each RNA molecule in the first set of RNA molecules.

[0009] In another embodiment, the method further includes determining translatability of the RNA molecules associated with each barcode sequence in the fraction by identifying the prevalence of each barcode in the fraction.

[0010] In a still further embodiment, the RNA molecules are transfected into a collection of cells.

[0011] In still another embodiment, the collection of cells is selected from mammalian cells, yeast cells, bacteria cells, and plant cells.

[0012] In a yet further embodiment, the RNA molecules are added to a cell lysate.

[0013] In yet another embodiment, polysome profiling comprises adding a cell lysate to a sucrose gradient and centrifuging the sucrose gradient to segregate the RNA molecules.

[0014] In a further embodiment again, the barcoding sequence is selected from SEQ ID NOs: 115-1380.

[0015] In another embodiment again, the profiling sequence is selected from SEQ ID NOs: 1381-1382.

[0016] In a further additional embodiment, the method further includes isolating a second fraction from the polysome profile to generate a second set of RNA molecules showing a second level of ribosomes bound to the RNA molecules in the set of RNA molecules, where the first level and second level represent different amounts of bound ribosomes.

[0017] In another additional embodiment, the method further includes sequencing the barcode sequence of each RNA molecule in the first set of RNA molecules and the second set of RNA molecules to identify the presence of each RNA molecule in the first set of RNA molecules and the second set of RNA molecules.

[0018] In a still yet further embodiment, isolating a first fraction from the polysome profile includes isolating a plurality of fractions of the polysome profile, where each fraction in the plurality of fractions generates a set of RNA molecules showing a different level of ribosomes bound to the RNA molecules in that set of RNA molecules.

[0019] In still yet another embodiment, the method further includes sequencing the barcode sequence of each RNA molecule in each set of RNA molecules to identify the presence of each RNA molecule in each set of RNA molecules.

[0020] In a still further embodiment again, the method further includes generating a distribution for each RNA molecule based on the prevalence of each RNA molecule in each fraction.

[0021] In still another embodiment again, isolating a first fraction further comprises introducing a known amount of spike-in RNA molecule, wherein the spike-in RNA molecule serves as an internal reference to allow for quantification of the first set of RNA molecules.

[0022] In a still further additional embodiment, an RNA molecule for increased translation includes a 5' untranslated region, a 3' untranslated region, and a coding sequence, where the 5' untranslated region is located 5' of the coding sequence and the 3' untranslated region is located 3' of the coding sequence.

[0023] In still another additional embodiment, wherein the coding sequence codes for a peptide of interest.

[0024] In a yet further embodiment again, the 5' untranslated region is selected from SEQ ID NOs: 1-55 and SEQ ID NOs: 81-111.

[0025] In yet another embodiment again, the 3' untranslated region is selected from SEQ ID NOs: 56-80.

[0026] In a yet further additional embodiment, the RNA molecule further includes a barcode sequence located 3' of the coding sequence and at least one profiling sequence adjacent to the barcode sequence.

[0027] In yet another additional embodiment, the barcode sequence is selected from SEQ ID NOs: 115-1380 and the profiling sequence is selected from SEQ ID NOs: 1381-1382.

[0028] In a further additional embodiment again, a method to determine RNA stability includes obtaining a pool of RNA molecules, where each RNA molecule is uniquely encoded with a barcoding sequence and each barcoding sequence is flanked by at least one profiling sequence, treating the pool of RNA molecules under an experimental condition, and isolating the pool of RNA molecules at a specified timepoint to generate a fraction of RNA molecules showing stability under the experimental condition for the specified timepoint.

[0029] In another additional embodiment again, the method further includes sequencing the barcode sequence of each RNA molecule in the fraction to identify the presence of each RNA molecule in the fraction of RNA molecules.

[0030] In a still yet further embodiment again, the method further includes determining stability of the RNA molecules associated with each barcode sequence in the fraction by identifying the prevalence of each barcode in the fraction.

[0031] In still yet another embodiment again, the treating step includes transfecting the pool of RNA molecules into a collection of cells.

[0032] In a still yet further additional embodiment, the collection of cells is selected from mammalian cells, yeast cells, bacteria cells, and plant cells.

[0033] In still yet another additional embodiment, the treating step includes adding the pool of RNA molecules to a cell lysate.

[0034] In a yet further additional embodiment again, the treatment condition is selected from temperature, pH, presence of certain molecules, presence of certain ions, concentration of certain molecules, concentration of certain ions, irradiation, buffer type, and buffer concentration.

[0035] In yet another additional embodiment again, the method further includes size selecting for full-length RNA molecules.

[0036] In a still yet further additional embodiment again, size selecting includes performing reverse transcription PCR to transcribe a region from each into cDNA, wherein the

region is selected from a full-length mRNA, a full-length CDS, a 5'UTR-CDS, a 3'UTR-CDS, and the barcode.

[0037] In still yet another additional embodiment again, the isolating step further includes isolating the pool of RNA molecules at a second specified timepoint to generate a second fraction of RNA molecules showing stability under the experimental condition for the specified timepoint.

[0038] In another further embodiment, isolating the pool of RNA molecules further includes introducing a known amount of spike-in RNA molecule, where the spike-in RNA molecule serves as an internal reference to allow for quantification of the fraction of RNA molecules.

[0039] In still another further embodiment, a method for identifying RNA molecules possessing increased translatability and stability includes obtaining a pool of RNA molecules, where each RNA molecule is uniquely encoded with a barcoding sequence and each barcoding sequence is flanked by at least one profiling sequence, assessing translatability of the pool of RNA molecules by transfecting a cell or cell lysate with a first subset of the pool of RNA molecules, performing polysome profiling on the first subset of the pool of RNA molecules to segregate RNA molecules based on the number of ribosomes bound to the RNA molecule, and isolating a fraction from the polysome profile to generate a first set of RNA molecules showing a first level of ribosomes bound to the RNA molecules in the set of RNA molecules, and assessing stability of the pool of RNA molecules by treating a second subset of the pool of RNA molecules under an experimental condition, and isolating a fraction from the second subset the pool of RNA molecules at a specified timepoint to generate a second set of RNA molecules showing stability under the experimental condition for the specified timepoint.

[0040] In yet another further embodiment, the method further includes sequencing the barcode sequence of the first set of RNA molecules and the second set of RNA molecules to identify the presence of each RNA molecule in each fraction of RNA molecules.

[0041] In another further embodiment again, the method further includes determining translatability and stability of the RNA molecules associated with each barcode sequence in the first set of RNA molecules and the second set of RNA molecules by identifying the prevalence of each barcode in each fraction of RNA molecules.

[0042] In another further additional embodiment, the barcoding sequence is selected from SEQ ID NOs: 115-1380.

[0043] In yet another further additional embodiment, the profiling sequence is selected from SEQ ID NOs: 1381-1382.

[0044] In yet again another further additional embodiment, a method to select for RNA elements includes obtaining a library of RNA molecules, where each RNA molecule comprises a coding sequence, a 5' untranslated region (5'UTR), and a 3' untranslated region (3'UTR), where one of the coding sequence, the 5'UTR, or the 3'UTR comprises a degenerate region, assessing a property of the library of RNA molecules, where the property is selected from translatability, in vivo stability, and in vitro stability, and selecting an RNA molecule from the library of RNA molecules showing increase in the property over other RNA molecules in the library of RNA molecules.

[0045] In yet another further additional embodiment again, the method further includes sequencing the selected RNA molecule.

[0046] In a yet further additional embodiment, the selected RNA molecule is a pool of RNA molecules.

[0047] In yet again another further embodiment, the method further includes reassessing the property of the pool of RNA molecules, and selecting an RNA molecule from the pool of RNA molecules showing increase in the property over other RNA molecules in the pool of RNA molecules.

[0048] In again another yet further additional embodiment, the method further includes sequencing the selected RNA molecule from the pool of RNA molecules.

[0049] In yet again another yet further additional embodiment, the property is translatability.

[0050] In yet another yet further additional embodiment again, the degenerate region is selected from a deletion, a random sequence, an ambiguous sequence, and a truncation.

[0051] The foregoing and other objects, features, and advantages of the disclosed technology will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0052] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0053] The description and claims will be more fully understood with reference to the following figures and data graphs, which are presented as exemplary embodiments of the invention and should not be construed as a complete recitation of the scope of the invention.

[0054] FIG. 1A illustrates a generalized structure of RNA molecules in accordance with various embodiments of the invention.

[0055] FIG. 1B illustrates a method for performing iterative selection of RNA elements to enhance translatability and/or stability in accordance with various embodiments of the invention.

[0056] FIG. 2 illustrates a method to screen RNAs for increased translatability in accordance with various embodiments of the invention.

[0057] FIG. 3 illustrates a method to screen RNAs for increased in vivo stability in accordance with various embodiments of the invention.

[0058] FIG. 4 illustrates a method to screen RNAs for increased in vitro stability in accordance with various embodiments of the invention.

[0059] FIG. 5A illustrates a method to screen a pool of RNAs for stability and/or translatability in accordance with various embodiments of the invention.

[0060] FIG. 5B illustrates a method to identify RNAs possessing increased translatability and/or stability in accordance with various embodiments of the invention.

[0061] FIGS. 6A-6C illustrate exemplary data of a heatmap showing RNA presence in various fractions after polysome profiling. FIG. 6A illustrates a full view of the heatmap; while FIGS. 6B-6C illustrate enlarged views of the heatmap of FIG. 6A.

[0062] FIGS. 7A-7C illustrate exemplary data in the form of box and whisker plots showing ribosome load (FIG. 7A), in cell half-life (FIG. 7B), and in solution half-life (FIG. 7C).

[0063] FIGS. 8A-8C illustrate exemplary data of correlations between in cell half-life and ribosome load (FIG. 8A), monosome-to-free-subunit ratio (FIG. 8B), and polysome-to-monomer ratio (FIG. 8C).

[0064] FIGS. 9A-9B illustrate exemplary data of in cell half-life (FIG. 9A) and ribosome load (FIG. 9B).

[0065] FIG. 9C illustrates an exemplary demonstration of how to determine or predict protein expression based on in cell half-life and ribosome load.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0066] Turning now to the drawings, systems and methods to enhance RNA translatability and uses thereof, and systems and methods to quantify RNA stability and translatability and uses thereof are provided. Many embodiments provide nucleic acid molecules (e.g., RNA molecules (including messenger RNA (mRNA)), DNA molecules, DNA/RNA hybrid molecules) that allow for an assessment of in vitro, in vivo, in cell, in solution, in storage, and/or any other form of molecular stability. Some embodiments are directed to RNA molecules, including mRNA, with increased translatability and/or stability. Certain embodiments provide RNA molecules used for RNA therapeutics, including vaccines, where one or more of 1) high and sustained expression of RNA (e.g., mRNA), 2) high stability of RNA inside of cells (e.g., in vivo), and 3) high stability of RNA in solution (e.g., in vitro) is desired.

[0067] Further embodiments provide methods that provide RNA molecules with increased translatability and/or stability, while additional embodiments provide methods to test translatability and/or stability of RNA molecules. Certain embodiments provide a multiplexed workflow to generate RNA molecules, including mRNA, having increased translatability and/or stability in a single assay. In many embodiments, the RNA molecules of various embodiments are generated via rational design, while certain embodiments generate RNA molecules via iterative selection.

RNA Molecules and Design

[0068] As noted above, some embodiments generate RNA molecules via rational design, while others utilize iterative selection. Rational design is a methodology that combines sequence components, such as a 5' UTR, a 3' UTR, and/or a coding region that exist in nature or are synthetically engineered for specific objective (e.g., increased stability or translatability). However, certain embodiments utilize iterative selection to generate RNA molecules, where various sequence components, such as a 5' UTR, a 3' UTR, and/or a coding region, comprise random sequences. Certain embodiments utilizing iterative selection optimize RNA molecules for translatability and/or stability over several rounds of sequence selection (e.g., selecting for sequences showing increased translation or stability).

[0069] Turning to FIG. 1A, an exemplary structure for an embodiment of an RNA molecule in accordance with various embodiments is illustrated. Certain embodiments of an RNA molecule possess a 5' cap moiety. Some embodiments utilize a 7-methyl guanosine triphosphate as the cap moiety, but various additional cap sequences are known in the art for a 5' cap moiety. Additional embodiments possess a cap-proximal sequence for an mRNA region located at the 5' end of the mRNA at the 3' end of the 5' cap moiety. Various cap

sequences are known in the art for a 5' cap-proximal sequence. Certain embodiments use a small triplet, such GGG as the cap-proximal sequence.

[0070] Additional embodiments of an RNA molecule possess a 5' untranslated region (5'UTR) sequence and/or a 3'UTR sequence. Certain embodiments place the 5'UTR near the 5' end of the RNA molecule, while the 3'UTR is located near the 3' end of the molecule. In some embodiments, the 5'UTR is located at the 3' end of a 5' cap moiety, while additional embodiments place the 5'UTR directly at the 5' end without a 5' cap moiety or cap sequence. Similarly, a 3'UTR can be placed at the 3' end of a molecule, while additional embodiments may have a tailing sequence placed 3' of the 3'UTR. Certain embodiments select a 5'UTR and/or a 3'UTR for a variety of factors to increase RNA translatability, stability, and/or other property based on an innate sequence, while others select a 5'UTR and/or a 3'UTR for that may pose improved translatability, stability, and/or other property based on a particular coding sequence of interest. Many possible 5'UTRs and 3'UTRs are known in the art, which are used in various embodiments. Some specific embodiments of rationally designed RNAs select the 5'UTR from natural or modified 5'UTR elements, including SEQ ID NOs: 1-55. And, certain specific embodiments select the 3'UTR from SEQ ID NOs: 56-80. Tables 1 and 2 list various 5'UTRs and 3'UTRs, respectively, with their respective SEQ ID NOs.

TABLE 1

5'UTR Sequences	
Name:	SEQ ID NO:
SynJ	1
hHBB30	2
CYP2E1	3
CYBA	4
mRpl18a	5
RpS25	6
scrUTR	7
TEV	8
hHBB	9
APOA2	10
TOP_hHBB	11
C3	12
hHBB_pA	13
TCV	14
PoV_pA_scrUTR	15
TMV	16
PoV_pA_hHBB	17
RpL38	18
CoV-2-TTG-dSL4-5	19
hACTB	20
RpL31	21
mRpl18a_hHBB	22
DEN2	23
RBCS3B	24
mActb	25
mActb_inv	26
TEV_CERT_hHBB	27
Tubb2b	28
P4_hACTB	29
hCOL1A2	30
BYDV	31
CoV-2-TTG-dSL5	32
P4_mActb	33
P4_mActb_inv	34
CoV-2-TTG-dSL5A-C	35
CoV-2-TTG-dSL1-3	36
mRpl18a_P4_mActb	37
TEV_P4_mActb	38

TABLE 1-continued

5'UTR Sequences	
Name:	SEQ ID NO:
P4_TEV_mActb	39
CoV-2-TTG-dSL4-full	40
CoV-2-TTG-dSL4-1	41
CoV-2-TTG-dSL5A	42
CoV-2-TTG-TTGfull-dSL1-3	43
CoV-2-TTG-dSL5B, C	44
CoV-2-TTG-dSL1	45
CoV-2-TTG-dSL4-2	46
CoV-2-TTG-dSL2	47
CoV-2-TTG-dSL3	48
CoV2	49
CoV2_TTG	50
CoV2_P4	51
CoV-2-TTG-TTGfull	52
mHoxa9_IRES	53
HCV_IRES	54
RBCS1A	55

TABLE 2

3'UTR Sequences	
Name:	SEQ ID NO:
SINV_URE	56
CYBA	57
PV	58
hHBA1	59
CYBA_1.5x	60
BMV	61
hHBB	62
AMV	63
ENE_Wilusz	64
ENE_Weissman	65
BYDV	66
TSV	67
hHBB_F30Pepper	68
P4P6	69
TCV	70
hHBBx2	71
CrPV	72
SINV	73
CoV2	74
DV	75
RV	76
WPRE	77
hActb	78
mActb	79
hCOL1A2	80

[0071] FIG. 1B illustrates a method **100** for iteratively selecting elements to increase RNA translatability and/or stability. Such embodiments identify sequences or segments of an expression-affecting region (e.g., 5'UTR, 3'UTR, and/or coding region) that increase translatability, stability, and/or other property of the RNA molecule.

[0072] At **102**, various embodiments obtain a library of RNA molecules. In certain embodiments, the library comprises RNA molecules with degenerate sequences in regions that affect RNA expression. In certain embodiments, the degenerate expression-affecting region are truncated at its 5'- and/or 3'-end. In some embodiments, the degenerate expression-affecting region contains internal deletions, such that the 5'- and/or 3'-end remain intact, but the overall region is smaller. In certain embodiments, the degenerate sequences are random, ambiguous, and/or mutated sequences to iden-

tify specific bases that may allow for an outsized role in translatability and/or stability.

[0073] At **104**, many embodiments assess stability and/or translatability of the molecules. Various methods to assess translatability and/or stability are described herein. At **106**, certain embodiments select for molecules having a minimum level of translatability and/or stability, such as through selection of a specific fraction of stability and/or translatability. For example, many embodiments select for fractions having high levels of stability (e.g., at longer time points) and/or translatability (e.g., higher polysome fractions).

[0074] Upon assessing stability and/or translatability, certain embodiments sequence the selected for molecules at **108**. Sequencing the selected molecules identifies the specific sequences that correlate to the tested characteristic (e.g., translatability and/or stability).

[0075] It should be noted that many embodiments may perform several features multiple times, such as the assessing **104** and selecting **106** features, in order to identify the sequences having the highest rates of translatability and/or stability. For example, numerous embodiments take the selected for molecules (e.g., ones having high levels of translatability and/or stability) and reassess the translatability and/or stability of these molecules, selecting for high levels of high levels of translatability and/or stability. Various embodiments repeat the assessing **104** and selecting **106** features 2 times, 3 times, 4 times 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, or more times to identify molecules having the highest levels of translatability and/or stability. Additionally, various embodiments repeat sequencing **108**, such as after each selection **106**, or just after every second selection **106**.

[0076] Methods, such as method **100**, allow for iterative selection of expression-affecting regions to further increase translatability, stability, and/or other property. Some exemplary embodiments utilize a pool 5'-UTR selected from SEQ ID NOs: 81-111. Table 3 identifies specific pools of 5'UTR sequences for iterative selection.

TABLE 3

5'UTR Sequence Pools	
Pool 1	SEQ ID NOs: 81-90
Pool 2	SEQ ID NOs: 91-101
Pool 3	SEQ ID NOs: 102-106
Pool 4	SEQ ID NOs: 107-111

[0077] Returning to FIG. 1A, many embodiments of an RNA molecule possess a coding sequence, or CDS, located 3' from the 5'UTR, and 5' of the 3'UTR. In many embodiments, the CDS begins (e.g., at its 5'-end) with a start codon (e.g., the canonical AUG and/or any other codon known to begin translation). In many embodiments, the CDS terminates (e.g., at its 3'-end) with a stop codon. In various embodiments the stop codon is a canonical stop codon (e.g., UAG, UAA, UGA), while further embodiments comprise a noncanonical stop codon or another sequence shown to terminate translation. Certain embodiments comprise more than one stop codon in the CDS.

[0078] The coding sequence is a designed sequence of interest to encode a protein or peptide of interest. In certain embodiments, the coding sequence encodes an epitope or other antigen to induce an immune response, thus allowing creation of a vaccine. In various embodiments, the protein or

peptide of interest is used as a therapeutic directly, such that the protein or peptide of interest replaces or supplements a dysfunctional protein or peptide. In some embodiments, the protein or peptide of interest corrects for dysfunction of another protein or peptide. While protein coding sequences are described in the context of this exemplary embodiment, additional embodiments possess sequences for non-coding RNAs, such as RNAs that guide genome editing and/or coat chromatin. Various embodiments possess a CDS encoding a reporter gene; for example, nanoluciferase ("Nluc", SEQ ID NO: 112), green fluorescence protein ("GFP", SEQ ID NO: 113), and/or any other reporter gene of interest. Various embodiments encode a therapeutic, such as a multi-epitope vaccine ("MEV", SEQ ID NO: 114).

[0079] Additional embodiments of an RNA molecule include a barcode to identify particular molecules based on unique sequences. Many barcode schemes are known in the art and range from 2 to 12 or more nucleotides. In many embodiments, the barcodes are 6-9 nucleotides in length. Certain embodiments select one or more barcodes from SEQ ID NOs: 115-1380.

[0080] To read barcodes, an RNA molecule can include one or more profiling sequences that can be used by PCR primers or sequencing primers to amplify and/or sequence the barcode region. In some embodiments profiling sequences are located at the 5' and/or 3' end of a barcode. In many embodiments, profiling sequences flank the barcode. In various embodiments profiling sequences are selected from profiling sequence 1 (SEQ ID NO: 1381) and profiling sequence 2 (SEQ ID NO: 1382).

[0081] As noted above, some embodiments of an RNA molecule possess a tailing sequence located at the 3' end of a molecule. In various embodiments the tailing sequence is used to add a poly-A tail or other structural sequence to an RNA molecule. In some embodiments, the tailing sequence is selected as SEQ ID NO: 1383.

[0082] Structures, such as those described above in regard to FIG. 1 allow for modular and combinatorial testing of various 5'UTRs, CDSs, and 3'UTRs.

Methods of Assessing RNA Translatability

[0083] Certain embodiments assess translatability of RNA molecules, such as those described elsewhere herein. An exemplary embodiment of a method **200** to assess translatability is illustrated in FIG. 2. In method **200**, an RNA molecule is obtained at **202** of many embodiments. In certain embodiments, the RNA molecule is generated via in vitro transcription. Additionally, certain embodiments generate an RNA transcript and/or further modify RNA transcripts to be used for translation (e.g., including a 5' cap and/or a 3' polyA tail). Some embodiments obtain DNA templates from a commercial vendor. In various embodiments, polymerase chain reaction (PCR) is used to amplify a full-length DNA template for the RNA molecule. Additional embodiments assess amplicon quality via electrophoresis, including gel (agarose and/or polyacrylamide) and/or capillary electrophoresis (e.g., ABI 3700 and/or Agilent Bioanalyzer). Further embodiments transcribe DNA amplicons to RNA using a DNA-dependent RNA polymerase. Certain embodiments perform the in vitro transcription using commercial kits, including Thermo's T7 MEGAScript kit. Various embodiments modify the RNA transcripts with a 5' cap and/or polyA tail. These modifications can be accomplished using kits, such as the Cellscript kit and/or any

other applicable and commercially available kit. Additional cleanups can be accomplished at various stages (e.g., after PCR, after transcription, and/or after modification), using columns or reagents, such as Thermo's MEGAClear columns. And, quality of the transcribed and/or modified RNAs can be accomplished via electrophoresis, including gel and capillary electrophoresis. Further embodiments quantify the RNA pool via various known means, such as spectrophotometry, fluorometry, or and/or any other known method for quantifying nucleic acids.

[0084] In various embodiments, the RNA molecule is obtained as a pool of RNA molecules, where each unique RNA sequence in the pool comprises a unique barcode, such as described herein. In certain embodiments, the RNA molecules within the pool are approximately the same length. In certain embodiments, the RNA molecules within the pool vary in length.

[0085] Various embodiments transfect RNA transcripts into cells or add the transcripts to a cellular lysate at **204**. In certain embodiments, transfection occurs on cultured cells or tissue, including mammalian cells, while other embodiments use yeast, bacteria, or plant cells. Some specific embodiments transfect HEK293T cells. Various embodiments incubate the transfected cells to allow for translation of the RNAs. Incubation can last between 1 hour and several days (e.g., 7-10 days) at temperatures and/or conditions to encourage cellular growth and translation. Culture media can include antibiotics or other selective reagents to prevent growth of non-transfected cells and/or contamination. Certain embodiments utilize a cellular lysate as a proxy of in vivo stress on RNA. In such embodiments, cultured cells are lysed via a known method, such as sonication, hydrodynamic stress, or any other method to generate cellular lysate. In various embodiments, the RNA molecule(s) are added to the lysate and allowed to react for a period of time, such as between 1 hour and several days (e.g., 7-10 days) and at temperatures commensurate with the operating temperature for the RNA (e.g., average body temperature, 37° C.).

[0086] At **206**, certain embodiments perform polysome profiling. In various embodiments, the polysome profiling separates RNA molecules or transcripts based on the number of ribosomes located on, or bound to, a transcript or RNA molecule. As ribosomes are the machinery for translation, the number of ribosomes located on a transcript is indicative of the translatability of a particular transcript.

[0087] In certain embodiments, polysome profiling uses a sucrose gradient (e.g., a continuous sucrose gradient) to fractionate RNA molecules based on the number of ribosomes (e.g., polysomes) located on the transcript. Various embodiments perform polysome profiling by lysing transfected cells and applying the lysate to a column containing a sucrose gradient. In embodiments, where RNA transcripts are applied to a cellular lysate, the lysate is directly added to a sucrose gradient column. Centrifugation is applied to the column to separate transcripts based on the number of ribosomes attached to a transcript.

[0088] At **208**, many embodiments isolate or extract one or more fractions of RNA molecules from the polysome profile. In certain embodiments, the fractions are isolated from the sucrose gradient. In various embodiments, the fractions are isolated as slices, drops, and/or other method of obtaining a fraction from a sucrose gradient. Actively translating RNA molecules have a higher number of ribosomes associated with them and are found in polysomal fractions

(e.g., more ribosomes bound to the RNA molecule) whereas non-translating/poorly-translating RNA molecules are present in a free RNA fraction or associated with ribosomal subunits (e.g., 40S ribosomal subunit). In certain embodiments, fractions representing higher amounts of ribosomes bound to RNA are isolated, while some embodiments isolate fractions representing a range of ribosomes bound to RNA in order to identify a distribution of ribosomes present for a particular transcript sequence. RNA molecules from an isolated fraction can be cleaned up via known procedures or kits, including columns.

[0089] Certain embodiments introduce a known amount of one or more RNA molecules as a spike-in. Spike-ins serve as an internal reference to allow for quantification of molecules within the assessed RNA library. Such spike-ins are unique RNA molecules that are not present in the analyzed RNA library. The spike-ins can be similar in length to the molecules in the library, and/or possess unique sequences or barcodes.

[0090] Various embodiments identify the RNA molecules located in the one or more fractions based on their barcodes at **210**. As noted above in relation to FIG. 1, many embodiments of RNA molecules contain a barcode sequence (e.g., SEQ ID NOs: 115-1360). The profiling sequences flanking the barcodes (e.g., SEQ ID NOs: 1381-1382) can be used to amplify the barcode or can be used as sequencing primers for barcoding reads of the RNA molecules of certain embodiments. Further embodiments utilize hybridization probes, quantitative PCR (qPCR), or any other known method with or without pooling strategies to identify which RNAs are present in each fraction.

Methods of Assessing In Vivo or In-Cell RNA Stability

[0091] Certain embodiments assess the stability of RNA molecules, including stability within in vivo and in vitro environments. An exemplary embodiment of a method **300** to assess stability is illustrated in FIG. 3. In method **300**, RNA is obtained at **302**. Obtaining RNA at **302** can be accomplished via many methods, including such steps as described in regard to method **200** (FIG. 2), including the obtention of a pool of RNA molecules, where each unique RNA sequence is identifiable by a unique barcode.

[0092] Various embodiments transfect RNA transcripts into cells or add the transcripts to a cellular lysate at **304**. In certain embodiments, transfection occurs on cultured cells or tissue, including mammalian cells, while other embodiments use yeast, bacteria, or plant cells. Some specific embodiments transfect HEK293T cells. Various embodiments incubate the transfected cells. Incubation can last between 1 hour and several days (e.g., 7-10 days) at temperatures and/or conditions to encourage cellular growth. Culture media can include antibiotics or other selective reagents to prevent growth of non-transfected cells and/or contamination. Certain embodiments utilize a cellular lysate as a proxy of in vivo stress on RNA. In such embodiments, cultured cells are lysed via a known method, such as sonication, hydrodynamic stress, or any other method to generate cellular lysate. Then, the RNAs are added to the lysate and allowed to react for a period of time, such as between 1 hour and several days (e.g., 7-10 days) and at temperatures commensurate with the operating temperature for the RNA (e.g., average body temperature, 37° C.).

[0093] At **306**, certain embodiments isolate RNAs based on in-cell stability. In various embodiments, RNAs are

isolated from transfected cells, while some embodiments isolate the RNAs from a cellular lysate. Certain embodiments isolate RNA from transfected cells at various time points (e.g., after 1 hour, 2 hours, 3 hours, 6 hours, 12 hours, 24 hours, etc.) to create time-based fractions of RNAs. Based on the relative amounts of an RNA at the different timepoints, assessment of RNA stability can be derived, and a RNA half-life can be calculated. Additionally, isolated RNA molecules can be cleaned up via known procedures or kits, including isolation protocols, kits, columns, or any other known method for isolating RNA from cells or a lysate.

[0094] Some embodiments select for stable RNAs by performing reverse transcription PCR (RT-PCR) to amplify long, full length RNA regions, for example the full-length mRNA, full-length CDS, 5' UTR-CDS, 3' UTR-CDS, or any other length covering functional region, or only the barcode region, into complimentary DNA (cDNA). By creating cDNAs, downstream amplifications can utilize DNA-dependent polymerases to create sequencing libraries or other molecules for analysis. Such embodiments select for full length or any longer functional length of RNAs rather than RNAs that may have been hydrolyzed but may still be of sufficient length that electrophoresis or other methods do not remove them.

[0095] Certain embodiments introduce a known amount of one or more RNA molecules as a spike-in. Spike-ins serve as an internal reference to allow for quantification of molecules within the assessed RNA library. Such spike-ins are unique RNA molecules that are not present in the analyzed RNA library. The spike-ins can be similar in length to the molecules in the library, and/or possess unique sequences or barcodes.

[0096] Various embodiments identify the RNAs based on their barcodes at **308**. As noted above in relation to FIG. 2, many embodiments of RNA molecules contain a barcode sequence (e.g., SEQ ID NOs: 115-1380). The profiling sequences flanking the barcodes (e.g., SEQ ID NOs: 1381-1382) can be used to amplify the barcode or can be used as sequencing primers for barcoding reads of the RNA molecules of certain embodiments. Further embodiments utilize hybridization probes, quantitative PCR (qPCR), or any other known method with or without pooling strategies to identify which RNAs are present in timepoint based fractions.

Determination of In Vitro or in Solution RNA Stability

[0097] An additional challenge for RNA therapeutics, including vaccines, include the stability in storage, such as between manufacture and actual treatment or delivery to an individual. Such stability is referred to as in vitro stability, as it emphasizes stability in non-biological environments, such as in vials, syringes, or other method of storage. Various embodiments provide a method to measure in vitro stability of RNAs. Turning to FIG. 4, a method **400** to determine in vitro RNA stability in accordance with various embodiments is illustrated. Within method **400**, RNA is obtained at **402**. Obtaining RNA at **402** can be accomplished via many methods, including such steps as described in regard to method **200** (FIG. 2), including the obtention of a pool of RNA molecules, where each unique RNA sequence is identifiable by a unique barcode.

[0098] At **404** of many embodiments, the RNA pool is treated or subjected to an experimental condition. The experimental conditions include any condition that may

cause degradation of an RNA molecule in a storage situation, including (but not limited to) temperature, pH, presence of certain molecules and/or ions, concentration of certain molecules and/or ions, irradiation, time, buffer type, buffer concentration, and/or any other condition that can affect RNA stability. Such conditions are meant to reproduce actual conditions that can induce one or more hydrolytic events within the RNA molecules. A hydrolytic event, in accordance with various embodiments, causes a break within the RNA molecule, resulting in a broken or incomplete RNA molecule. Incomplete or broken RNA molecules may be insufficient for use as a therapeutic, as they may be prone to degradation or ineffective in protein production, thus incomplete or broken RNA molecules may limit the efficacy of the molecule as a therapeutic.

[0099] Further embodiments further select for stable RNAs in the pool at **406**. In some embodiments, the selection occurs by size selecting for full length RNAs, such as through electrophoresis, including (but not limited to) agarose gel electrophoresis, polyacrylamide electrophoresis, and capillary electrophoresis.

[0100] Some embodiments select for stable RNAs by performing reverse transcription PCR (RT-PCR) to amplify long RNA regions, for example the full-length mRNA, full-length CDS, 5' UTR-CDS, 3' UTR-CDS, or any other length covering functional region, or only the barcode region, into complimentary DNA (cDNA). By creating cDNAs, downstream amplifications can utilize DNA-dependent polymerases to create sequencing libraries or other molecules for analysis. Such embodiments select for full length or any longer functional length of RNAs rather than RNAs that may have been hydrolyzed but may still be of sufficient length that electrophoresis or other methods do not remove them.

[0101] Certain embodiments introduce a known amount of one or more RNA molecules as a spike-in. Spike-ins serve as an internal reference to allow for quantification of molecules within the assessed RNA library. Such spike-ins are unique RNA molecules that are not present in the analyzed RNA library. The spike-ins can be similar in length to the molecules in the library, and/or possess unique sequences or barcodes.

[0102] Many embodiments isolate RNAs based on in vitro or in solution stability at **408**. Certain embodiments isolate RNA from a solution at various time points (e.g., after 1 hour, 2 hours, 3 hours, 6 hours, 12 hours, 24 hours, etc.) to create time-based fractions of RNAs from a solution. Based on the amount of an RNA at the timepoint 0, relative assessment of RNA stability can be derived, and a RNA half-life can be calculated. Additionally, isolated RNA molecules can be cleaned up via known procedures or kits, including isolation protocols, kits, columns, or any other known method for isolating RNA from cells or a lysate.

[0103] At **410**, stable RNAs are identified. In various embodiments, the undigested or gel-extracted RNAs are sequenced using the barcode to identify the particular molecules that are stable. In many embodiments, cDNAs created in **406** are utilized as templates to create a sequencing library to avoid the amplification of RNAs that may be near full length.

Identifying RNAs Having Enhanced Translatability, Stability, and/or Other Property

[0104] Turning to FIG. 5A, certain embodiments are capable of simultaneously assessing one or more of trans-

latability, stability, and/or any other property. Such embodiments assess one or more of translatability, in vivo (or in cell) stability, in vitro (or in solution) stability, and/or any other property. Within method **500**, RNA is obtained at **502**. Obtaining RNA at **502** can be accomplished via many methods, including such steps as described in regard to method **200** (FIG. 2), including the obtention of a pool of RNA molecules, where each unique RNA sequence is identifiable by a unique barcode. Many embodiments perform one or more of assessing translatability **504**, assessing in vivo (or in cell) stability **506**, and/or assessing in vitro (or in solution) stability **508**. Assessing translatability **504** can be performed via methods, such as method **200** (FIG. 2), while in vivo stability **506** can be performed via method **300** (FIG. 3), and assessing in vitro stability can be performed via method **400** (FIG. 4). Upon obtaining fractions from the one or more of assessing translatability **504**, assessing in vivo stability **506**, and/or assessing in vitro stability **508**, various embodiments can identify RNAs at **510**.

[**0105**] Turning to FIG. 5B, various embodiments identify RNA molecules possessing increased translatability in method **550**. At **552**, many embodiments obtain identities of RNA molecules present in various fractions of translatability (e.g., RNAs assessed via methods **200**, **300**, **400**, and/or **500**). In various embodiments, these identities include the barcode or barcodes that identify each of the RNA molecules in a fraction and a read count of each barcode in each fraction.

[**0106**] At **554**, various embodiments determine the translatability of each RNA molecule by identifying prevalence of each barcode in each fraction. Certain embodiments perform statistical analyses to relative prevalence of the barcode in each fraction. The presence of RNAs in fractions correlating to more ribosomes, indicate increased translatability of that particular RNA molecule as compared to other fractions across the whole polysome profile gradient.

[**0107**] Some embodiments filter RNA molecules based on particular characteristics at **556**. Particular characteristics may be specific cutoffs, minimum levels of translatability, or a statistical distribution of a particular barcode. For example, certain embodiments may select barcodes that have a narrower distribution with a lower average ribosomal load (e.g., fewer ribosomes on RNA molecules), while other embodiments may select for a higher average with a broader overall distribution.

[**0108**] Various embodiments deconvolve the barcodes at **558**, where deconvolution involves matching the specific RNA sequence or sequence name with the barcode sequence comprised within that RNA molecule.

[**0109**] Additional embodiments output results of translatability, stability, and/or other property at **560**. Certain embodiments provide lists of each of the sequences providing a specific cutoff or parameter for minimum translatability, stability, and/or other property. Various embodiments produce a graphical display or visualization, such as a dot plot, heat map, or other graph or chart to visualize stability (e.g., in vivo, in vitro, in cell, in solution, etc.), translatability, and/or any other property of a particular RNA molecule.

[**0110**] Additional embodiments output results of predicted protein expression at a given time or total protein expression over time, from experimentally determined stability and translatability. For this, additional embodiments can use modelling of the empirical data to estimate the predicted

protein expression in a pool of hundreds of different RNA molecules based on measurements of a selected number of RNA designs.

Enhanced Translatability of RNA Molecules

[**0111**] Turning to FIGS. 6A-6C, exemplary results of embodiments showing translation efficiency are illustrated, where FIG. 6A illustrates a heatmap and FIGS. 6B-6C show enlarged portions of FIG. 6A. FIGS. 6A-6C illustrate the relative prevalence of 64 unique RNA molecules in accordance with various embodiments based on polysome fraction. Darker cells indicate a lower relative prevalence of the molecule in a particular fraction, while lighter colors indicate a higher relative prevalence of the molecule in a particular fraction.

[**0112**] Additionally, FIGS. 7A-7C illustrate exemplary data plotting ribosomal load (FIG. 7A), half-lives for in-cell (or in vivo) stability (FIG. 7B) and in solution (or in vitro) stability (FIG. 7C) of various mRNA molecules, including mRNA molecules having 5'UTR variants, 3'UTR variants, both 5'UTR and 3'UTR variants, and various CDS sequences, including from Nluc, eGFP, and MEV. For ribosomal load in FIG. 7A, ribosomal load is determined by the equation listed in FIG. 7A.

[**0113**] FIGS. 8A-8C illustrate exemplary data showing correlations of in cell mRNA half-life to ribosomal load (FIG. 8A), in cell mRNA half-life to monosome-to-free-subunit ratio (FIG. 8B), and in cell mRNA half-life to polysome-to-monomosome ratio (FIG. 8C).

[**0114**] Given the assessment of in cell stability and translatability in accordance with various embodiments, further embodiments determine protein expression levels of proteins encoded in a CDS of the molecule. Certain embodiments determine protein expression via the equation:

$$P(t) \sim k_t \frac{e^{-k_p t} - e^{-k_m t}}{k_m - k_p}$$

Where P(t) is protein quantity at time t; k_t is translation rate; and k_m and k_p are rates of mRNA and protein decay, respectively.

[**0115**] An exemplary demonstration of predicted expression is illustrated in FIGS. 9A-9C, where FIG. 9A illustrates in cell half-life of various mRNA constructs, FIG. 9B illustrates ribosomal load of various constructs, and FIG. 9C illustrates the predicted expression.

EXEMPLARY EMBODIMENTS

[**0116**] Although the following embodiments provide details on certain embodiments of the inventions, it should be understood that these are only exemplary in nature, and are not intended to limit the scope of the invention.

Example 1: In Vitro Transcription of Reporter mRNAs

[**0117**] Method: Preparation of mRNAs were based on in vitro transcription from DNA templates. DNA templates were amplified by PCR using AccuPrime Pfx (Life Technologies, 12344024) and purified using the Monarch PCR & DNA Cleanup Kit (NEB, T1030L). The source of the 3×HA-Nluc starting CDS (“Nluc start”) is derived from the

pcDNA3.1-5'UTR-3×HA-Nluc plasmid encoding the HA-tagged Nanoluc CDS. Individual template DNA or the 233-mRNA library was amplified from linear DNA synthesized on a BioXP 3200 system (Codex DNA) or by Twist Bioscience, using the fixed forward (T7_F_28nt) and reverse (const3_R) primer. The forward primer binds to the T7 RNA polymerase promoter common in DNA template for all mRNA designs; the reverse primer is complementary to a common “const3” region at the end of all tested mRNA 3' UTRs. For the IVT template pool, individual DNA templates were pooled for a template pool of hundreds of constructs at an equimolar concentration and are amplified with outer primers in a pooled format. For the pooled template, 1 μ L of each construct (~20 ng/ μ L stock concentration) was pooled to be used as the PCR template. The Pfx PCR contained the following: 2.5 μ L 10×Pfx buffer, 0.25 μ L forward primer (100 μ M), 0.25 μ L reverse primer (100 μ M), 0.75 μ L DMSO (NEB), 0.25 μ L Pfx Polymerase (Thermo), 20.5 water, and 0.5 μ L template DNA (~20-50 ng/ μ L), in a total 25 μ L reaction with the following program: 2 min at 95° C.; 10 sec at 95° C.; 30 sec at 58° C.; 30 s or 1 min at 68° C.; cycled 9×; final extension of 5 min at 68° C. PCR reactions were purified with Monarch PCR & DNA Cleanup Kit (NEB, T1030L). For the hHBB-Fluc control mRNA, the DNA template was amplified from the pGL3-HBB plasmid using the primers KL588/KL589 which yielded a PCR product of 1,750 kb in length. For cloning the MALAT1 ENE 3' UTR stem-loop, we first amplified the ENE region using primers ENE-1/ENE-2 with flanking constant regions. The resulting amplicon was assembled with a hHBB-Nluc sequence that lacked a 3' UTR but maintained a unique barcode using a NEBuilder HiFi Assembly Kit (NEB, ES2621).

[0118] In vitro transcription was performed with the MEGAscript T7 kit (Ambion, AM1333) according to the manufacturer's instructions. A 20 μ L transcription reaction contained max. 5 μ g linear DNA template, 4 mM of each NTP (Ambion), 2 μ L/200 U MEGAscript T7 RNA polymerase (Ambion) and 1×T7 MEGAscript Transcription Buffer (Ambion). After a total incubation for 3 hours at 37° C., the DNA was digested by addition of 1 μ L/2 U Turbo DNase (Ambion, AM2238) for 15 min at 37° C. For pseudouridylated mRNAs, pseudouridine triphosphate (Trilink Biotechnologies, N1019-5) was substituted for uridine triphosphate at an equivalent concentration. mRNA was purified using MegaClear columns (Thermo Scientific, Ambion, AM1908). A 20 μ L reaction usually yielded 100-150 μ g of RNA.

[0119] For mRNA transfection of HEK293T cells, m⁷G-capped and polyadenylated mRNAs were generated as follows. In vitro transcribed mRNA was then m⁷G-capped and polyadenylated using the ScriptCap m7G Capping System (CellScript, C-SCCE0625) and A-Plus Poly(A) Polymerase Tailing Kit (CellScript, C-PAP5104H), respectively, according to the manufacturer's instruction with the following modifications. Aliquots of 30 μ g of each RNA were processed in parallel, diluted to 34.25 μ L in water and heated for 5 min at 65° C. to denature and placed on ice. The 50 μ L capping reaction contained 5 μ L 10× ScriptCap buffer (Cellscript), 5 μ L 10 mM GTP (Cellscript), 2.5 μ L 2 mM S-adenosyl-methionine (SAM, 20 mM stock, Cellscript), 1.25 μ L ScriptGuard RNase Inhibitor (Cellscript), and 2 μ L Capping enzyme (20 U, Cellscript, 10 U/ μ L). For the capping step, the 37° C. incubation was performed for 1 hour and the capped RNA was placed on ice. Polyadenylation was

performed from the resulting RNAs without purification in between. The polyA reaction contained 30 μ g of capped mRNA in 50 μ L, 6.6 μ L 10× A-Plus polyA tailing buffer (Cellscript), 6.6 μ L 10 mM ATP (Cellscript), 0.3 μ L ScriptGuard RNase Inhibitor (Cellscript), and 2.5 μ L A-Plus PolyA Polymerase (10 U, 4 U/ μ L, Cellscript) in a total reaction volume of 66 μ L. We aimed to add a 150 nt-long polyA-tail for which we incubated the capped mRNA for 30 min at 37° C. with 10 U of polyA enzyme, after which the reaction was placed on ice. The mRNA was again purified using MegaClear columns. mRNA concentration was determined on a Nanodrop 2000 (Thermo Fisher). This usually yields 30-40 μ g of capped and polyadenylated mRNA. mRNA quality was determined by 4% urea-PAGE, 1% formaldehyde agarose gel or capillary electrophoresis with an Agilent 2100 Bioanalyzer (Agilent Technologies).

Example 2: Cell Culture and Transfections

[0120] Method: HEK293T (ATCC: CRL-3216) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, 11965-118) containing 2 mM L-glutamine, supplemented with 10% fetal bovine serum (EMD Millipore, TMS-013-B), 100 U/ml penicillin and 0.1 mg/ml streptomycin (EmbryoMax ES Cell Qualified Penicillin-Streptomycin Solution 100×; EMD Millipore, TMS-AB2-C or Gibco, 15140-122) at 37° C. in 5% CO₂-buffered incubators. For transfection of pooled 5' m⁷G-capped and poly(A)-tailed RNAs, 5.0×10⁶ HEK293T cells were seeded in a 10 cm plate 24 h before transfection. 10 μ g of pooled RNAs were transfected using Lipofectamine MessengerMax as per manufacturer's instructions (Life Technologies). Media was changed 3 h after transfection and replaced with complete DMEM supplemented with 10% FBS and Pen/Strep. For transfections of individual m⁷G-capped RNAs, 3.0×10⁴ HEK293T cells were seeded per well 24 h before transfection in a 96-well plate. Subsequently, 10 ng of Nluc RNA was co-transfected with 20 ng of m⁷G-capped HBB-Fluc control RNA using Lipofectamine MessengerMax as per manufacturer's instructions (Life Technologies).

Example 3: Sucrose Gradient Fractionation Analysis

[0121] Method: Cell culture media was replaced with cycloheximide (MilliporeSigma, C7698-1G) containing media at 100 μ g/mL. After 2 minutes, cells were washed, trypsinized and harvested using PBS, trypsin, and culture media containing 100 μ g/mL cycloheximide. ~10×10⁶ cells were resuspended in 400 μ L of following lysis buffer on ice for 30 min, vortexing every 10 min: 25 mM Tris-HCl pH 7.5, 150 mM NaCl, 15 mM MgCl₂, 1 mM DTT, 8% glycerol, 1% Triton X-100, 100 μ g/mL cycloheximide, 0.2 U/ μ L Superase-In RNase inhibitor (ThermoFisher Scientific, AM2694), 1× Halt protease inhibitor cocktail (ThermoFisher Scientific, 78430), 0.02 U/ μ L TURBO DNase (ThermoFisher Scientific, AM2238). After lysis, nuclei were removed by two step centrifuging, first at 1300 g for 5 min and second at 10000 g for 5 min, taking the supernatants from each. 25%-50% sucrose gradient was prepared in 13.2 mL ultracentrifuge tubes (Beckman Coulter, 331372) using Biocomp Gradient Master with the following recipe: 25 or 50% sucrose (w/v), 25 mM Tris-HCl pH 7.5, 150 mM NaCl, 15 mM MgCl₂, 1 mM DTT, 100 μ g/mL cycloheximide. The lysate was layered onto the sucrose gradient and ultracentrifuged.

trifuged on Beckman Coulter SW-41Ti rotor at 40000 rpm for 150 min at 4° C. The gradient was density fractionated using Brandel BR-188 into 16×750 μL fractions, and in vitro transcribed spike-in RNA mix (120002B1, 120010B1, 220023B1, 310333T3; 1000, 100, 10, 1-fold dilutions respectively) were added to each fraction. 700 μL of each fraction was mixed with 100 μL 10% SDS, 200 μL 1.5 M sodium acetate, and 900 μL acid phenol-chloroform, pH 4.5 (ThermoFisher Scientific, AM9720), heated at 65° C. for 5 min, and centrifuged at 20.000 g for 15 min at 4° C. for phase separation. 600 μL aqueous phase was mixed with 600 μL 100% ethanol and RNA was purified on silica columns (Zymo, R1013).

Example 4: Polysome Selection and Library Preparation

[0122] Method: The variant 5' UTR is composed of: fixed first 29 nt of hHBB, variable 35 nt (initially degenerate) and 6 nt Kozak consensus. To generate the reporter mRNA pool containing the variant 5' UTR library, IVT template was first assembled by PCR under the following conditions: 4 μL 10× AccuPrime Pfx Reaction Mix, 0.4 pmol HBB29_N35 amplicon, 0.4 pmol Nluc_HBB_3UTR, 0.4 μL AccuPrime Pfx Polymerase in 40 μL of total reaction volume. Cycling conditions are: 95° C. for 120 sec, and 19 cycles of 95° C. for 15 sec, 66° C. for 30 sec, 68° C. for 75 sec. PCR product was purified on silica columns (NEB T1034) and amplified with under the following conditions: 4 uL 10× AccuPrime Pfx Reaction Mix, 4 μL 10 μM T7_28_HBB_30_F, 4 μL 10 μM Nanoluc_ORF_R, 0.4 μL AccuPrime Pfx Polymerase in 40 μL total reaction volume. Cycling conditions are: 95° C. for 120 sec, and 4 cycles of 95° C. for 15 sec, 66° C. for 30 sec, 68° C. for 75 sec. The mRNA was in vitro transcribed, capped and polyadenylated as described above. This yields an estimated initial starting degenerate pool complexity of $\sim 2.4 \times 10^{11}$.

[0123] Transfection of HEK-293 cells and sucrose gradient fractionation were performed as described above. Equal volumes of fractions 10-16 were pooled and RNA was by acidic phenol chloroform extraction followed by column purification (Zymo Research, R1013) as described above. 1/3 lysate volume was kept as input before layering onto the sucrose gradient and RNA was extracted from the input lysate by Trizol extraction followed by column purification. 1.5 μg RNA in 5.5 μL was mixed with 0.5 μL 2 uM RT_Nluc26_UM112_Read1Partial and 0.5 μL 10 mM dNTPs each. The RNA samples were then denatured at 65° C. for 5 min and chilled to 4° C. 3.5 μL reverse transcription mix was added to 10 μL total reaction volume: 2 μL 5× Superscript IV buffer, 0.5 μL 10 mM DTT, 0.5 μL Superase-In (ThermoFisher Scientific, AM2694), 0.5 μL Superscript IV (Thermo 18091050). The reaction was incubated at 55° C. for 45 min and inactivated at 80° C. for 10 min. Variant 5' UTR amplicon was amplified from the reverse transcription reaction via PCR under the following reaction conditions: 4 μL RT reaction, 40 μL 2× Q5 Hot Start Master Mix (NEB M0494S), 0.8 μL 100×SYBR (Thermo S7563), 4 μL 10 μM T7_28_HBB_29_F, 4 μL 10 μM Nanoluc_ORF_R, in 80 μL total reaction volume. Cycling conditions were as follows: 98° C. for 60 sec, and 15 cycles of 98° C. for 10 sec, 68° C. for 10 sec, 72° C. for 10 sec. PCR product was purified on silica columns (NEB T1034) and assembly with Nluc_HBB_3UTR fragment was performed as described above for initial preparation of IVT template using HBB29_

N35 amplicon. The mRNA was in vitro transcribed, capped and polyadenylated as described above. The same process of transfection, fractionation, reverse transcription, PCR amplification, assembly and in vitro transcription was repeated.

[0124] For sequencing library preparation, the RT reaction was PCR amplified under the following conditions: 1 μL RT reaction, 10 μL 2× Q5 Hot Start Master Mix (NEB M0494S), 0.2 μL 100×SYBR (Thermo S7563), 1 μL 10 μM Read1, 1 μL 10 μM Read2Partial_HBB29 in 20 μL total reaction volume. Cycling conditions were as follows: 98° C. for 60 sec, and 15 cycles of 98° C. for 10 sec, 68° C. for 10 sec, 72° C. for 10 sec. Sequencing adaptors were added using the following conditions for final round PCR: 1 μL first round PCR reaction, 10 μL 2× Q5 Hot Start Master Mix, 0.2 μL 100×SYBR, 1 μL 10 uM NEBNext Index Primer (NEB E7335, NEB E7500, NEB E7710, NEB E7730, NEB E6609), 1 μL 10 uM NEBNext Universal PCR Primer in 20 μL total volume. Cycling conditions are: 98° C. for 60 sec, and 5 cycles of 98° C. for 10 sec, 72° C. for 10 sec. All barcoded samples were then pooled at equal volumes and purified with 1.1×SPRiselect beads Beckman Coulter B23317). Sequencing was performed at the Stanford Functional Genomics Facility (SFGF) at Stanford University, on the Illumina NextSeq 550 instrument, using a high output kit, 1×81 cycles.

Example 5: In Cell and In-Solution RNA Degradation Time Courses

[0125] Method: For in-cell RNA stability, the 233-member in vitro transcribed mRNA pool (m⁷G-capped and polyA) was transfected into HEK293T cells as described above and RNA was harvested at 1, 7, 12, and 24 h in Trizol (ThermoFisher Scientific, 15596026). RNA was extracted from the aqueous phase on silica columns (Zymo, R1013).

[0126] For in-solution RNA degradation experiments, 750 ng of the 233-mRNA pool (not m⁷G-capped or polyA) was incubated in 30 μL of Degradation Buffer (50 mM CHES at pH 10 and 10 mM MgCl₂) and collected over 10 time points: 0, 0.5, 1, 2, 3, 4, 5, 6, 16 and 24 h. To each sample, 15 μL of 0.5 M Tris-HCl pH 7 and 3 μL of 0.5 M EDTA-Na was added to quench the degradation. The integrity of each sample was checked by loading 5 μL of total RNA alongside a spike-in control (P4P62HP, 50 ng) onto a PAGE-Urea-TBE gel and visualized by SYBR Gold (Thermo Fisher). Subsequently, RNA was purified using Ampure beads +40% polyethylene glycol 8000 (7:3) and checked again by PAGE-Urea-TBE gel and visualized by SYBR Gold.

Example 6: Measurement of In-Solution mRNA Stability by Capillary Electrophoresis

[0127] Method: For one-by-one measurement of in-solution mRNA stability, in vitro transcribed mRNA was incubated in a degradation buffer over ten time points (0, 0.5, 1.0, 1.5, 2, 3, 4, 5, 18, and 24 hours), then analyzed by capillary electrophoresis.

[0128] For each time point, 1.6 pmol of mRNA brought to 10 μL in a buffer containing 50 mM Na-CHES at pH 10 with 10 mM MgCl₂, and the reaction was incubated at 25° C. When the incubation period was reached for each time point, 5 μL of Tris-HCl at pH 7 and 1 μL of 500 mM EDTA in nuclease free water was added to quench the degradation reaction, and frozen for further analysis. After the final time

point (24 hours), 4 μL of each mRNA degradation sample (out of a total stored volume of 16 μL) was taken, and mixed with 1 μL of a control RNA at a concentration of 50 ng/ μL . For these experiments we opted to use the P4-P6 domain of the Tetrahymena ribozyme with two added hairpins (~239 nt) as a control. The RNA mixture was then purified using a mixture of AMPure XP beads (Beckman Coulter) with 40% polyethylene glycol (mixed in a 7:3 ratio). The resulting RNA was eluted into 4.5 μL of RNase-free water for analysis on the 2100 Bioanalyzer (Agilent) using the RNA-Nano Eukaryote protocol.

[0129] The data from the Bioanalyzer were analyzed using a custom script that performs the following analysis. We first converted elution times to nucleotides based on a ladder control (25, 200, 500, 1000, 2000, and 4000 nts). Relative mRNA amounts were estimated based on peak areas at expected band lengths (for example, ~900 nucleotides for the mRNAs of interest and ~265 nucleotides for the control). When calculating peak areas, background subtraction was performed, where the background was defined as the area under a linear line in the range of nucleotides used for the peak area. Normalization was performed using two different methods used to cross-validate. First, the peak areas of full-length mRNA were normalized to the control P4-P6 domain RNA that was spiked into the samples after degradation was performed. Second, peak areas of full-length mRNAs were also normalized to the total amount of RNA in the lane less the peak area of the bands of interest (between ~20-1000 nucleotides in our case), assuming that the majority of the other RNA in the lane were degradation products from the mRNA of interest. These distinct approaches to normalizing the data gave the same results within estimated error (see below). After calculations of normalized peak areas, fraction intact values were then calculated for each mRNA by dividing the normalized area across the ten timepoints by the normalized area at the start (0 hours).

$$\text{Fraction Intact}_i = \frac{\text{Normalized Area}_i}{\text{Normalized Area}_0 \text{ hours}}$$

[0130] For each sample, fraction intact values were fit across the different timepoints to an exponential function:

$$F_i = Ae^{-t/\tau}$$

[0131] Where F_i is an array of fraction intact values across multiple time points, A is the amplitude of the exponential decay function, x is the time constant, and t is an array of time points in hours. The time constant was then used to calculate the in vitro half-life of mRNA:

$$\text{Half-life} = \ln(2)\tau$$

Example 7: Library Preparation and Amplicon Sequencing

[0132] Method: Up to 250 ng RNA in 2.75 μL was mixed with 0.25 μL 2 μM RT_Const2_N12_Read1Partial and 0.25 μL 10 mM dNTPs each. The RNA samples were then denatured at 65° C. for 5 min and chilled to 4° C. 1.75 μL reverse transcription mix was added to 5 μL total reaction volume: 1 μL 5 \times Superscript IV buffer, 0.25 μL 10 mM DTT, 0.25 μL Superase-In (ThermoFisher Scientific, AM2694),

0.25 μL Superscript IV (Thermo 18091050). The reaction was incubated at 55° C. for 45 min and inactivated at 80° C. for 10 min.

[0133] First round PCR was performed under following conditions: 1 μL RT reaction, 10 μL 2 \times Q5 Hot Start Master Mix (NEB M0494S), 0.2 μL 100 \times SYBR (Thermo S7563), 1 μL 10 uM Read1Partial_F, 1 μL 10 uM 50:50 Hbb_Fwd: Nluc_Fwd mix in 20 μL total volume. Cycling conditions were: 98° C. for 60 sec, and 15 cycles of 98° C. for 10 sec, 68° C. for 10 sec and 72° C. Second round PCR was performed under the following conditions: 1 μL first round PCR, 10 μL 2 \times Q5 Hot Start Master Mix, 0.2 μL 100 \times SYBR, 1 μL 10 uM Read1Partial_F, 1 μL 10 uM Read2Partial_Const1_R in 20 μL total volume. Cycling conditions were: 98° C. for 60 sec, and 5 cycles of 98° C. for 10 sec, 72° C. for 5 sec. Sequencing adaptors were added using the following conditions for final round PCR: 1 μL second round PCR, 10 μL 2 \times Q5 Hot Start Master Mix, 0.2 μL 100 \times SYBR, 1 μL 10 μM NEBNext Index Primer (NEB E7335, NEB E7500, NEB E7710, NEB E7730, NEB E6609), 1 μL 10 μM NEBNext Universal PCR Primer in 20 μL total volume. Cycling conditions were: 98° C. for 60 sec, and 5 cycles of 98° C. for 10 sec, 72° C. for 5 sec. All barcoded samples were then pooled at equal volumes and purified with 1.1 \times SPRIselect beads (Beckman Coulter B23317). Sequencing was performed at the Stanford Functional Genomics Facility (SFGF) at Stanford University, on an Illumina NextSeq 550 instrument, using a high output kit, 1 \times 76 cycles. The SEQ ID NOs for the various PCR primers are listed in Table 4.

TABLE 4

Primer Sequences	
Name:	SEQ ID NO:
RT_Const2_N12_Read1Partial	1384
Const3_R	1385
Hbb_Fwd	1386
Nluc_Fwd	1387
Read1Partial_F	1388
Read2Partial_Const1_R	1389
T7_F_28nt (forward)	1390

Example 8: Amplicon Sequencing Data Analysis

[0134] Method: After bcl conversion and demultiplexing with Illumina bcl2fastq, the constant regions were trimmed using cutadapt. The trimmed reads were aligned to the indexed reference of barcode sequences using Bowtie2 with the following options: -L 11-N 0--nofw. The alignments were deduplicated based on UMIs using Umicollapse with -p 0.05 and counted using samtools idxstats. This pipeline yields a matrix of barcode read counts where rows are the different constructs in the library and columns are the different samples.

[0135] The count matrix was log transformed and normalized column-wise using a linear fit on the dilution series of spike-in constructs in each sample. For the calculation of RNA degradation coefficients in cells, we carried out a linear fit to log RNA abundance from the time course data, i.e. we fit an expression of $Y = \beta_0 + \beta_1 t$ where Y is the normalized log RNA abundance and t is the number of hours after transfection; π is the degradation constant. For the calculation of in solution degradation coefficients, sufficient data points were available to carry out a nonlinear fit directly to an

exponential model, i.e. an expression of $y=A \exp(-\tau/t)$ was fit, where y is the fraction intact (RNA abundance normalized to initial abundance), A is the amplitude, t is the time of incubation in degradation buffer in hours, and T is the degradation time constant. Time courses in which the observed fraction intact exceeded the fitted exponential by more than 0.05 in the last time point signaled RT-PCR amplification of misprimed, non-full-length products and were filtered out of downstream analysis.

[0136] For polysome profiles, percent RNA abundances for each fraction were first calculated by scaling per-fraction values by the sum of all fractions. For the heatmap displays in the figures, column medians were also subtracted from each percent RNA value. For the calculation of ribosome load, the matrix of percent RNA abundances in fractions 4-9 (1-3 are free RNP fractions, and >9 have negligible abundance) were first multiplied by a weight vector representing the number of ribosomes in each fraction as determined by the A260 trace from the fractionator, then the weighted abundances were summed across the row. For the calculation of polysome to monosome ratio, the sum of fractions 7-9 (>3 ribosomes) abundances were divided by fraction 4 (80S) abundance. For the calculation of monosome to 40S/60S ratio, fraction 4 (80S) abundance was divided by the sum of fraction 2 (40S/60S) abundance.

[0137] To calculate the expected protein levels assuming first order kinetics of mRNA translation and mRNA/protein decay, the following differential equations were used:

$$\frac{dM}{dt} = -k_m \cdot M(t)$$

$$\frac{dP}{dt} = k_t \cdot M(t) - k_p \cdot P(t)$$

where dM/dt and dP/dt are rates of change in mRNA and protein levels, respectively; $M(t)$ and $P(t)$ are moles of mRNA and protein at time t , respectively; k_t is the translation rate constant; and k_m and k_p are rate constants of mRNA and protein decay, respectively. The analytical solution for $P(t)$ is proportional to:

$$P(t) \sim k_t \frac{e^{-k_p t} - e^{-k_m t}}{k_m - k_p}$$

where m_0 is the mass of mRNA present at $t=0$, and l is the mRNA length in nucleotides. k_p is set to 0 since Nluc protein has negligible degradation as measured by luciferase activity in transiently Nluc-expressing HEK293 cells for at least 6 hours after cycloheximide treatment, which allows assessment of protein degradation in the absence of further translation⁹⁹. k_m is the degradation constant obtained from the linear fit of in-cell time course RNA data ($-\beta_1$ above). k_t is the ribosome load calculated by summing weighted RNA abundances from polysome profile data.

Example 9: Luciferase Activity Assay after mRNA Transfection

[0138] Method: Media from transiently transfected HEK293T cells was aspirated and cells were lysed in 40 μ L of 1 \times passive lysis buffer from the Dual-Luciferase Reporter Assay System (Promega, E1980) and either directly assayed

or frozen at -20° C. After thawing, 20 μ L of supernatant was transferred to a new plate and assayed for luciferase activity using the Nano-Glo Dual-Luciferase Reporter Assay System (Promega, N1610) to measure Firefly (Fluc) and NanoLuc (Nluc) luciferase activities. In particular, 50 μ L of ONE-Glo Ex Reagent was added to each well of lysate and incubated for 3 minutes at room temperature before measuring Fluc activities. Subsequently, 50 μ L of NanoDLR Stop & Glo reagent was added to each well, and incubated for 10 min at room temperature before measuring luciferase activities on a GloMax-Multi (Promega) plate reader. Luciferase reporter activity is expressed as a ratio between Nluc and Fluc. Each experiment was performed a minimum of three independent times. Because this assay relies on accumulation of luciferase in the cytosol, any signal peptide sequences were removed from the CDS for templates and mRNA for these transfection and luciferase activity experiments.

Example 10: Polysome Selection Library Sequencing Data Analysis

[0139] Method: Following adapter trimming, 670440 sequences with at least 10 summed read count across all libraries combined were set as the reference. Each library was aligned to this indexed reference using Bowtie2. Only uniquely mapping reads with edit distance ≤ 3 were retained. Alignments were further deduplicated using UMIcollapse ($-p$ 0.05, $-k$ 1). This results in the matrix of read count where rows are different sequence variants and columns are the samples.

[0140] Normalized counts were obtained by dividing the matrix column-wise by total read counts per sample. For sequence variants with at least 15 reads in any one of the samples, a regression model was fitted on normalized read counts with the sequential selection rounds as ordinal predictors, penalizing differences between coefficients of adjacent groups (R package ordPens). False discovery rate was estimated by Benjamini-Hochberg procedure. For choosing the final set of candidates, the criteria of 15 read counts in the final round polysome selection library and 2 fold enrichment over input in the final round was also required.

DOCTRINE OF EQUIVALENTS

[0141] Having described several embodiments, it will be recognized by those skilled in the art that various modifications, alternative constructions, and equivalents may be used without departing from the spirit of the invention. Additionally, a number of well-known processes and elements have not been described in order to avoid unnecessarily obscuring the present invention. Accordingly, the above description should not be taken as limiting the scope of the invention.

[0142] Those skilled in the art will appreciate that the foregoing examples and descriptions of various preferred embodiments of the present invention are merely illustrative of the invention as a whole, and that variations in the components or steps of the present invention may be made within the spirit and scope of the invention. Accordingly, the present invention is not limited to the specific embodiments described herein, but, rather, is defined by the scope of the appended claims.

 SEQUENCE LISTING

Sequence total quantity: 1390

SEQ ID NO: 1 moltype = DNA length = 28
 FEATURE Location/Qualifiers

misc_feature 1..28
 note = Modified 5'UTR

source 1..28
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 1

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SEQ ID NO: 2 moltype = DNA length = 29
 FEATURE Location/Qualifiers

misc_feature 1..29
 note = Modified 5'UTR

source 1..29
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 2

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SEQ ID NO: 3 moltype = DNA length = 33
 FEATURE Location/Qualifiers

misc_feature 1..33
 note = Modified 5'UTR

source 1..33
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 3

ctcccgggct ggcagcaggc ccccagcggc acc 33

SEQ ID NO: 4 moltype = DNA length = 36
 FEATURE Location/Qualifiers

misc_feature 1..36
 note = Modified 5'UTR

source 1..36
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 4

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SEQ ID NO: 5 moltype = DNA length = 39
 FEATURE Location/Qualifiers

source 1..39
 mol_type = genomic DNA
 organism = Mus musculus

SEQUENCE: 5

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SEQ ID NO: 6 moltype = DNA length = 41
 FEATURE Location/Qualifiers

source 1..41
 mol_type = genomic DNA
 organism = Homo sapiens

SEQUENCE: 6

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 FEATURE Location/Qualifiers

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 note = Modified 5'UTR

source 1..46
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 7

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SEQ ID NO: 8 moltype = DNA length = 47
 FEATURE Location/Qualifiers

source 1..47
 mol_type = genomic DNA
 organism = Tobacco etch virus

SEQUENCE: 8

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source               1..50
                    mol_type = other DNA
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SEQ ID NO: 10         moltype = DNA length = 58
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                    note = Modified 5'UTR
source               1..58
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 10
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FEATURE              Location/Qualifiers
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                    note = Modified 5'UTR
source               1..61
                    mol_type = other DNA
                    organism = synthetic construct

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c                                                                61

SEQ ID NO: 12         moltype = DNA length = 61
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                    note = Modified 5'UTR
source               1..61
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 12
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c                                                                61

SEQ ID NO: 13         moltype = DNA length = 62
FEATURE              Location/Qualifiers
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                    note = Modified 5'UTR
source               1..62
                    mol_type = other DNA
                    organism = synthetic construct

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aa                                                                62

SEQ ID NO: 14         moltype = DNA length = 63
FEATURE              Location/Qualifiers
misc_feature         1..63
                    note = Modified 5'UTR
source               1..63
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 14
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acg                                                                63

SEQ ID NO: 15         moltype = DNA length = 65
FEATURE              Location/Qualifiers
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                    note = Modified 5'UTR
source               1..65
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 15
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agacc                                                                65

SEQ ID NO: 16         moltype = DNA length = 68

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FEATURE	Location/Qualifiers	
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	mol_type = genomic DNA	
	organism = Tobacco mosaic virus	
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caattaca		68
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	note = Modified 5'UTR	
source	1..72	
	mol_type = other DNA	
	organism = synthetic construct	
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caaacagaca cc		72
SEQ ID NO: 18	moltype = DNA length = 78	
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	mol_type = genomic DNA	
	organism = Homo sapiens	
SEQUENCE: 18		
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agccgagccc gcgtcgcc		78
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	note = Modified 5'UTR	
source	1..83	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 19		
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gttctctaaa cgaactttaa aat		83
SEQ ID NO: 20	moltype = DNA length = 84	
FEATURE	Location/Qualifiers	
source	1..84	
	mol_type = genomic DNA	
	organism = Homo sapiens	
SEQUENCE: 20		
accgccgaga ccgctccgc cccgcgagca cagagcctcg cctttgccga tccgccgccc		60
gtccacacc gccgacagct cacc		84
SEQ ID NO: 21	moltype = DNA length = 84	
FEATURE	Location/Qualifiers	
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	mol_type = genomic DNA	
	organism = Homo sapiens	
SEQUENCE: 21		
cccgtgacct ggaagttgta cggtacgcg actttccctc ccacaaacct tcgcccctt		60
cctttcctac ttggcccgg caga		84
SEQ ID NO: 22	moltype = DNA length = 89	
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	note = Modified 5'UTR	
source	1..89	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 22		
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gtgttcaacta gcaacctcaa acagacacc		89
SEQ ID NO: 23	moltype = DNA length = 96	
FEATURE	Location/Qualifiers	
source	1..96	
	mol_type = genomic DNA	
	organism = Dengue virus	
SEQUENCE: 23		
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gttctaacag tttttaatt agagagcaga tctctg		96

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SEQ ID NO: 24      moltype = DNA  length = 110
FEATURE           Location/Qualifiers
source           1..110
                 mol_type = genomic DNA
                 organism = Arabidopsis thaliana

SEQUENCE: 24
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agtcagtaag taaacgagca aaagaagaag agaaacaaca agaagtagta 110

SEQ ID NO: 25      moltype = DNA  length = 111
FEATURE           Location/Qualifiers
source           1..111
                 mol_type = genomic DNA
                 organism = Mus musculus

SEQUENCE: 25
tataaaaccc ggcggcgcaa cgcgcagcca ctgtcgagtc gcgtccaccg gcgagcacag 60
cttctttgca gctccttcgt tgccgggtcca cacccgccac cagttegcc c 111

SEQ ID NO: 26      moltype = DNA  length = 111
FEATURE           Location/Qualifiers
misc_feature     1..111
                 note = Modified 5'UTR
source           1..111
                 mol_type = other DNA
                 organism = synthetic construct

SEQUENCE: 26
ggcgaactgg tggcggtgt ggaccggcaa cgaaggagct gcaaagaagc tgtgctcgcg 60
ggtggacgcg actcgacagt ggctgcgctg tgcgcccgcg ggttttatac c 111

SEQ ID NO: 27      moltype = DNA  length = 112
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                 note = Modified 5'UTR
source           1..112
                 mol_type = other DNA
                 organism = synthetic construct

SEQUENCE: 27
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ccacatttgc ttctgacaca actgtgttca ctagcaacct caaacagaca cc 112

SEQ ID NO: 28      moltype = DNA  length = 121
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misc_feature     1..121
                 note = Modified 5'UTR
source           1..121
                 mol_type = other DNA
                 organism = synthetic construct

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aggggaggat catcagacct actgacacag acccaagaca gcaagaagct aaccagggcac 120
c 121

SEQ ID NO: 29      moltype = DNA  length = 125
FEATURE           Location/Qualifiers
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                 note = Modified 5'UTR
source           1..125
                 mol_type = other DNA
                 organism = synthetic construct

SEQUENCE: 29
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cccgcgagc acagagcctc gcctttgccc atccgccgccc cgteccacacc cgccgccagc 120
tcacc 125

SEQ ID NO: 30      moltype = DNA  length = 137
FEATURE           Location/Qualifiers
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                 mol_type = genomic DNA
                 organism = Homo sapiens

SEQUENCE: 30
agcaccacgg cagcaggagg ttccggctaa gttggaggta ctggccacga ctgcatgccc 60
gcgcccgcga ggtgatacct ccgcccgggtga cccaggggct ctgcgacaca aggagtctgc 120
atgtctaagt gctagac 137

SEQ ID NO: 31      moltype = DNA  length = 140

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FEATURE                Location/Qualifiers
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                      mol_type = genomic DNA
                      organism = Barley yellow dwarf virus

SEQUENCE: 31
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tgtattcggg aagtagttgc gaaaacggtc cccttattgc ctgacaagct aagggccacc 120
cttctttccc caccgcatc                                     140

SEQ ID NO: 32          moltype = DNA length = 149
FEATURE                Location/Qualifiers
misc_feature          1..149
                      note = Modified 5'UTR
source                1..149
                      mol_type = other DNA
                      organism = synthetic construct

SEQUENCE: 32
attaaagggt tataccttcc caggtaacaa accaaccaac ttctgatctc ttgtagatct 60
gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcttgc ttagtgcact 120
cacgcagtat aattaataac taattactg                                     149

SEQ ID NO: 33          moltype = DNA length = 152
FEATURE                Location/Qualifiers
misc_feature          1..152
                      note = Modified 5'UTR
source                1..152
                      mol_type = other DNA
                      organism = synthetic construct

SEQUENCE: 33
cgcgttattg ttctgcccgg cggacacgtg acgcgaagct ttataaaacc cggcgggcgca 60
acgcgcagcc actgtcgagt cgcgtccacc cgcgagcaca gcttctttgc agctccttgc 120
ttgcccgttc acacccgcca ccagttcgcc cc                                     152

SEQ ID NO: 34          moltype = DNA length = 152
FEATURE                Location/Qualifiers
misc_feature          1..152
                      note = Modified 5'UTR
source                1..152
                      mol_type = other DNA
                      organism = synthetic construct

SEQUENCE: 34
cgcgttattg ttctgcccgg cggacacgtg acgcgaagct tggcgaactg gtggcggggtg 60
tggaccggca acgaaggagc tgcaaagaag ctgtgctcgc ggggtggacgc gactcgcagc 120
tggctgcgcg ttgcgcccgc gggttttata cc                                     152

SEQ ID NO: 35          moltype = DNA length = 185
FEATURE                Location/Qualifiers
misc_feature          1..185
                      note = Modified 5'UTR
source                1..185
                      mol_type = other DNA
                      organism = synthetic construct

SEQUENCE: 35
attaaagggt tataccttcc caggtaacaa accaaccaac ttctgatctc ttgtagatct 60
gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcttgc ttagtgcact 120
cacgcagtat aattaataac taattactgt cgttgacagg acacgagtaa ctctgtctatc 180
ttaag                                               185

SEQ ID NO: 36          moltype = DNA length = 190
FEATURE                Location/Qualifiers
misc_feature          1..190
                      note = Modified 5'UTR
source                1..190
                      mol_type = other DNA
                      organism = synthetic construct

SEQUENCE: 36
tttaaatct gtgtggctgt cactcggctg cttgcttagt gcactcacgc agtataatta 60
ataactaatt actgtcgttg acaggacacg agtaactcgt ctatcttctg caggctgctt 120
acggtttcgt ccgtgttgca gccgatcatc agcacatcta ggtttcgtcc ggggtgtgacc 180
gaaaggtaag                                               190

SEQ ID NO: 37          moltype = DNA length = 191
FEATURE                Location/Qualifiers
misc_feature          1..191
                      note = Modified 5'UTR
source                1..191

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mol_type = other DNA
organism = synthetic construct

SEQUENCE: 37
cttccttttg tgactggcgg tgaacgagtg cgcagtgcc gcgttattgt tctgcccggg 60
ggacacgtga cgcgaagctt tataaaaccc ggccggcga cgcgcagcca ctgtcgagtc 120
gcgccacccc gcgagcacag cttctttgca gtccttcgt tgccgggtcca caccgccac 180
cagttcgccc c 191

SEQ ID NO: 38      moltype = DNA length = 199
FEATURE           Location/Qualifiers
misc_feature      1..199
                  note = Modified 5'UTR
source            1..199
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 38
gggaaataag agagaaaaga agagtaagaa gaaatataag agccaccgc gttattgttc 60
tgccggggcg acacgtgacg cgaagcttta taaaaccgg cgccgcaacg cgcagccact 120
gtcgagtcgc gtccaccgc gagcacagct tctttgcagc tccttcgttg ccggtccaca 180
cccgccacca gttcgccc 199

SEQ ID NO: 39      moltype = DNA length = 199
FEATURE           Location/Qualifiers
misc_feature      1..199
                  note = Modified 5'UTR
source            1..199
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 39
cgcgttattg ttctgcccgg cggacacgtg acgcgaagct tgggaaataa gagagaaaag 60
aagagtaaga agaaatataa gagccaccta taaaaccgg cgccgcaacg cgcagccact 120
gtcgagtcgc gtccaccgc gagcacagct tctttgcagc tccttcgttg ccggtccaca 180
cccgccacca gttcgccc 199

SEQ ID NO: 40      moltype = DNA length = 202
FEATURE           Location/Qualifiers
misc_feature      1..202
                  note = Modified 5'UTR
source            1..202
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 40
attaaagggt tataccttcc caggtaacaa accaaccaac ttctgatctc ttgtagatct 60
gttctctaaa cgaactttaa aatctgtcgt tgacaggaca cgagtaactc gtctatcttc 120
tgcaggctgc ttacggtttc gtccgtgttg cagccgatca tcagcacatc taggtttcgt 180
ccgggtgtga ccgaaaggta ag 202

SEQ ID NO: 41      moltype = DNA length = 221
FEATURE           Location/Qualifiers
misc_feature      1..221
                  note = Modified 5'UTR
source            1..221
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 41
attaaagggt tataccttcc caggtaacaa accaaccaac ttctgatctc ttgtagatct 60
gttctctaaa cgaactttaa aattataatt aataactaat tactgtcgtt gacaggacac 120
gagtaactcg tctatcttct gcaggctgct tacggtttcg tccgtgttgc agccgatcat 180
cagcacatct aggtttcgtc cgggtgtgac cgaaaggtaa g 221

SEQ ID NO: 42      moltype = DNA length = 222
FEATURE           Location/Qualifiers
misc_feature      1..222
                  note = Modified 5'UTR
source            1..222
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 42
attaaagggt tataccttcc caggtaacaa accaaccaac ttctgatctc ttgtagatct 60
gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcttgc ttagtgact 120
cagcagatg aattaataac taattactgt cgttgacagg acacagtaa ctcgtctatc 180
ttcgcacatc taggtttcgt cgggtgtgta ccgaaaggta ag 222

SEQ ID NO: 43      moltype = DNA length = 225
FEATURE           Location/Qualifiers
misc_feature      1..225

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source          note = Modified 5'UTR
                1..225
                mol_type = other DNA
                organism = synthetic construct

SEQUENCE: 43
tttaaaatct gtgtggctgt cactcggctg cttgcttagt gcactcacgc agtataatta 60
ataactaatt actgtcgttg acaggacacg agtaactcgt ctatcttctg caggctgctt 120
acggtttcgt ccgtggtgca gccgatcatc agcacatcta ggtttcgtcc ggggtgtgacc 180
gaaaggtaag ttggagagcc ttgtccctgg tttcaacgag aaaaac 225

SEQ ID NO: 44      moltype = DNA length = 230
FEATURE           Location/Qualifiers
misc_feature      1..230
                  note = Modified 5'UTR
source           1..230
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 44
attaaagggtt tataccttcc caggtaacaa accaaccaac tttegatctc ttgtagatct 60
gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcttgc ttagtgcact 120
cacgcagtat aattaataac taattactgt cgttgacagg acacgagtaa ctcgtctatc 180
ttctgcaggc tgcttacggt ttcgtccgtg ttgcagccga tcatcagaag 230

SEQ ID NO: 45      moltype = DNA length = 238
FEATURE           Location/Qualifiers
misc_feature      1..238
                  note = Modified 5'UTR
source           1..238
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 45
attaaaaaac aactttcgat ctctttaga tctgttctct aaacgaactt taaaatctgt 60
gtggctgtca ctcggctgct tgcttagtgc actcacgcag tataattaat aactaattac 120
tgctgttgac aggacacgag taactcgtct atcttctgca ggctgcttac ggtttcgtcc 180
gtgttgacgc cgatcatcag cacatctagg ttcgtccggg gtgtgaccga aaggtaag 238

SEQ ID NO: 46      moltype = DNA length = 246
FEATURE           Location/Qualifiers
misc_feature      1..246
                  note = Modified 5'UTR
source           1..246
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 46
attaaagggtt tataccttcc caggtaacaa accaaccaac tttegatctc ttgtagatct 60
gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcttgc ttagtgcact 120
cacgcagctg tcggtgacag gacacgagta actcgtctat cttctgcagg ctgcttacgg 180
ttcgtccgtg gttgcagccg atcatcagca catctagggt tcgtccgggt gtgaccgaaa 240
ggtaag 246

SEQ ID NO: 47      moltype = DNA length = 250
FEATURE           Location/Qualifiers
misc_feature      1..250
                  note = Modified 5'UTR
source           1..250
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 47
attaaagggtt tataccttcc caggtaacaa accaaccaac tttctgttct ctaaacgaac 60
tttaaaatct gtgtggctgt cactcggctg cttgcttagt gcactcacgc agtataatta 120
ataactaatt actgtcgttg acaggacacg agtaactcgt ctatcttctg caggctgctt 180
acggtttcgt ccgtggtgca gccgatcatc agcacatcta ggtttcgtcc ggggtgtgacc 240
gaaaggtaag 250

SEQ ID NO: 48      moltype = DNA length = 250
FEATURE           Location/Qualifiers
misc_feature      1..250
                  note = Modified 5'UTR
source           1..250
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 48
attaaagggtt tataccttcc caggtaacaa accaaccaac tttegatctc ttgtagatct 60
tttaaaatct gtgtggctgt cactcggctg cttgcttagt gcactcacgc agtataatta 120
ataactaatt actgtcgttg acaggacacg agtaactcgt ctatcttctg caggctgctt 180
acggtttcgt ccgtggtgca gccgatcatc agcacatcta ggtttcgtcc ggggtgtgacc 240

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gaaaggtaag 250

SEQ ID NO: 49      moltype = DNA length = 265
FEATURE           Location/Qualifiers
misc_feature      1..265
                  note = Modified 5'UTR
source           1..265
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 49
attaaagggtt tataccttcc caggtaacaa accaaccaac tttcgatctc ttgtagatct 60
gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcatgc ttagtgact 120
cacgcagtat aattaataac taattactgt cgttgacagg acacgagtaa ctcgctatc 180
ttctgcaggc tgcttacggt ttcgtccgtg ttgcagccga tcatcagcac atctagggtt 240
cgctccgggtg tgaccgaaag gtaag 265

SEQ ID NO: 50      moltype = DNA length = 265
FEATURE           Location/Qualifiers
misc_feature      1..265
                  note = Modified 5'UTR
source           1..265
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 50
attaaagggtt tataccttcc caggtaacaa accaaccaac tttcgatctc ttgtagatct 60
gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcttgc ttagtgact 120
cacgcagtat aattaataac taattactgt cgttgacagg acacgagtaa ctcgctatc 180
ttctgcaggc tgcttacggt ttcgtccgtg ttgcagccga tcatcagcac atctagggtt 240
cgctccgggtg tgaccgaaag gtaag 265

SEQ ID NO: 51      moltype = DNA length = 270
FEATURE           Location/Qualifiers
misc_feature      1..270
                  note = Modified 5'UTR
source           1..270
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 51
attaaagggtt tataccttcc caggtaacaa accaaccaac tttcgatctc ttgtagatct 60
gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcccgc ttattgttct 120
gccgggcccga cacgtgacgc gtaactaatt actgtcgttg acaggacacg agtaactcgt 180
ctatcttctg caggctgctt acggtttcgt ccgtgttgca gccgatcatc agcacatcta 240
ggtttcgtcc ggggtgtgacc gaaaggtaag 270

SEQ ID NO: 52      moltype = DNA length = 300
FEATURE           Location/Qualifiers
misc_feature      1..300
                  note = Modified 5'UTR
source           1..300
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 52
attaaagggtt tataccttcc caggtaacaa accaaccaac tttcgatctc ttgtagatct 60
gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcttgc ttagtgact 120
cacgcagtat aattaataac taattactgt cgttgacagg acacgagtaa ctcgctatc 180
ttctgcaggc tgcttacggt ttcgtccgtg ttgcagccga tcatcagcac atctagggtt 240
cgctccgggtg tgaccgaaag gtaagttgga gagccttgtc cctggtttca acgagaaaac 300

SEQ ID NO: 53      moltype = DNA length = 323
FEATURE           Location/Qualifiers
misc_feature      1..323
                  note = Modified 5'UTR
source           1..323
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 53
ttgatctttt aatcttcggt ggccacaatt aaaacaaacc agatcgtgga gctgcgcat 60
ccctttgcat aaaaacatat ggcttttgct ataaaaatta tgactgcaa acaccgggcc 120
attaatagcg tgcggagtga tttacgcggt attgttctgc cgggcccaga cgtgacgcgc 180
gtggccaatg ggggcccggg cgccggcaac ttattagggtg actgtacttc accccccct 240
ggtgccacca agttgttaca tgaaatctgc agtttcataa tttcggcggg tcgggctggg 300
cggccagggc ggggctact gca 323

SEQ ID NO: 54      moltype = DNA length = 398
FEATURE           Location/Qualifiers
misc_feature      1..398

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source          note = Modified 5'UTR
                1..398
                mol_type = other DNA
                organism = synthetic construct

SEQUENCE: 54
ttgggggga cactccacca tagatcactc ccctgtgagg aactactgtc ttcacgcaga 60
aagcgtctag ccatggcgtt agtatgagtg tcgtgcagcc tccaggacc ccctcccgg 120
gagagccata gtggctcgcg gaaccgggta gtacaccgga attgccagga cgaccgggtc 180
ctttcttgga ttaaccgct caatgcctgg agatttgggc gtgccccgc gagactgcta 240
gccgagtagt gttgggtcgc gaaaggcctt gtggactgc ctgatagggt gcttgcgagt 300
gccccgggag gtctcgtaga ccgtgcatca tgagcaciaa tcctaacct caaagaaaa 360
caaacgtaa caagggcga ttcgttgta aagccacc 398

SEQ ID NO: 55      moltype = DNA length = 418
FEATURE           Location/Qualifiers
source           1..418
                mol_type = genomic DNA
                organism = Arabidopsis thaliana

SEQUENCE: 55
ccaaggtaaa aaaaaggtat gaaagctcta tagtaagtaa aatataaatt ccccataagg 60
aaagggccaa gtccaccagg caagtaaaat gagcaagcac cactccacca tcacacaatt 120
tcaactcatag ataacgataa gattcatgga attatcttcc acgtggcatt attccagcgg 180
ttcaagccga taagggtctc aacacctctc cttaggcctt tgtggccgtt accaagtaaa 240
attaacctca cacatatcca cactcaaaat ccaacgggtg agatcctagt ccaattgaat 300
ctcatgtatc ctagaccctc cgatcactcc aaagcttgtt ctattgttg ttatcattat 360
atatagatga ccaaagcact agaccaaacc tcagtccacac aaagagtaaa gaagaaca 418

SEQ ID NO: 56      moltype = DNA length = 60
FEATURE           Location/Qualifiers
source           1..60
                mol_type = genomic DNA
                organism = Sindbis virus

SEQUENCE: 56
cgctgcata acttttatta tttcttttat taatcaacaa aattttgttt ttaacatttc 60

SEQ ID NO: 57      moltype = DNA length = 64
FEATURE           Location/Qualifiers
misc_feature     1..64
                note = Modified 3'UTR
source          1..64
                mol_type = other DNA
                organism = synthetic construct

SEQUENCE: 57
cctcgccccg gacctgcct cccgccaggt gcacccacct gcaataaatg cagcgaagcc 60
ggga 64

SEQ ID NO: 58      moltype = DNA length = 68
FEATURE           Location/Qualifiers
misc_feature     1..68
                note = Modified 3'UTR
source          1..68
                mol_type = other DNA
                organism = synthetic construct

SEQUENCE: 58
taaccctacc tcagtcgaat tggattgggt catactgttg taggggtaaa tttttcttta 60
attcggag 68

SEQ ID NO: 59      moltype = DNA length = 111
FEATURE           Location/Qualifiers
source          1..111
                mol_type = genomic DNA
                organism = Homo sapiens

SEQUENCE: 59
gctggagcct cgggtggccat gcttcttgcc ccttgggct cccccagcc cctcctcccc 60
ttcctgcacc cgtacccccg tggcttttga ataaagtctg agtgggcggc a 111

SEQ ID NO: 60      moltype = DNA length = 115
FEATURE           Location/Qualifiers
misc_feature     1..115
                note = Modified 3'UTR
source          1..115
                mol_type = other DNA
                organism = synthetic construct

SEQUENCE: 60
cctcgccccg gacctgcct cccgccaggt gcacccacct gcaataaatg cagcgaagcc 60
gggacctcgc cccggacctg cctccccgcc aggtgcaccc acctgcaata aatgc 115

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ttaaaccacc ttcaggtgta gaccggtcat tgtgacgctg gggttgaggt gccatgaatt 60
tgtcattcat ggtgcattta tctcaacagt tttccctaac cgcgcggtgc gcggcagggg 120
tttactctg agagataaat gcctgctcac taaggtctat tagagacatt agtacgatcc 180
ggctaatagt cgctttgat gacctcaaaa gcggcggatt cct 223

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SEQ ID NO: 68          moltype = DNA length = 235
FEATURE              Location/Qualifiers
misc_feature         1..235
                    note = Modified 3'UTR
source               1..235
                    mol_type = other DNA
                    organism = synthetic construct

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SEQUENCE: 68
gctcgctttc ttgctgtcca atttctatta aaggttcctt tgttcctaa gtccaactac 60
taaactgggg gatattatga agggccttga gcatctggat tctgcctaataaaaaacatt 120
tattttcatt gcaattgcca tgtgtatgtg ggctcgccca catactctga tgatcccaaa 180
tcgtggcgtg tcggcctgct tcggcaggca ctggcgccgg gatcattcat ggcaa 235

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SEQ ID NO: 69          moltype = DNA length = 239
FEATURE              Location/Qualifiers
misc_feature         1..239
                    note = Modified 3'UTR
source               1..239
                    mol_type = other DNA
                    organism = synthetic construct

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SEQUENCE: 69
ggccaaaggc gtcgagtaga cgccaacaac ggaattgctg gaaaggggtc aacagccgtt 60
cagtaccaag tctcagggga aactttgaga tggccttgca aagggtatgg taataagctg 120
acggacatgg tcctaaccac gcagccaagt cctaagtcaa cagatcttct gttgatatgg 180
atgcagttca aaaccaaacc gtcagcgagt agctgacaaa aagaacaac aacaacaac 239

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SEQ ID NO: 70          moltype = DNA length = 255
FEATURE              Location/Qualifiers
misc_feature         1..255
                    note = Modified 3'UTR
source               1..255
                    mol_type = other DNA
                    organism = synthetic construct

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SEQUENCE: 70
tacggtaata gtgtagtctt ctcacttag tagttagctc tctcttatat taagaaaaga 60
aaacaaaaac ccccaggtcg ctttattttg acctgtgta gggaccaaaa acgggtggcag 120
cactgtctag ctgcccggcat tagactggaa aactagtgtc ctttgggtaa cactaaaat 180
cccgaagggt tgggctgtgg tgaccttccg aactaaaaga tagcctccct cctcgcgctg 240
ggggggggcc tgccc 255

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SEQ ID NO: 71          moltype = DNA length = 268
FEATURE              Location/Qualifiers
source               1..268
                    mol_type = genomic DNA
                    organism = Homo sapiens

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SEQUENCE: 71
gctcgctttc ttgctgtcca atttctatta aaggttcctt tgttcctaa gtccaactac 60
taaactgggg gatattatga agggccttga gcatctggat tctgcctaataaaaaacatt 120
tattttcatt gcaagctcgc tttcttctg tccaatttct attaaagggt cctttgttcc 180
ctaagtccaa ctactaaact gggggatatt atgaagggcc ttgagcatct ggattctgcc 240
taataaaaaa catttatttt cattgcaa 268

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SEQ ID NO: 72          moltype = DNA length = 281
FEATURE              Location/Qualifiers
misc_feature         1..281
                    note = Modified 3'UTR
source               1..281
                    mol_type = other DNA
                    organism = synthetic construct

```

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SEQUENCE: 72
ctaactattt gctttgtatt ttaagatfff gtaaataaaa aaatatataa cccactcgt 60
aggtaaggat ttattgtata ttttatttag ttagttattc agtacttacg gccctattac 120
caacgggtat taatcaciaa cactttatcc ccataggatt cttttaaatt taaaatttta 180
aataattaac gtcagagtcc catcggggct aacaggtttt tcgcactttt cctgctaact 240
gacagaagtg caatttggtt tttgattaat agttgttttc t 281

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SEQ ID NO: 73          moltype = DNA length = 319
FEATURE              Location/Qualifiers
source               1..319
                    mol_type = genomic DNA
                    organism = Sindbis virus

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SEQUENCE: 73
 ccgctacgcc ccaatgatcc gaccagcaaa actcgatgta cttccgagga actgatgtgc 60
 ataatgcac aggctggtag attagatccc cgcttaccgc gggcaatata gcaacactaa 120
 aaactcgatg tacttccgag gaagcgcagt gcataatgct gcgcagtgtt gccacataac 180
 cactatatta accatttacc tagcggagcg caaaaactca atgtatttct gaggaagcgt 240
 ggtgcataat gccacgcagc gtctgcataa cttttattat ttcttttatt aatcaacaaa 300
 atttgtttt taacatttc 319

SEQ ID NO: 74 moltype = DNA length = 328
 FEATURE Location/Qualifiers
 misc_feature 1..328
 note = Modified 3'UTR
 source 1..328
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 74
 gaccacacaa ggcagatggg ctatataaac gttttcgctt ttccgtttac gatatatagt 60
 ctactcttgg gcagaatgaa ttctcgtaac tacatagcac aagtagatgt agttaacttt 120
 aatctcacat agcaatcttt aatcagtggt taacattagg gaggacttga aagagccacc 180
 acattttcac cgaggccacg cggagtagca tcgagtgtag agtgaacaat gctagggaga 240
 gctgcctata tggaagagcc ctaatgtgta aaattaattt tagtagtgct atccccatgt 300
 gattttaata gcttcttagg agaagtagc 328

SEQ ID NO: 75 moltype = DNA length = 451
 FEATURE Location/Qualifiers
 misc_feature 1..451
 note = Modified 3'UTR
 source 1..451
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 75
 aaagcaaaac taacatgaaa caaggctaga agtcaggctg gattaagcca tagtacggaa 60
 aaaactatgc tacctgtgag ccccgctcaa ggacgttaa agaagtcagg ccatcataaa 120
 tgccatagct tgagtaaaact atgcagcctg tagtccacc tgagaagggtg taaaaaatcc 180
 gggaggccac aaaccatgga agctgtacgc atggcgtagt ggactagcgg ttagaggaga 240
 ccctccctt acaaatcgca gcaacaatgg gggcccaagg cgagatgaag ctgtagtctc 300
 gctggaagga ctagaggtta gaggagacc ccccgaaaca aaaaacagca tattgacgct 360
 gggaaagacc agagatcctg ctgtctctc agcatcattc caggcacaga acgccagaaa 420
 atggaatggt gctggtgaat caacaggttc t 451

SEQ ID NO: 76 moltype = DNA length = 457
 FEATURE Location/Qualifiers
 misc_feature 1..457
 note = Modified 3'UTR
 source 1..457
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 76
 aggcgggtca tccttttgac acttcaagtc ccgaggataa cctcctctcg gggttggggg 60
 gaatcttggg atccagtagt cctccttgaa ctccatccaa cagggtagat ttaagagtca 120
 tgagactttc attaatcatc tcagttgatc agacatgggt gtgtagattc tcataacacg 180
 ggagatcttc tagcagtttc agtgaccaac ggtgctttcc ttctccagga agtataaccg 240
 aagttggttg acaagccaag ggggtgcttc gattactctg tgcttgggca cagaaagagg 300
 tcgtagtttg ccccttgata gcagattcaa catgaattaa ctaagaaagg cgatctgcct 360
 cccatgaagg acataagcaa tagttcacia tcatcttgca tctcagtgaa gtgtacataa 420
 ctataaaggg ctgggtcatc taagcatttc agtcgag 457

SEQ ID NO: 77 moltype = DNA length = 597
 FEATURE Location/Qualifiers
 source 1..597
 mol_type = genomic DNA
 organism = Woodchuck hepatitis virus

SEQUENCE: 77
 tcgacaatca acctctggat tacaaaattt gtgaaagatt gactggattt cttaactatg 60
 ttgctccttt tacgctatgt ggatacgtg ctttaatgcc tttgtatcat gctattgctt 120
 cccgatggc tttcattttc tcctccttgt ataaatcctg gttgctgtct ctttatgagg 180
 agttgtggcc cgttgtcagg caacgtggcg tggtgtgcac tgtgtttgct gacgcaaccc 240
 ccactggttg gggcattgcc accacctgtc agctccttcc cgggactttc gctttcccc 300
 tcctattgc cacggcggaa ctcatcgccg cctgccttgc ccgctgctgg acaggggctc 360
 ggctgttggg cactgacaat tccgtggtgt tgcgggggaa gctgacgtcc tttccatggc 420
 tgctgccttg tgttgccacc tggattctgc gcgggacgct cttctgctac gtcccttcgg 480
 cctcaatcc ageggacctt ccttcccggc gctgctgccc ggctctgccc cctcttcggc 540
 gtcttcgctc tcgcctcag acgagtcgga tctccctttg ggccgctcc ccgctc 597

SEQ ID NO: 78 moltype = DNA length = 600
 FEATURE Location/Qualifiers

-continued

```

source                1..600
                      mol_type = genomic DNA
                      organism = Homo sapiens

SEQUENCE: 78
gcggaactatg acttagttgc gttacacctt ttcttgacaa aacctaactt ggcgagaaaa 60
caagatgaga ttggcatggc tttatattgtt ttttttggtt tgttttgggt tttttttttt 120
ttttggcttg actcaggatt taaaaactgg aacgggtgaag gtgacagcag tcggttggag 180
cgagcatccc ccaaagttca caatgtggcc gaggactttg attgcacatt gttgtttttt 240
taatagtcat tccaaatatg agatgcgttg ttacaggaag tcccttgcca tcctaaaagc 300
caccaccactt ctctctaagg agaatggccc agtcctctcc caagtccaca caggggaggt 360
gatagcattg ctttcgtgta aattatgtaa tgcaaaattt ttttaattct cgccttaata 420
cttttttatt ttgttttatt ttgaatgatg agccttcgtg ccccccttc cccctttttt 480
gtcccccaac ttgagatgta tgaaggcttt tggctctcct gggagtgggt ggagggcagcc 540
agggcttacc tgtacactga cttgagacca gttgaataaa agtgcacacc ttaaaaatga 600

SEQ ID NO: 79          moltype = DNA length = 682
FEATURE               Location/Qualifiers
source                1..682
                      mol_type = genomic DNA
                      organism = Mus musculus

SEQUENCE: 79
gcggaactggt actgagctgc gttttacacc ctttctttga caaaacctaa cttgcgcaga 60
aaaaaaaaaa ataagagaca acattggcat ggctttggtt ttttaaattt tttttaaagt 120
tttttttttt tttttttttt ttttttttaa gttttttgtt tttgttttgg cgcttttgac 180
tcaggattta aaaactggaa cgggtgaaggc gacagcagtt ggttggagca aacatcccc 240
aaagtctctac aaatgtggct gaggactttg tacattggtt tgtttttttt tttttttggt 300
ttgtcttttt tttaatagtc attccaagta tccatgaaat aagtggttac aggaagtccc 360
tcaccctccc aaaagccacc cccactccta agaggaggat ggtcgcgtcc atgccctgag 420
tccaccocgg ggaaggtgac agcattgctt ctgtgtaaat tatgtactgc aaaaattttt 480
ttaaactctc cgccttaata cttcattttt gtttttaatt tctgaatggc ccaggctctga 540
ggcctccctt tttttgttcc ccccaacttg atgtatgaag gctttggtct ccctgggagg 600
gggttgagggt gttgaggcag ccagggtctg cctgtacact gacttgagac caataaaaagt 660
gcacacctta ccttacacaa ac 682

SEQ ID NO: 80          moltype = DNA length = 839
FEATURE               Location/Qualifiers
source                1..839
                      mol_type = genomic DNA
                      organism = Homo sapiens

SEQUENCE: 80
atgaactcaa tctaaattaa aaaagaaaga aatttgaaaa aactttctct ttgccatttc 60
ttcttcttct tttttaactg aaagctgaat ccttccattt cttctgcaca tctacttgct 120
taaattgtgg gcaaaagaga aaaagaagga ttgatcagag cattgtgcaa tacagtttca 180
ttaactcctt cccccgctcc cccaaaaatt tgaatttttt tttcaacact cttacacctg 240
ttatggaaaa tgtcaacctt tgtaagaaaa ccaaaataaa aattgaaaaa taaaaacat 300
aacatttgc accacttgtg gcttttgaat atcttcaca gaggggaagt taaaacccaa 360
acttccaaag gtttaacta cctcaaaaca ctttccatg agtgtgatcc acattgtag 420
gtgctgacct agacagagat gaactgaggt cctgtttttg ttttgttcat aatacaaagg 480
tgtaattaa tagtatttca gatacttgaa gaatgttgat ggtgctagaa gaatttgaga 540
agaaatactc ctgtattgag ttgtatcgtg tgggtgattt tttaaaaaat ttgatttagc 600
attcatattt tccatcttat tcccaattaa aagtatgcag attatttgcc caaatcttct 660
tcagattcag catttgttct ttgccagtct caatttcatc ttcttccatg gtccacaga 720
agctttgttt cttgggcaag cagaaaaatt aaattgtacc tattttgtat atgtgagatg 780
tttaataaaa ttgtgaaaaa aatgaaataa agcatgtttg gttttccaaa agaacatat 839

SEQ ID NO: 81          moltype = DNA length = 70
FEATURE               Location/Qualifiers
misc_feature         1..70
                      note = Modified 5'UTR
source                1..70
                      mol_type = other DNA
                      organism = synthetic construct

SEQUENCE: 81
acatttgctt ctgacacaac tgtgttcaca gttggaacaa tcgtgaaggt gggttgtaat 60
cagtgccacc 70

SEQ ID NO: 82          moltype = DNA length = 70
FEATURE               Location/Qualifiers
misc_feature         1..70
                      note = Modified 5'UTR
source                1..70
                      mol_type = other DNA
                      organism = synthetic construct

SEQUENCE: 82
acatttgctt ctgacacaac tgtgttcact ttgctaaccg gggaatctac gtctatagcg 60
atcagccacc 70

```


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```

gtagccacc 70

SEQ ID NO: 90      moltype = DNA length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct

SEQUENCE: 90
acatttgctt ctgacacaac tgtgttcact gcacagcatg gtcaaggagt actgtgtacg 60
tcttgccact 70

SEQ ID NO: 91      moltype = DNA length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct

SEQUENCE: 91
acatttgctt ctgacacaac tgtgttcact agtcttgaag aagccttctt gagacctcgt 60
tcttgccacc 70

SEQ ID NO: 92      moltype = DNA length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct

SEQUENCE: 92
acatttgctt ctgacacaac tgtgttcact ggtccattgc cttggttgat ctggttgata 60
ggcagccacc 70

SEQ ID NO: 93      moltype = DNA length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct

SEQUENCE: 93
acatttgctt ctgacacaac tgtgttcacc agtacagtct ctgaagccac tacctaata 60
cgttgccacc 70

SEQ ID NO: 94      moltype = DNA length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct

SEQUENCE: 94
acatttgctt ctgacacaac tgtgttcact ggtgttctgt ggtgtggcaa tggctttgac 60
ttgtgccacc 70

SEQ ID NO: 95      moltype = DNA length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct

SEQUENCE: 95
acatttgctt ctgacacaac tgtgttcacc aggcttctgt taggttcgag tcgtgtaatt 60
acagccacc 70

SEQ ID NO: 96      moltype = DNA length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct

SEQUENCE: 96

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```
acatttgctt ctgacacaac tgtgttcact ctggcacaga ctgcacttcc gtatacgcta 60
cattgccacc 70
```

```
SEQ ID NO: 97      moltype = DNA  length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct
```

```
SEQUENCE: 97
acatttgctt ctgacacaac tgtgttcaca gtgggtcagt agcatgccgg ctaacgttct 60
gtatgccacc 70
```

```
SEQ ID NO: 98      moltype = DNA  length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct
```

```
SEQUENCE: 98
acatttgctt ctgacacaac tgtgttcact gacttggggg tggctcttga tgaggttgtg 60
ttctgccacc 70
```

```
SEQ ID NO: 99      moltype = DNA  length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct
```

```
SEQUENCE: 99
acatttgctt ctgacacaac tgtgttcacc aacagttttg gtttccttg tccgattcct 60
tctgccacc 70
```

```
SEQ ID NO: 100     moltype = DNA  length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct
```

```
SEQUENCE: 100
acatttgctt ctgacacaac tgtgttcact ggtacgcagc accatgggag tgcattggtg 60
tgttgccagt 70
```

```
SEQ ID NO: 101     moltype = DNA  length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct
```

```
SEQUENCE: 101
acatttgctt ctgacacaac tgtgttcacc tgatcgtgta ggattgagac ttgtcgtgtaag 60
ttcagccacc 70
```

```
SEQ ID NO: 102     moltype = DNA  length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct
```

```
SEQUENCE: 102
acatttgctt ctgacacaac tgtgttcacg ggcatgtgag tggtaacat gtggctattg 60
ttccgccacc 70
```

```
SEQ ID NO: 103     moltype = DNA  length = 70
FEATURE          Location/Qualifiers
misc_feature     1..70
                 note = Modified 5'UTR
source          1..70
                 mol_type = other DNA
                 organism = synthetic construct
```

-continued

SEQUENCE: 103
acatttgctt ctgacacaac tgtgttcact gtgtcattgg gcgaatagct ggaacttcgc 60
gccagccacc 70

SEQ ID NO: 104 moltype = DNA length = 70
FEATURE Location/Qualifiers
misc_feature 1..70
note = Modified 5'UTR
source 1..70
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 104
acatttgctt ctgacacaac tgtgttcact ggtgggctaa ggcgtcagaa atcaaagcgt 60
ttttgccacc 70

SEQ ID NO: 105 moltype = DNA length = 70
FEATURE Location/Qualifiers
misc_feature 1..70
note = Modified 5'UTR
source 1..70
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 105
acatttgctt ctgacacaac tgtgttcact ggttcgaatt tggacgtcgc ctgcatggtc 60
ttctgccacc 70

SEQ ID NO: 106 moltype = DNA length = 70
FEATURE Location/Qualifiers
misc_feature 1..70
note = Modified 5'UTR
source 1..70
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 106
acatttgctt ctgacacaac tgtgttcact gcgccttggc aggcaactgc aaagtcttgg 60
tagcaccacc 70

SEQ ID NO: 107 moltype = DNA length = 70
FEATURE Location/Qualifiers
misc_feature 1..70
note = Modified 5'UTR
source 1..70
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 107
acatttgctt ctgacacaac tgtgttcact tagtgagcac gtgagctggt ttcaggtttg 60
taaagccacc 70

SEQ ID NO: 108 moltype = DNA length = 70
FEATURE Location/Qualifiers
misc_feature 1..70
note = Modified 5'UTR
source 1..70
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 108
acatttgctt ctgacacaac tgtgttcacg agttgtgcac ttgggtgata tttggcctca 60
gaaagccacc 70

SEQ ID NO: 109 moltype = DNA length = 70
FEATURE Location/Qualifiers
misc_feature 1..70
note = Modified 5'UTR
source 1..70
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 109
acatttgctt ctgacacaac tgtgttcacg ctgcaactgc cattctcgct tggatgggtca 60
gaatgccacc 70

SEQ ID NO: 110 moltype = DNA length = 70
FEATURE Location/Qualifiers
misc_feature 1..70
note = Modified 5'UTR
source 1..70
mol_type = other DNA

-continued

```

                                organism = synthetic construct
SEQUENCE: 110
acatttgctt ctgacacaac tgtgttcacg ggcggttggtg tttgtgatgt catgttcgca 60
ttcagccacc                                                    70

SEQ ID NO: 111                moltype = DNA length = 70
FEATURE                        Location/Qualifiers
misc_feature                    1..70
                                note = Modified 5'UTR
source                          1..70
                                mol_type = other DNA
                                organism = synthetic construct

SEQUENCE: 111
acatttgctt ctgacacaac tgtgttcact cgacacgggt ctgagtccag aacatgagag 60
taatgccacc                                                    70

SEQ ID NO: 112                moltype = DNA length = 621
FEATURE                        Location/Qualifiers
misc_feature                    1..621
                                note = Nanoluciferase reporter gene
source                          1..621
                                mol_type = other DNA
                                organism = synthetic construct

SEQUENCE: 112
atggccggtt acccatacga tgttcctgac tatgcccggct atccctatga cgtcccggac 60
tatgcaggct cctatccata tgacgttcca gattacgctg gatctggcgt cttcacactc 120
gaagatttcg ttggggactg ggcacagaca gccggctaca acctggacca agtccttgaa 180
cagggagggtg tgtccagttt gtttcagaat ctccgggtgt ccgtaactcc gatccaaagg 240
attgtcctga gcggtgaaaa tgggctgaag atcgacatcc atgtcatcat cccgtatgaa 300
ggtctgagcg gcgaccaaat gggccagatc gaaaaaattt ttaaggtggt gtaccctgtg 360
gatgatcatc actttaaggt gatcctgcac tatggcacac tggtaatcga cgggggttacg 420
ccgaacatga tcgactattt cggacggccg tatgaaggca tcgccgtgtt cgacggcaaa 480
aagatcactg taacagggac cctgtggaac ggcaacaaaa ttatcgacga gcgcctgatc 540
aaccocgacg gctccctgct gttccgagta accatcaacg gagtgaccgg ctggcggctg 600
tcggaacgca ttctggcgta a                                                    621

SEQ ID NO: 113                moltype = DNA length = 855
FEATURE                        Location/Qualifiers
misc_feature                    1..855
                                note = GFP reporter gene
source                          1..855
                                mol_type = other DNA
                                organism = synthetic construct

SEQUENCE: 113
atggttttcta agggagaaga actgttcact ggtgtagtac ctatcctggt agaactggat 60
ggagatgtta acggccataa gttcagtggt tctggagaag gagaagggtga tgctacttac 120
ggtaagctga cactgaagtt catctgtaca actggaaagc tgccagttcc ttggcctaca 180
ctggtaaaca ctctgactta cggagtacaa tgtttctctc gataccaga tcatatgaag 240
caacatgatt tcttcaagtc agcaatgect gaaggttacg tacaagaaag aactatcttc 300
ttcaaggatg atggttaacta caagactaga gctgaagtaa agttcgaagg agatactctg 360
gttaacagaa tcgaactgaa gggatcagat ttcaaggaag atggaaacat cctgggtcat 420
aagctggaat acaactaaa ctcacataac gtatacatca tggcagataa gcaaaaagaac 480
ggaatcaagg taaacttaa gatcagacat aacatcgaag atggttcagt acaactggca 540
gatcattacc aacaaaacac acctatcgga gatggacctg tactgctgcc agataacat 600
tacctgtcaa ctcaatctgc actgtcaaag gatcctaacg aaaagagaga tcatatggta 660
ctgctggaat tcgtaacagc tgcaggaatc actctgggta tggatgaact gtacaagaga 720
agtagagata tctctcatgg tttcccacct gctgtagctg cacaagatga tggtagactg 780
cctatgagtt gtgctcaaga atctggaatg gatagacatc ctgcagcttg tgcacagca 840
agaatcaacg tataa                                                    855

SEQ ID NO: 114                moltype = DNA length = 144
FEATURE                        Location/Qualifiers
misc_feature                    1..144
                                note = Multi-epitope vaccine
source                          1..144
                                mol_type = other DNA
                                organism = synthetic construct

SEQUENCE: 114
atgggggggct ccggtggttc ggggtaccag ccgtaccgcg tcgtggtgct gggcggggagc 60
ggcggcagcc cgtaccgggt tgctggtctc tcgttcgggg ggtctggggg atcccttagc 120
ccccgctggt acttctacta ctaa                                                    144

SEQ ID NO: 115                moltype = length =
SEQUENCE: 115
000

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SEQ ID NO: 116 SEQUENCE: 116 000	moltype =	length =
SEQ ID NO: 117 SEQUENCE: 117 000	moltype =	length =
SEQ ID NO: 118 SEQUENCE: 118 000	moltype =	length =
SEQ ID NO: 119 SEQUENCE: 119 000	moltype =	length =
SEQ ID NO: 120 SEQUENCE: 120 000	moltype =	length =
SEQ ID NO: 121 SEQUENCE: 121 000	moltype =	length =
SEQ ID NO: 122 SEQUENCE: 122 000	moltype =	length =
SEQ ID NO: 123 SEQUENCE: 123 000	moltype =	length =
SEQ ID NO: 124 SEQUENCE: 124 000	moltype =	length =
SEQ ID NO: 125 SEQUENCE: 125 000	moltype =	length =
SEQ ID NO: 126 SEQUENCE: 126 000	moltype =	length =
SEQ ID NO: 127 SEQUENCE: 127 000	moltype =	length =
SEQ ID NO: 128 SEQUENCE: 128 000	moltype =	length =
SEQ ID NO: 129 SEQUENCE: 129 000	moltype =	length =
SEQ ID NO: 130 SEQUENCE: 130 000	moltype =	length =
SEQ ID NO: 131 SEQUENCE: 131 000	moltype =	length =
SEQ ID NO: 132 SEQUENCE: 132 000	moltype =	length =
SEQ ID NO: 133 SEQUENCE: 133 000	moltype =	length =
SEQ ID NO: 134 SEQUENCE: 134 000	moltype =	length =

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SEQ ID NO: 135 SEQUENCE: 135 000	moltype =	length =
SEQ ID NO: 136 SEQUENCE: 136 000	moltype =	length =
SEQ ID NO: 137 SEQUENCE: 137 000	moltype =	length =
SEQ ID NO: 138 SEQUENCE: 138 000	moltype =	length =
SEQ ID NO: 139 SEQUENCE: 139 000	moltype =	length =
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SEQ ID NO: 146 SEQUENCE: 146 000	moltype =	length =
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SEQ ID NO: 150 SEQUENCE: 150 000	moltype =	length =
SEQ ID NO: 151 SEQUENCE: 151 000	moltype =	length =
SEQ ID NO: 152 SEQUENCE: 152 000	moltype =	length =
SEQ ID NO: 153 SEQUENCE: 153 000	moltype =	length =

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SEQ ID NO: 154 SEQUENCE: 154 000	moltype =	length =
SEQ ID NO: 155 SEQUENCE: 155 000	moltype =	length =
SEQ ID NO: 156 SEQUENCE: 156 000	moltype =	length =
SEQ ID NO: 157 SEQUENCE: 157 000	moltype =	length =
SEQ ID NO: 158 SEQUENCE: 158 000	moltype =	length =
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SEQ ID NO: 169 SEQUENCE: 169 000	moltype =	length =
SEQ ID NO: 170 SEQUENCE: 170 000	moltype =	length =
SEQ ID NO: 171 SEQUENCE: 171 000	moltype =	length =
SEQ ID NO: 172 SEQUENCE: 172 000	moltype =	length =

-continued

SEQ ID NO: 173 SEQUENCE: 173 000	moltype =	length =
SEQ ID NO: 174 SEQUENCE: 174 000	moltype =	length =
SEQ ID NO: 175 SEQUENCE: 175 000	moltype =	length =
SEQ ID NO: 176 SEQUENCE: 176 000	moltype =	length =
SEQ ID NO: 177 SEQUENCE: 177 000	moltype =	length =
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SEQ ID NO: 187 SEQUENCE: 187 000	moltype =	length =
SEQ ID NO: 188 SEQUENCE: 188 000	moltype =	length =
SEQ ID NO: 189 SEQUENCE: 189 000	moltype =	length =
SEQ ID NO: 190 SEQUENCE: 190 000	moltype =	length =
SEQ ID NO: 191 SEQUENCE: 191 000	moltype =	length =

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SEQ ID NO: 192 SEQUENCE: 192 000	moltype =	length =
SEQ ID NO: 193 SEQUENCE: 193 000	moltype =	length =
SEQ ID NO: 194 SEQUENCE: 194 000	moltype =	length =
SEQ ID NO: 195 SEQUENCE: 195 000	moltype =	length =
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SEQ ID NO: 207 SEQUENCE: 207 000	moltype =	length =
SEQ ID NO: 208 SEQUENCE: 208 000	moltype =	length =
SEQ ID NO: 209 SEQUENCE: 209 000	moltype =	length =
SEQ ID NO: 210 SEQUENCE: 210 000	moltype =	length =

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SEQ ID NO: 211 SEQUENCE: 211 000	moltype =	length =
SEQ ID NO: 212 SEQUENCE: 212 000	moltype =	length =
SEQ ID NO: 213 SEQUENCE: 213 000	moltype =	length =
SEQ ID NO: 214 SEQUENCE: 214 000	moltype =	length =
SEQ ID NO: 215 SEQUENCE: 215 000	moltype =	length =
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SEQ ID NO: 629 SEQUENCE: 629 000	moltype =	length =
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SEQ ID NO: 631 SEQUENCE: 631 000	moltype =	length =
SEQ ID NO: 632 SEQUENCE: 632 000	moltype =	length =
SEQ ID NO: 633 SEQUENCE: 633 000	moltype =	length =
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SEQ ID NO: 635 SEQUENCE: 635 000	moltype =	length =
SEQ ID NO: 636 SEQUENCE: 636 000	moltype =	length =
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SEQ ID NO: 639 SEQUENCE: 639 000	moltype =	length =
SEQ ID NO: 640 SEQUENCE: 640 000	moltype =	length =
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SEQ ID NO: 642 SEQUENCE: 642 000	moltype =	length =
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SEQ ID NO: 645 SEQUENCE: 645 000	moltype =	length =
SEQ ID NO: 646 SEQUENCE: 646 000	moltype =	length =
SEQ ID NO: 647 SEQUENCE: 647 000	moltype =	length =

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SEQ ID NO: 648 SEQUENCE: 648 000	moltype =	length =
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SEQ ID NO: 650 SEQUENCE: 650 000	moltype =	length =
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SEQ ID NO: 659 SEQUENCE: 659 000	moltype =	length =
SEQ ID NO: 660 SEQUENCE: 660 000	moltype =	length =
SEQ ID NO: 661 SEQUENCE: 661 000	moltype =	length =
SEQ ID NO: 662 SEQUENCE: 662 000	moltype =	length =
SEQ ID NO: 663 SEQUENCE: 663 000	moltype =	length =
SEQ ID NO: 664 SEQUENCE: 664 000	moltype =	length =
SEQ ID NO: 665 SEQUENCE: 665 000	moltype =	length =
SEQ ID NO: 666 SEQUENCE: 666 000	moltype =	length =

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SEQ ID NO: 667 SEQUENCE: 667 000	moltype =	length =
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SEQ ID NO: 675 SEQUENCE: 675 000	moltype =	length =
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SEQ ID NO: 683 SEQUENCE: 683 000	moltype =	length =
SEQ ID NO: 684 SEQUENCE: 684 000	moltype =	length =
SEQ ID NO: 685 SEQUENCE: 685 000	moltype =	length =

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SEQ ID NO: 686 SEQUENCE: 686 000	moltype =	length =
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SEQ ID NO: 688 SEQUENCE: 688 000	moltype =	length =
SEQ ID NO: 689 SEQUENCE: 689 000	moltype =	length =
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SEQ ID NO: 701 SEQUENCE: 701 000	moltype =	length =
SEQ ID NO: 702 SEQUENCE: 702 000	moltype =	length =
SEQ ID NO: 703 SEQUENCE: 703 000	moltype =	length =
SEQ ID NO: 704 SEQUENCE: 704 000	moltype =	length =

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SEQ ID NO: 705 SEQUENCE: 705 000	moltype =	length =
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SEQ ID NO: 707 SEQUENCE: 707 000	moltype =	length =
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SEQ ID NO: 709 SEQUENCE: 709 000	moltype =	length =
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SEQ ID NO: 720 SEQUENCE: 720 000	moltype =	length =
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SEQ ID NO: 723 SEQUENCE: 723 000	moltype =	length =

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SEQ ID NO: 724 SEQUENCE: 724 000	moltype =	length =
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SEQ ID NO: 727 SEQUENCE: 727 000	moltype =	length =
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SEQ ID NO: 742 SEQUENCE: 742 000	moltype =	length =

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SEQ ID NO: 743 SEQUENCE: 743 000	moltype =	length =
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SEQ ID NO: 762 SEQUENCE: 762 000	moltype =	length =
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SEQ ID NO: 777 SEQUENCE: 777 000	moltype =	length =
SEQ ID NO: 778 SEQUENCE: 778 000	moltype =	length =
SEQ ID NO: 779 SEQUENCE: 779 000	moltype =	length =
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SEQ ID NO: 781 SEQUENCE: 781 000	moltype =	length =
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SEQ ID NO: 787 SEQUENCE: 787 000	moltype =	length =
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SEQ ID NO: 797 SEQUENCE: 797 000	moltype =	length =
SEQ ID NO: 798 SEQUENCE: 798 000	moltype =	length =
SEQ ID NO: 799 SEQUENCE: 799 000	moltype =	length =

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SEQ ID NO: 835 SEQUENCE: 835 000	moltype =	length =
SEQ ID NO: 836 SEQUENCE: 836 000	moltype =	length =
SEQ ID NO: 837 SEQUENCE: 837 000	moltype =	length =

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SEQ ID NO: 841 SEQUENCE: 841 000	moltype =	length =
SEQ ID NO: 842 SEQUENCE: 842 000	moltype =	length =
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SEQ ID NO: 845 SEQUENCE: 845 000	moltype =	length =
SEQ ID NO: 846 SEQUENCE: 846 000	moltype =	length =
SEQ ID NO: 847 SEQUENCE: 847 000	moltype =	length =
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SEQ ID NO: 849 SEQUENCE: 849 000	moltype =	length =
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SEQ ID NO: 854 SEQUENCE: 854 000	moltype =	length =
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SEQ ID NO: 856 SEQUENCE: 856 000	moltype =	length =

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SEQ ID NO: 861 SEQUENCE: 861 000	moltype =	length =
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SEQ ID NO: 863 SEQUENCE: 863 000	moltype =	length =
SEQ ID NO: 864 SEQUENCE: 864 000	moltype =	length =
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SEQ ID NO: 873 SEQUENCE: 873 000	moltype =	length =
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SEQ ID NO: 892 SEQUENCE: 892 000	moltype =	length =
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SEQ ID NO: 910 SEQUENCE: 910 000	moltype =	length =
SEQ ID NO: 911 SEQUENCE: 911 000	moltype =	length =
SEQ ID NO: 912 SEQUENCE: 912 000	moltype =	length =
SEQ ID NO: 913 SEQUENCE: 913 000	moltype =	length =

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SEQ ID NO: 932 SEQUENCE: 932 000	moltype =	length =

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SEQ ID NO: 948 SEQUENCE: 948 000	moltype =	length =
SEQ ID NO: 949 SEQUENCE: 949 000	moltype =	length =
SEQ ID NO: 950 SEQUENCE: 950 000	moltype =	length =
SEQ ID NO: 951 SEQUENCE: 951 000	moltype =	length =

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SEQ ID NO: 968 SEQUENCE: 968 000	moltype =	length =
SEQ ID NO: 969 SEQUENCE: 969 000	moltype =	length =
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SEQ ID NO: 987 SEQUENCE: 987 000	moltype =	length =
SEQ ID NO: 988 SEQUENCE: 988 000	moltype =	length =
SEQ ID NO: 989 SEQUENCE: 989 000	moltype =	length =

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SEQ ID NO: 996 SEQUENCE: 996 000	moltype =	length =
SEQ ID NO: 997 SEQUENCE: 997 000	moltype =	length =
SEQ ID NO: 998 SEQUENCE: 998 000	moltype =	length =
SEQ ID NO: 999 SEQUENCE: 999 000	moltype =	length =
SEQ ID NO: 1000 SEQUENCE: 1000 000	moltype =	length =
SEQ ID NO: 1001 SEQUENCE: 1001 000	moltype =	length =
SEQ ID NO: 1002 SEQUENCE: 1002 000	moltype =	length =
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SEQ ID NO: 1006 SEQUENCE: 1006 000	moltype =	length =
SEQ ID NO: 1007 SEQUENCE: 1007 000	moltype =	length =
SEQ ID NO: 1008 SEQUENCE: 1008 000	moltype =	length =

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SEQ ID NO: 1009 SEQUENCE: 1009 000	moltype =	length =
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SEQ ID NO: 1012 SEQUENCE: 1012 000	moltype =	length =
SEQ ID NO: 1013 SEQUENCE: 1013 000	moltype =	length =
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SEQ ID NO: 1015 SEQUENCE: 1015 000	moltype =	length =
SEQ ID NO: 1016 SEQUENCE: 1016 000	moltype =	length =
SEQ ID NO: 1017 SEQUENCE: 1017 000	moltype =	length =
SEQ ID NO: 1018 SEQUENCE: 1018 000	moltype =	length =
SEQ ID NO: 1019 SEQUENCE: 1019 000	moltype =	length =
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SEQ ID NO: 1025 SEQUENCE: 1025 000	moltype =	length =
SEQ ID NO: 1026 SEQUENCE: 1026 000	moltype =	length =
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SEQ ID NO: 1032 SEQUENCE: 1032 000	moltype =	length =
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SEQ ID NO: 1037 SEQUENCE: 1037 000	moltype =	length =
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SEQ ID NO: 1039 SEQUENCE: 1039 000	moltype =	length =
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SEQ ID NO: 1044 SEQUENCE: 1044 000	moltype =	length =
SEQ ID NO: 1045 SEQUENCE: 1045 000	moltype =	length =
SEQ ID NO: 1046 SEQUENCE: 1046 000	moltype =	length =

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SEQ ID NO: 1047 SEQUENCE: 1047 000	moltype =	length =
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SEQ ID NO: 1051 SEQUENCE: 1051 000	moltype =	length =
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SEQ ID NO: 1062 SEQUENCE: 1062 000	moltype =	length =
SEQ ID NO: 1063 SEQUENCE: 1063 000	moltype =	length =
SEQ ID NO: 1064 SEQUENCE: 1064 000	moltype =	length =
SEQ ID NO: 1065 SEQUENCE: 1065 000	moltype =	length =

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SEQ ID NO: 1069 SEQUENCE: 1069 000	moltype =	length =
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SEQ ID NO: 1079 SEQUENCE: 1079 000	moltype =	length =
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SEQ ID NO: 1082 SEQUENCE: 1082 000	moltype =	length =
SEQ ID NO: 1083 SEQUENCE: 1083 000	moltype =	length =
SEQ ID NO: 1084 SEQUENCE: 1084 000	moltype =	length =

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SEQ ID NO: 1100 SEQUENCE: 1100 000	moltype =	length =
SEQ ID NO: 1101 SEQUENCE: 1101 000	moltype =	length =
SEQ ID NO: 1102 SEQUENCE: 1102 000	moltype =	length =
SEQ ID NO: 1103 SEQUENCE: 1103 000	moltype =	length =

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SEQ ID NO: 1104 SEQUENCE: 1104 000	moltype =	length =
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SEQ ID NO: 1106 SEQUENCE: 1106 000	moltype =	length =
SEQ ID NO: 1107 SEQUENCE: 1107 000	moltype =	length =
SEQ ID NO: 1108 SEQUENCE: 1108 000	moltype =	length =
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SEQ ID NO: 1120 SEQUENCE: 1120 000	moltype =	length =
SEQ ID NO: 1121 SEQUENCE: 1121 000	moltype =	length =
SEQ ID NO: 1122 SEQUENCE: 1122 000	moltype =	length =

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SEQ ID NO: 1127 SEQUENCE: 1127 000	moltype =	length =
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SEQ ID NO: 1136 SEQUENCE: 1136 000	moltype =	length =
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SEQ ID NO: 1139 SEQUENCE: 1139 000	moltype =	length =
SEQ ID NO: 1140 SEQUENCE: 1140 000	moltype =	length =
SEQ ID NO: 1141 SEQUENCE: 1141 000	moltype =	length =

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SEQ ID NO: 1142 SEQUENCE: 1142 000	moltype =	length =
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SEQ ID NO: 1144 SEQUENCE: 1144 000	moltype =	length =
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SEQ ID NO: 1149 SEQUENCE: 1149 000	moltype =	length =
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SEQ ID NO: 1158 SEQUENCE: 1158 000	moltype =	length =
SEQ ID NO: 1159 SEQUENCE: 1159 000	moltype =	length =
SEQ ID NO: 1160 SEQUENCE: 1160 000	moltype =	length =

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SEQ ID NO: 1163 SEQUENCE: 1163 000	moltype =	length =
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SEQ ID NO: 1165 SEQUENCE: 1165 000	moltype =	length =
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SEQ ID NO: 1168 SEQUENCE: 1168 000	moltype =	length =
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SEQ ID NO: 1177 SEQUENCE: 1177 000	moltype =	length =
SEQ ID NO: 1178 SEQUENCE: 1178 000	moltype =	length =
SEQ ID NO: 1179 SEQUENCE: 1179 000	moltype =	length =

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SEQ ID NO: 1184 SEQUENCE: 1184 000	moltype =	length =
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SEQ ID NO: 1187 SEQUENCE: 1187 000	moltype =	length =
SEQ ID NO: 1188 SEQUENCE: 1188 000	moltype =	length =
SEQ ID NO: 1189 SEQUENCE: 1189 000	moltype =	length =
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SEQ ID NO: 1192 SEQUENCE: 1192 000	moltype =	length =
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SEQ ID NO: 1196 SEQUENCE: 1196 000	moltype =	length =
SEQ ID NO: 1197 SEQUENCE: 1197 000	moltype =	length =
SEQ ID NO: 1198 SEQUENCE: 1198 000	moltype =	length =

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SEQ ID NO: 1199 SEQUENCE: 1199 000	moltype =	length =
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SEQ ID NO: 1201 SEQUENCE: 1201 000	moltype =	length =
SEQ ID NO: 1202 SEQUENCE: 1202 000	moltype =	length =
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SEQ ID NO: 1206 SEQUENCE: 1206 000	moltype =	length =
SEQ ID NO: 1207 SEQUENCE: 1207 000	moltype =	length =
SEQ ID NO: 1208 SEQUENCE: 1208 000	moltype =	length =
SEQ ID NO: 1209 SEQUENCE: 1209 000	moltype =	length =
SEQ ID NO: 1210 SEQUENCE: 1210 000	moltype =	length =
SEQ ID NO: 1211 SEQUENCE: 1211 000	moltype =	length =
SEQ ID NO: 1212 SEQUENCE: 1212 000	moltype =	length =
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SEQ ID NO: 1215 SEQUENCE: 1215 000	moltype =	length =
SEQ ID NO: 1216 SEQUENCE: 1216 000	moltype =	length =
SEQ ID NO: 1217 SEQUENCE: 1217 000	moltype =	length =

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SEQ ID NO: 1218 SEQUENCE: 1218 000	moltype =	length =
SEQ ID NO: 1219 SEQUENCE: 1219 000	moltype =	length =
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SEQ ID NO: 1222 SEQUENCE: 1222 000	moltype =	length =
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SEQ ID NO: 1224 SEQUENCE: 1224 000	moltype =	length =
SEQ ID NO: 1225 SEQUENCE: 1225 000	moltype =	length =
SEQ ID NO: 1226 SEQUENCE: 1226 000	moltype =	length =
SEQ ID NO: 1227 SEQUENCE: 1227 000	moltype =	length =
SEQ ID NO: 1228 SEQUENCE: 1228 000	moltype =	length =
SEQ ID NO: 1229 SEQUENCE: 1229 000	moltype =	length =
SEQ ID NO: 1230 SEQUENCE: 1230 000	moltype =	length =
SEQ ID NO: 1231 SEQUENCE: 1231 000	moltype =	length =
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SEQ ID NO: 1234 SEQUENCE: 1234 000	moltype =	length =
SEQ ID NO: 1235 SEQUENCE: 1235 000	moltype =	length =
SEQ ID NO: 1236 SEQUENCE: 1236 000	moltype =	length =

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SEQ ID NO: 1241 SEQUENCE: 1241 000	moltype =	length =
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SEQ ID NO: 1244 SEQUENCE: 1244 000	moltype =	length =
SEQ ID NO: 1245 SEQUENCE: 1245 000	moltype =	length =
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SEQ ID NO: 1247 SEQUENCE: 1247 000	moltype =	length =
SEQ ID NO: 1248 SEQUENCE: 1248 000	moltype =	length =
SEQ ID NO: 1249 SEQUENCE: 1249 000	moltype =	length =
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SEQ ID NO: 1252 SEQUENCE: 1252 000	moltype =	length =
SEQ ID NO: 1253 SEQUENCE: 1253 000	moltype =	length =
SEQ ID NO: 1254 SEQUENCE: 1254 000	moltype =	length =
SEQ ID NO: 1255 SEQUENCE: 1255 000	moltype =	length =

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SEQ ID NO: 1256 SEQUENCE: 1256 000	moltype =	length =
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SEQ ID NO: 1258 SEQUENCE: 1258 000	moltype =	length =
SEQ ID NO: 1259 SEQUENCE: 1259 000	moltype =	length =
SEQ ID NO: 1260 SEQUENCE: 1260 000	moltype =	length =
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SEQ ID NO: 1262 SEQUENCE: 1262 000	moltype =	length =
SEQ ID NO: 1263 SEQUENCE: 1263 000	moltype =	length =
SEQ ID NO: 1264 SEQUENCE: 1264 000	moltype =	length =
SEQ ID NO: 1265 SEQUENCE: 1265 000	moltype =	length =
SEQ ID NO: 1266 SEQUENCE: 1266 000	moltype =	length =
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SEQ ID NO: 1268 SEQUENCE: 1268 000	moltype =	length =
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SEQ ID NO: 1270 SEQUENCE: 1270 000	moltype =	length =
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SEQ ID NO: 1272 SEQUENCE: 1272 000	moltype =	length =
SEQ ID NO: 1273 SEQUENCE: 1273 000	moltype =	length =
SEQ ID NO: 1274 SEQUENCE: 1274 000	moltype =	length =

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SEQ ID NO: 1279 SEQUENCE: 1279 000	moltype =	length =
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SEQ ID NO: 1281 SEQUENCE: 1281 000	moltype =	length =
SEQ ID NO: 1282 SEQUENCE: 1282 000	moltype =	length =
SEQ ID NO: 1283 SEQUENCE: 1283 000	moltype =	length =
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SEQ ID NO: 1285 SEQUENCE: 1285 000	moltype =	length =
SEQ ID NO: 1286 SEQUENCE: 1286 000	moltype =	length =
SEQ ID NO: 1287 SEQUENCE: 1287 000	moltype =	length =
SEQ ID NO: 1288 SEQUENCE: 1288 000	moltype =	length =
SEQ ID NO: 1289 SEQUENCE: 1289 000	moltype =	length =
SEQ ID NO: 1290 SEQUENCE: 1290 000	moltype =	length =
SEQ ID NO: 1291 SEQUENCE: 1291 000	moltype =	length =
SEQ ID NO: 1292 SEQUENCE: 1292 000	moltype =	length =
SEQ ID NO: 1293 SEQUENCE: 1293 000	moltype =	length =

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SEQ ID NO: 1294 SEQUENCE: 1294 000	moltype =	length =
SEQ ID NO: 1295 SEQUENCE: 1295 000	moltype =	length =
SEQ ID NO: 1296 SEQUENCE: 1296 000	moltype =	length =
SEQ ID NO: 1297 SEQUENCE: 1297 000	moltype =	length =
SEQ ID NO: 1298 SEQUENCE: 1298 000	moltype =	length =
SEQ ID NO: 1299 SEQUENCE: 1299 000	moltype =	length =
SEQ ID NO: 1300 SEQUENCE: 1300 000	moltype =	length =
SEQ ID NO: 1301 SEQUENCE: 1301 000	moltype =	length =
SEQ ID NO: 1302 SEQUENCE: 1302 000	moltype =	length =
SEQ ID NO: 1303 SEQUENCE: 1303 000	moltype =	length =
SEQ ID NO: 1304 SEQUENCE: 1304 000	moltype =	length =
SEQ ID NO: 1305 SEQUENCE: 1305 000	moltype =	length =
SEQ ID NO: 1306 SEQUENCE: 1306 000	moltype =	length =
SEQ ID NO: 1307 SEQUENCE: 1307 000	moltype =	length =
SEQ ID NO: 1308 SEQUENCE: 1308 000	moltype =	length =
SEQ ID NO: 1309 SEQUENCE: 1309 000	moltype =	length =
SEQ ID NO: 1310 SEQUENCE: 1310 000	moltype =	length =
SEQ ID NO: 1311 SEQUENCE: 1311 000	moltype =	length =
SEQ ID NO: 1312 SEQUENCE: 1312 000	moltype =	length =

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SEQ ID NO: 1313 SEQUENCE: 1313 000	moltype =	length =
SEQ ID NO: 1314 SEQUENCE: 1314 000	moltype =	length =
SEQ ID NO: 1315 SEQUENCE: 1315 000	moltype =	length =
SEQ ID NO: 1316 SEQUENCE: 1316 000	moltype =	length =
SEQ ID NO: 1317 SEQUENCE: 1317 000	moltype =	length =
SEQ ID NO: 1318 SEQUENCE: 1318 000	moltype =	length =
SEQ ID NO: 1319 SEQUENCE: 1319 000	moltype =	length =
SEQ ID NO: 1320 SEQUENCE: 1320 000	moltype =	length =
SEQ ID NO: 1321 SEQUENCE: 1321 000	moltype =	length =
SEQ ID NO: 1322 SEQUENCE: 1322 000	moltype =	length =
SEQ ID NO: 1323 SEQUENCE: 1323 000	moltype =	length =
SEQ ID NO: 1324 SEQUENCE: 1324 000	moltype =	length =
SEQ ID NO: 1325 SEQUENCE: 1325 000	moltype =	length =
SEQ ID NO: 1326 SEQUENCE: 1326 000	moltype =	length =
SEQ ID NO: 1327 SEQUENCE: 1327 000	moltype =	length =
SEQ ID NO: 1328 SEQUENCE: 1328 000	moltype =	length =
SEQ ID NO: 1329 SEQUENCE: 1329 000	moltype =	length =
SEQ ID NO: 1330 SEQUENCE: 1330 000	moltype =	length =
SEQ ID NO: 1331 SEQUENCE: 1331 000	moltype =	length =

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SEQ ID NO: 1334 SEQUENCE: 1334 000	moltype =	length =
SEQ ID NO: 1335 SEQUENCE: 1335 000	moltype =	length =
SEQ ID NO: 1336 SEQUENCE: 1336 000	moltype =	length =
SEQ ID NO: 1337 SEQUENCE: 1337 000	moltype =	length =
SEQ ID NO: 1338 SEQUENCE: 1338 000	moltype =	length =
SEQ ID NO: 1339 SEQUENCE: 1339 000	moltype =	length =
SEQ ID NO: 1340 SEQUENCE: 1340 000	moltype =	length =
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SEQ ID NO: 1342 SEQUENCE: 1342 000	moltype =	length =
SEQ ID NO: 1343 SEQUENCE: 1343 000	moltype =	length =
SEQ ID NO: 1344 SEQUENCE: 1344 000	moltype =	length =
SEQ ID NO: 1345 SEQUENCE: 1345 000	moltype =	length =
SEQ ID NO: 1346 SEQUENCE: 1346 000	moltype =	length =
SEQ ID NO: 1347 SEQUENCE: 1347 000	moltype =	length =
SEQ ID NO: 1348 SEQUENCE: 1348 000	moltype =	length =
SEQ ID NO: 1349 SEQUENCE: 1349 000	moltype =	length =
SEQ ID NO: 1350 SEQUENCE: 1350 000	moltype =	length =

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SEQ ID NO: 1353 SEQUENCE: 1353 000	moltype =	length =
SEQ ID NO: 1354 SEQUENCE: 1354 000	moltype =	length =
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SEQ ID NO: 1356 SEQUENCE: 1356 000	moltype =	length =
SEQ ID NO: 1357 SEQUENCE: 1357 000	moltype =	length =
SEQ ID NO: 1358 SEQUENCE: 1358 000	moltype =	length =
SEQ ID NO: 1359 SEQUENCE: 1359 000	moltype =	length =
SEQ ID NO: 1360 SEQUENCE: 1360 000	moltype =	length =
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SEQ ID NO: 1362 SEQUENCE: 1362 000	moltype =	length =
SEQ ID NO: 1363 SEQUENCE: 1363 000	moltype =	length =
SEQ ID NO: 1364 SEQUENCE: 1364 000	moltype =	length =
SEQ ID NO: 1365 SEQUENCE: 1365 000	moltype =	length =
SEQ ID NO: 1366 SEQUENCE: 1366 000	moltype =	length =
SEQ ID NO: 1367 SEQUENCE: 1367 000	moltype =	length =
SEQ ID NO: 1368 SEQUENCE: 1368 000	moltype =	length =
SEQ ID NO: 1369 SEQUENCE: 1369 000	moltype =	length =

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SEQ ID NO: 1370 SEQUENCE: 1370 000	moltype = length =	
SEQ ID NO: 1371 SEQUENCE: 1371 000	moltype = length =	
SEQ ID NO: 1372 SEQUENCE: 1372 000	moltype = length =	
SEQ ID NO: 1373 SEQUENCE: 1373 000	moltype = length =	
SEQ ID NO: 1374 SEQUENCE: 1374 000	moltype = length =	
SEQ ID NO: 1375 SEQUENCE: 1375 000	moltype = length =	
SEQ ID NO: 1376 SEQUENCE: 1376 000	moltype = length =	
SEQ ID NO: 1377 SEQUENCE: 1377 000	moltype = length =	
SEQ ID NO: 1378 SEQUENCE: 1378 000	moltype = length =	
SEQ ID NO: 1379 SEQUENCE: 1379 000	moltype = length =	
SEQ ID NO: 1380 SEQUENCE: 1380 000	moltype = length =	
SEQ ID NO: 1381 FEATURE misc_feature source	moltype = DNA length = 22 Location/Qualifiers 1..22 note = Profiling Sequence 1 1..22 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 1381 ttctagagcg gccgcttcga gc		22
SEQ ID NO: 1382 FEATURE misc_feature source	moltype = DNA length = 22 Location/Qualifiers 1..22 note = Profiling Sequence 2 1..22 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 1382 cgctgatctc acgccgtggt ga		22
SEQ ID NO: 1383 FEATURE misc_feature source	moltype = DNA length = 22 Location/Qualifiers 1..22 note = Poly A Tailing Sequence 1..22 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 1383 agttccgcgt acgtacggcg tc		22
SEQ ID NO: 1384 FEATURE	moltype = DNA length = 63 Location/Qualifiers	

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misc_feature      1..63
                  note = PCR Primer
misc_feature      30..41
                  note = n is a, c, g, or t
source            1..63
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 1384
tctttcccta cagcagctc ttccgatctn nnnnnnnnnn ntcaccacgg cgtgagatca 60
gcg                                                                63

SEQ ID NO: 1385      moltype = DNA length = 22
FEATURE             Location/Qualifiers
misc_feature        1..22
                  note = PCR Primer
source              1..22
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 1385
gacgccgtac gtacgcggaa ct                                          22

SEQ ID NO: 1386      moltype = DNA length = 29
FEATURE             Location/Qualifiers
misc_feature        1..29
                  note = PCR Primer
source              1..29
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 1386
acatttgctt ctgacacaac tgtgttcac                                  29

SEQ ID NO: 1387      moltype = DNA length = 30
FEATURE             Location/Qualifiers
misc_feature        1..30
                  note = PCR Primer
source              1..30
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 1387
atggccgttt acccatacga tgttctgac                                  30

SEQ ID NO: 1388      moltype = DNA length = 29
FEATURE             Location/Qualifiers
misc_feature        1..29
                  note = PCR Primer
source              1..29
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 1388
tctttcccta cagcagctc ttccgatct                                  29

SEQ ID NO: 1389      moltype = DNA length = 50
FEATURE             Location/Qualifiers
misc_feature        1..50
                  note = PCR Primer
source              1..50
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 1389
ggagttcaga cgtgtgctct tccgatcttt ctagagcggc cgcttcgagc      50

SEQ ID NO: 1390      moltype = DNA length = 28
FEATURE             Location/Qualifiers
misc_feature        1..28
                  note = PCR Primer
source              1..28
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 1390
ggcaaccta atacgactca ctataggg                                  28

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- 1-33.** (canceled)
- 34.** A method for identifying RNA molecules possessing increased translatability and stability, comprising:
 obtaining a pool of RNA molecules, wherein each RNA molecule is uniquely encoded with a barcoding sequence and each barcoding sequence is flanked by at least one profiling sequence;
 assessing translatability of the pool of RNA molecules by:
 transfecting a cell or cell lysate with a first subset of the pool of RNA molecules;
 performing polysome profiling on the first subset of the pool of RNA molecules to segregate RNA molecules based on the number of ribosomes bound to the RNA molecule; and
 isolating a fraction from the polysome profile to generate a first set of RNA molecules showing a first level of ribosomes bound to the RNA molecules in the set of RNA molecules; and
 assessing stability of the pool of RNA molecules by:
 treating a second subset of the pool of RNA molecules under an experimental condition; and
 isolating a fraction from the second subset the pool of RNA molecules at a specified timepoint to generate a second set of RNA molecules showing stability under the experimental condition for the specified timepoint.
- 35.** The method of claim **34**, further comprising sequencing the barcode sequence of the first set of RNA molecules and the second set of RNA molecules to identify the presence of each RNA molecule in each fraction of RNA molecules.
- 36.** The method of claim **35**, further comprising determining translatability and stability of the RNA molecules associated with each barcode sequence in the first set of RNA molecules and the second set of RNA molecules by identifying the prevalence of each barcode in each fraction of RNA molecules.
- 37.** The method of claim **34**, wherein the barcoding sequence is selected from SEQ ID NOs: 115-1380.
- 38.** The method of claim **34**, wherein the profiling sequence is selected from SEQ ID NOs: 1381-1382.
- 39.** A method to select for RNA elements, comprising:
 obtaining a library of RNA molecules, wherein each RNA molecule comprises a coding sequence, a 5' untranslated region (5'UTR), and a 3' untranslated region (3'UTR), wherein one of the coding sequence, the 5'UTR, or the 3'UTR comprises a degenerate region;
 assessing a property of the library of RNA molecules, wherein the property is selected from the group consisting of translatability, in vivo stability, and in vitro stability; and
 selecting an RNA molecule from the library of RNA molecules showing increase in the property over other RNA molecules in the library of RNA molecules.
- 40.** The method of claim **39**, further comprising sequencing the selected RNA molecule.
- 41.** The method of claim **39**, wherein the selected RNA molecule is a pool of RNA molecules.
- 42.** The method of claim **41**, further comprising:
 reassessing the property of the pool of RNA molecules; and
 selecting an RNA molecule from the pool of RNA molecules showing increase in the property over other RNA molecules in the pool of RNA molecules.
- 43.** The method of claim **42**, further comprising sequencing the selected RNA molecule from the pool of RNA molecules.
- 44.** The method of claim **39**, wherein the property is translatability.
- 45.** The method of claim **39**, wherein the degenerate region is selected from the group consisting of: a deletion, a random sequence, an ambiguous sequence, and a truncation.
- 46.** The method of claim **34**, wherein the RNA molecules are transfected into a collection of cells.
- 47.** The method of claim **46**, wherein the collection of cells is selected from mammalian cells, yeast cells, bacteria cells, and plant cells.
- 48.** The method of claim **34**, wherein the RNA molecules are added to a cell lysate.
- 49.** The method of claim **34**, wherein polysome profiling comprises adding a cell lysate to a sucrose gradient and centrifuging the sucrose gradient to segregate the RNA molecules.
- 50.** The method of claim **34**, further comprising isolating a second fraction from the polysome profile to generate a second set of RNA molecules showing a second level of ribosomes bound to the RNA molecules in the set of RNA molecules, wherein the first level and second level represent different amounts of bound ribosomes.
- 51.** The method of claim **50**, further comprising sequencing the barcode sequence of each RNA molecule in the first set of RNA molecules and the second set of RNA molecules to identify the presence of each RNA molecule in the first set of RNA molecules and the second set of RNA molecules.
- 52.** The method of claim **34**, wherein the treatment condition is selected from temperature, pH, presence of certain molecules, presence of certain ions, concentration of certain molecules, concentration of certain ions, irradiation, buffer type, and buffer concentration.
- 53.** The method of claim **34**, further comprising size selecting for full-length RNA molecules.

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