

CIRCULAR RNA SYNTHESIS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This PCT application claims the priority benefit of U.S. Provisional Application No. 63/605,896, filed December 4, 2023; the entire disclosure is incorporated herein by reference for all purposes.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

VIA EFS-WEB

[0002] The content of the sequence listing is submitted electronically (Name: 4597_034PC01_SequenceListing_ST26.xml; Size: 203,648 bytes; and Date of Creation: December 4, 2024) and is filed with the application is herein incorporated by reference in its entirety.

TECHNICAL FIELD

[0003] The following relates to methods and compositions for the production circular RNAs (circRNAs).

BACKGROUND

[0004] Circular RNAs (circRNAs) are covalently closed single-stranded RNA molecules lacking free 5' and 3' ends, and therefore lacking a 5' cap and a 3' polyA tail.

[0005] In nature, circRNAs have a wide range of origins and tissue specificity and play a variety of roles in the development and homeostasis of organisms, in aging, and have also been linked to the occurrence and development of various human diseases (*e.g.*, cardiovascular diseases, diabetes mellitus, and cancer). circRNAs are more stable than linear RNAs, do not need a 5' cap or a 3' poly(A) tail to keep them stable, and are not easily degraded by RNA exonucleases, making circRNAs great candidates as therapeutics.

[0006] Nevertheless, the use of circRNAs as therapeutics has been hampered by technical limitation in their synthesis. Linear RNA can be produced by chemical synthesis or enzymatic strategy, and circRNAs can be produced by ligating the ends of linear RNA precursors to produce a covalently closed circle. The use of chemical synthesis is limited by the high cost of purification and low yield, and can only produce RNAs of less than 50 to 70 nucleotides in length. Enzymatic strategy is usually realized through an *in vitro* transcription (IVT) reaction, which includes a DNA template, a reaction buffer, and a phage RNA polymerase. IVT reaction allows for longer RNA

synthesis at a lower cost. However, the run-off nature of phage polymerases may result in incomplete RNA. Additionally, the high immunogenicity of the synthesized circRNAs further hinders the applicability of circRNAs as therapeutic molecules.

[0007] Given the strong therapeutic potential of circRNAs, there is an immediate need for methods and composition for producing circRNAs with high efficiency, and for producing circRNAs having low immunogenicity.

SUMMARY

[0008] Aspects of the present disclosure address the above-referenced problems and/or others.

[0009] In some aspects, the disclosure provides a genetic construct comprising a formula of:

[0010] **5'-H-A-E-B-H'-3'**,

wherein H comprises a 5' homology arm; A comprises a 3' intronic sequence that is optionally derived from a Group II intron; E comprises an exonic sequence (i.e., one or more exons or fragments thereof); B comprises a 5' intronic sequence that is optionally derived from a Group II intron; and H' comprises a 3' homology arm; wherein at least one of the 3' intronic sequence and the 5' intronic sequence is derived from a Group II intron and wherein the genetic construct is capable of forming a circular RNA.

[0011] In some aspects, the disclosure provides a genetic construct comprising a formula of:

[0012] **5'-P-H-A-3S-E-5S-B-H'-3'**,

wherein P comprises a promoter (e.g., T7, T3, SP6, etc.); H comprises a 5' homology arm; A comprises a 3' intronic sequence that is optionally derived from a Group II intron; 3S comprises a 3' splice site; E comprises an exonic sequence (i.e., one or more exons or fragments thereof); 5S comprises a 5' splice site; B comprises a 5' intronic sequence that is optionally derived from a Group II intron; H' comprises a 3' homology arm; wherein the promoter (P) is to be understood to be comprised in the DNA genetic construct, but not in the RNA genetic construct, and wherein at least one of the 3' intronic sequence and the 5' intronic sequence is derived from a Group II intron.

[0013] In some aspects, the 3' intronic sequence and the 5' intronic sequence are derived from a Group II intron. In some aspects, the 3' intronic sequence and the 5' intronic sequence are derived from the same Group II intron. In some aspects, the 3' intronic sequence and the 5' intronic

sequence are derived from different Group II introns. In some aspects, the 3' intronic sequence and the 5' intronic sequence together are less than the full intron.

[0014] In some aspects, the circular RNA has a reduced immunogenicity compared to a circular RNA that contains an excision scar.

[0015] In some aspects, the 5' intronic sequence and/or the 3' intronic sequence are derived from an intron of *Pylaiella littoralis* 1-2, *Amoebidium parasiticum*, *Mesostigma viridae*, *Lactococcus lactis*, or *Oceanobacillus iheyensis*, or any combination thereof.

[0016] In some aspects, the 5' splice site comprises a site between two nucleotides comprising A and N, where N is any nucleotide. In some aspects, the N is A, T, U, G, or C, e.g., G.

[0017] In some aspects, the 3' splice site comprises a site between two nucleotides comprising T or U and N, wherein N is any nucleotide. In some aspects, the N is A or C.

[0018] In some aspects, the catalytic core comprises AGC, CGC, or both.

[0019] In some aspects, the circRNA formed by the genetic construct comprises an excision scar. In some aspects, the circRNA formed by the genetic construct does not comprise an excision scar.

[0020] In some aspects, the 3' intronic sequence comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to a 3' intronic sequence set forth in SEQ ID NOs: 17, 18, 19, or 20.

[0021] In some aspects, the 5' intronic sequence comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 95%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to a 5' intronic sequence set forth in SEQ ID NO: 21, 22, 23, or 24.

[0022] In some aspects, the genetic construct is an RNA genetic construct. In some aspects, the genetic construct is a DNA genetic constructs.

[0023] In some aspects, the genetic construct further comprises a promoter. In some aspects, the promoter comprises a T7 promoter, a T3 promoter, an SP6 promoter, or any combination thereof.

[0024] In some aspects, the genetic construct further comprises an internal ribosomal entry site (“IRES”). In some aspects, the IRES comprises a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to any sequence set forth in SEQ ID NO: 44 to 67 if the genetic construct is DNA and to any sequence set forth in SEQ ID NO: 68 to 91 if the genetic construct is RNA.

[0025] In some aspects, the exonic sequence further comprises a binding site for a polyA binding protein at the 5' terminus.

[0026] In some aspects, the 5' homology arm comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the sequence as set forth in SEQ ID NO: 27 or 29. In some aspects, the 3' homology arm comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the sequence as set forth in SEQ ID NO: 28 or 30.

[0027] In some aspects, the exonic sequence is linked to one or more untranslated regions at the 3' and/or 5' of the exonic sequence.

[0028] In some aspects, the genetic construct further comprises a polyA. In some aspects, the polyA comprises a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the sequence as set forth in SEQ ID NO: 42, 43, or any combination thereof.

[0029] In some aspects, the genetic construct further comprises a spacer sequence. In some aspects, the spacer comprises a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 95%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to any sequence as set forth in SEQ ID NO: 31, 32, 33, 34, or any combination thereof.

[0030] In some aspects, the exonic sequence encodes a protein. In some aspects, the circular RNA exhibits an increased expression of the protein, a prolonged expression of the protein in vitro and/or in vivo, a reduced activation of an immune signaling pathway, an increased immune activation, or any combination thereof. In some aspects, the protein comprises a therapeutic

protein. In some aspects, the protein comprises a cytokine, a receptor, a ligand, an immunomodulatory, a growth factor, an antigen, an RNA binding domain, or any combination thereof. In some aspects, the protein comprises an antibody, a fusion protein, or any combination thereof. In some aspects, the protein comprises CD3, CD4, CDS, CD19, CD20, CD22, CD30, CD34, HER2, HER3, HER4, LFA-I, MoI, p150, 95, VLA-4, ICAM-I, VCAM, alpha v/beta 3 integrin, human macrophage inflammatory protein (MIP-1 -alpha), erythropoietin (EPO), NGF-beta, platelet-derived growth factor (PDGF), fibroblast growth factors, epidermal growth factor (EGF), transforming growth factors (TGF), insulin-like growth factors-I and -II (IGF-I and IGF-II), des(1-3)-IGF-I (brain IGF-1), factor VIII, tissue factor, von Willebrands factor, protein C, alpha-1-antitrypsin, plasminogen activators, such as urokinase and tissue plasminogen activator ("t-PA"), bombazine, thrombin, thrombopoietin, M-CSF, GM-CSF, G-CSF, flk2/flt3 receptor, obesity (OB) receptor, LDL receptor, growth hormone receptors, thrombopoietin receptors ("TPO-R," "c-mpl"), glucagon receptors, interleukin receptors, interferon receptors, T-cell receptors, stem cell factor receptors, OX40L, bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), relaxin A-chain, relaxin B-chain, prorelaxin, interleukins and interleukin receptors, including IL-1 to IL-33 and IL-1 to IL-33 receptors, such as the IL-8 receptor, viral antigens, lipoproteins, calcitonin, glucagon, atrial natriuretic factor, lung surfactant, tumor necrosis factor-alpha and -beta, enkephalinase, RANTES (regulated on activation normally T-cell expressed and secreted), mouse gonadotropin-associated peptide, DNase, inhibin, activin, PUF domain, Cas protein, or any combination thereof.

[0031] In some aspects, the disclosure provides a composition comprising the genetic construct of the disclosure.

[0032] In some aspects, the disclosure provides a method of making a circular RNA, comprising initiating the genetic construct of the disclosure to form a circular RNA.

[0033] In some aspects, the disclosure provides a circular RNA prepared by the method of the disclosure.

[0034] In some aspects, the disclosure provides a method of treating a disease or condition in a subject in need thereof, comprising administering the circular RNA of the disclosure to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] Aspects of the present disclosure may take form in various components and arrangements of components, and in various steps and arrangements of steps. The drawings are

only for illustration purpose of preferred aspects of the present disclosure and are not to be considered as limiting.

[0036] Features of various aspects of the present disclosure will be more readily understood from the following detailed description taken in conjunction with the accompanying drawings.

[0037] **Fig. 1** is a schematic representation of a genetic construct (DNA or RNA) having the formula:

5'-P-H-A-3S-E-5S-B-H'-3'

wherein:

P comprises a promoter;

H comprises a 5' homology arm;

A comprises a 3' intronic sequence comprising a catalytic core (triad) (CC) that is derived from a Group II intron;

3S comprises a 3' splice site;

E comprises an exon;

5S comprises a 5' splice site;

B comprises a 5' intronic sequence comprising an intron-binding site (IBS) that is derived from a Group II intron; and

H' comprises a 3' homology arm;

wherein the promoter (P) is to be understood to be comprised in the DNA genetic construct, but not in the RNA genetic construct.

[0038] **Fig. 2** is a plasmid map of the genetic construct depicted in **Fig. 1**.

[0039] **Fig. 3** is a schematic representation of a circularization mechanism.

[0040] **Fig. 4** is an exemplary representation of a circularization mechanism.

DETAILED DESCRIPTION

[0041] The present disclosure generally relates to methods for synthesizing circular RNA (circRNA). The present disclosure includes RNA genetic constructs capable of forming circular RNAs (*i.e.*, pre-circRNAs), DNA genetic constructs from which the pre-circRNAs can be transcribed, methods of synthesizing circular RNAs (*i.e.*, circRNAs) using the pre-circRNAs, the circRNAs and/or DNA genetic constructs disclosed herein, pharmaceutical compositions comprising the pre-circRNAs, the circRNAs and/or DNA genetic constructs disclosed herein, and method of treating a disease in a subject in need thereof comprising the pre-circRNAs, the circRNAs and/or DNA genetic constructs or the pharmaceutical compositions disclosed herein.

[0042] Aspects of the present disclosure include synthesizing circRNA from a genetic construct that includes group II introns.

I. Definitions

[0043] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In case of conflict, the present application, including the definitions, will control. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0044] Throughout this disclosure, the term "**a**" or "**an**" entity refers to one or more of that entity; for example, "a polynucleotide," is understood to represent one or more polynucleotides. As such, the terms "a" (or "an"), "**one or more**," and "**at least one**" can be used interchangeably herein.

[0045] Furthermore, "**and/or**" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0046] It is understood that wherever aspects are described herein with the language "**comprising**," otherwise analogous aspects described in terms of "**consisting of**" and/or "**consisting essentially of**" are also provided. As used herein, "**comprising**" is synonymous with "**including**," "**containing**," or "**characterized by**," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. As used herein, "**consisting of**" excludes any element, step, or ingredient not specified in the claim element. As used herein, "**consisting essentially of**" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim.

[0047] The term "**about**" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 10 percent, up or down (higher or lower), unless indicated otherwise. Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used

in the specification, including claims, are to be understood as being modified in all instances by the term "about." Accordingly, unless otherwise indicated to the contrary, the numerical parameters are approximations and can vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches.

[0048] The term "**at least**" prior to a number or series of numbers is understood to include the number adjacent to the term "at least," and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, "at least 18 nucleotides of a 21-nucleotide nucleic acid molecule" means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range. "At least" is also not limited to integers (*e.g.*, "at least 5%" includes 5.0%, 5.1%, 5.18% without consideration of the number of significant figures).

[0049] "**Nucleic acid**," "**nucleic acid molecule**," "**nucleotide sequence**," "**nucleic acid sequence**," "**polynucleotide**," and grammatical variants thereof are used interchangeably and refer to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Single stranded nucleic acid sequences refer to single-stranded DNA (ssDNA) or single-stranded RNA (ssRNA). Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, supercoiled DNA and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences can be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the non-transcribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation. DNA includes, but is not limited to, cDNA, genomic DNA, plasmid DNA, synthetic DNA, and semi-synthetic DNA. A "nucleic acid composition" of the disclosure comprises one or

more nucleic acids as described herein. As described herein, a polynucleotide of the present disclosure comprises DNA, RNA, or both. In some aspects, the term "polynucleotide" includes polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), including tRNA, rRNA, shRNA, siRNA, miRNA and mRNA, whether spliced or unspliced, any other type of polynucleotide which is an N- or C-glycoside of a purine or pyrimidine base, and other polymers containing normucleotidic backbones, for example, polyamide (*e.g.*, peptide nucleic acids "PNAs") and polymorpholino polymers, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA.

[0050] The symbol "-" in the formula of any genetic constructs disclosed herein can be a phosphodiester bond, or one or more nucleotides inserted between two nucleotides. For example, when a formula requires 5'-A-E-B-3', wherein A comprises a 3' intronic sequence, E comprises an exonic sequence (*i.e.*, one or more exons or fragments thereof), and B comprises a 5' intronic sequence, and wherein "-" between A and E and/or "-" between E and B can be a phosphodiester bond or one or more nucleotides between A and E and/or E and B.

[0051] As used herein, the term "**polypeptide**" encompasses both peptides and proteins, unless indicated otherwise.

[0052] The term "**coding region**" refers to a DNA or RNA region (the transcribed region) which "encodes" a particular protein, *e.g.*, such as a payload.

[0053] The term "**RNA**" is used herein to mean a molecule which comprises at least one ribonucleotide residue. "**Ribonucleotide**" relates to a nucleotide with a hydroxyl group at the 2'-position of a β -D-ribofuranosyl group. The term comprises double-stranded RNA, single-stranded RNA, isolated RNA such as partially or completely purified RNA, essentially pure RNA, synthetic RNA, and recombinantly generated RNA such as modified RNA which differs from naturally occurring RNA by addition, deletion, substitution and/or alteration of one or more nucleotides. The term "**mRNA**" means "**messenger-RNA**" and relates to a "**transcript**" which is generated by using a DNA template and encodes a peptide or protein. Typically, an mRNA comprises a 5'-UTR, a protein coding region and a 3'-UTR. mRNA only possesses limited half-life in cells and *in vitro*. In the context of the present disclosure, mRNA can be generated by *in vitro* transcription from a DNA template. The *in vitro* transcription methodology is known to the skilled person. For example, there is a variety of *in vitro* transcription kits commercially available. As further described herein,

in some aspects, a RNA is a linear RNA. In some aspects, a RNA is a circular RNA. In some aspects, a RNA is a self-replicating RNA. In some aspects, a RNA is a non-replicating RNA.

[0054] As used herein, the term "**pre-circular RNA**" refers to a RNA (*e.g.*, mRNA) that is capable of directing its own circularization to form a circular RNA (also referred to herein as "**pre-circRNA**").

[0055] As used herein, the term "**circular RNA**," or "**circRNA**" refers to a RNA (*e.g.*, mRNA) that forms a circular structure through covalent bonds. CircRNAs are single-stranded RNA molecules lacking free 5' and 3' ends, and therefore lacking a 5' cap and a 3' polyA tail.

[0056] As used herein, the term "**payload sequence**" refers to a nucleotide sequence encoding a payload. As used herein, the term "**payload**" refers to any protein that can be encoded by the payload sequence. In some aspects, a payload comprises a therapeutic protein. Non-limiting examples of payloads are provided elsewhere in the present disclosure.

[0057] The term "**sequence identity**" is used herein to mean a relationship between two or more amino acid (polypeptide or protein) sequences or two or more nucleic acid (polynucleotide) sequences, as determined by comparing the sequences. In certain aspects, sequence identity is calculated based on the full length of two given SEQ ID NO or on part thereof. Part thereof can mean at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of both SEQ ID NO, or any other specified percentage. The term "**identity**" can also mean the degree of sequence relatedness between amino acid or nucleic acid sequences, as the case can be, as determined by the match between strings of such sequences.

[0058] In some aspects, methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs.

[0059] As used herein, the terms "**effective amount**" or "**therapeutically effective amount**" of, *e.g.*, a circRNA disclosed herein, refers to a quantity sufficient to, when administered to the subject, including a human, effect beneficial or desired results, including clinical results, and, as such, an "effective amount" or synonym thereto depends on the context in which it is being applied.

[0060] As used herein, the term "**target cell**" refers to a cell in which a payload (*e.g.*, encoded by the payload sequence) is desired to be expressed. As used herein, the term "**non-target cell**" refers to a cell in which a payload is not intended to be expressed.

[0061] As used herein, the terms "**genetic construct**" refers to an artificially-designed nucleic acid molecule, which can be borne on a vector. A genetic construct can be an RNA nucleic acid molecule (*e.g.*, a pre-circRNA) or a DNA nucleic acid molecule (*e.g.*, an expression cassette from which the pre-circRNA can be transcribed, or a vector comprising the expression cassette).

[0062] As used herein, the term or "**vector**" refers to any nucleic acid molecule for the cloning of a nucleic acid, such as a plasmid, phage, transposon, cosmid, chromosome, artificial chromosome, virus, virion, etc. A vector can be a replicon to which another nucleic acid segment can be attached so as to bring about the replication of the attached segment. A "replicon" refers to any genetic element (*e.g.*, plasmid, phage, cosmid, chromosome, virus) that functions as an autonomous unit of replication *in vivo*, *i.e.*, capable of replication under its own control. A vector can be a "delivery vector" includes both viral and nonviral vehicles for introducing the nucleic acid into a cell *in vitro*, *ex vivo* or *in vivo*. A vector can be also used *in vitro* in the absence of cells to perform any of the procedures known in the art. For example, a vector can be used for *in vitro* transcription. A large number of vectors are known and used in the art including, for example, plasmids, modified eukaryotic viruses, or modified bacterial viruses. In some aspects, insertion of a polynucleotide into a suitable vector can be accomplished by ligating the appropriate polynucleotide fragments into a chosen vector that has complementary cohesive termini. Vectors can be engineered to encode selectable markers or reporters that provide for the selection or identification of cells that have incorporated the vector. Expression of **selectable markers** or reporters allows identification and/or selection of host cells that incorporate and express other coding regions contained on the vector. Examples of selectable marker genes known and used in the art include: genes providing resistance to ampicillin, streptomycin, gentamycin, kanamycin, hygromycin, bialaphos herbicide, sulfonamide, and the like; and genes that are used as phenotypic markers, *i.e.*, anthocyanin regulatory genes, isopentenyl transferase gene, and the like. Examples of **reporters** known and used in the art include: luciferase (Luc), green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), β -galactosidase (LacZ), β -glucuronidase (Gus), and the like. Selectable markers can also be considered to be reporters. In some aspects, the delivery vector is selected from the group consisting of a viral vector (*e.g.*, an AAV vector), a plasmid, a lipid, a protein particle, a bacterial vector, and a lysosome.

[0063] As used herein the term "**splicing**" refers to the process by which exons are joined while introns are removed from primary transcripts (pre-RNAs) to form the mature RNAs.

[0064] "Exons" are the coding sections of a DNA molecule, or of an RNA molecule which is transcribed from a DNA molecule that are then translated into protein, or are part of a mature structural RNA (e.g., tRNA or rRNA). In most of eukaryotic genes exons are separated by "introns," intervening non-coding sections of DNA. Therefore, introns are segments of nucleic acid that are transcribed into RNA, but are excised via splicing and therefore are not present in the mature RNA transcript, which comprises only exons. The new, immature strands of messenger or structural RNAs, before splicing occurs are called pre-RNAs, and may contain both introns and exon, the RNA molecules resulting from splicing are called RNAs, or mature RNAs. pre-RNA molecules go through splicing and only the exons remain in the "mature RNA," which is then, in the case of mRNA, translated into a protein. "Structural RNAs" or "non-coding RNAs" are RNA molecule that are not translated into proteins and exert a function in the form of RNA molecules. Exemplary structural RNA molecules are rRNAs and tRNAs. During splicing introns are removed from the pre-RNA by cleavage at conserved sequences called "splice sites," which are located at the 5' and 3' ends of the exons.

[0065] As used herein, the term "exonic sequence" refers to a nucleotide sequence which is comprised in the mature RNA after splicing. As used herein, the term "intronic sequence" refers to a nucleotide sequence which is removed from the RNA molecule during splicing and it is thus not comprised in the mature RNA after splicing. An exonic sequence can comprise one or more exons, or portions thereof, can comprise a nucleotide sequence derived from a naturally occurring nucleotide sequence, or can be an artificial sequence. An intronic sequence can comprise one or more introns, or portions thereof, can comprise a nucleotide sequence derived from a naturally occurring nucleotide sequence, or can be an artificial sequence.

[0066] As used herein, the term "5' intronic sequence" and "3' intronic sequence" refer to intronic sequences that are located at the 5' and at the 3', respectively, of an intronic sequence. In some aspects, the constructs disclosed herein comprise "5' intronic sequence" and "3' intronic sequence" that are in a reverse position. Thus, in some aspects, a "3' intronic sequence" can be located at the 5', and a "5' intronic sequence" can be located at the 3' of the constructs disclosed herein.

[0067] As used herein, the term "catalytic nucleotide" refers to a nucleotide that facilitates the circularization of pre-circRNA by interacting with a catalytic core (triad) of an intronic sequence.

[0068] As used herein, the term "**splice site**" refers to a sequence at the border between an exon and an intron. A splice site comprises a pair of nucleotides linked by a phosphodiester bond that is cleaved during a splicing reaction.

[0069] A 5' splice site is located at the border between the 3' end of an exon and the 5' end of an intron. A 5' splice site comprises a pair of nucleotides between which cleavage of the phosphodiester bond occurs during a splicing reaction. The nucleotide at the 5' of the pair of nucleotides is comprised in the exon, the nucleotide at the 3' of the pair of nucleotides is comprised in the intron.

[0070] A 3' splice site is located at the border between the 5' end of an exon and the 3' end of an intron. A 3' splice site comprises a pair of nucleotides between which cleavage of the phosphodiester bond occurs during a splicing reaction. The nucleotide at the 5' of the pair of nucleotides is comprised in the intron, the nucleotide at the 3' of the pair of nucleotides is comprised in the exon.

[0071] As used herein, the term "**catalytic nucleotide**" refers to a nucleotide that facilitates the circularization of pre-circRNA by interacting with a catalytic core (triad) of an intronic sequence.

[0072] "**Linear nucleic acid molecules**" are said to have a "5'-terminus" (5' end) and a "3'-terminus" (3' end) because nucleic acid phosphodiester linkages occur at the 5' carbon and 3' carbon of the sugar moieties of the substituent mononucleotides. The end nucleotide of a polynucleotide at which a new linkage would be to a 5' carbon is its 5' terminal nucleotide. The end nucleotide of a polynucleotide at which a new linkage would be to a 3' carbon is its 3' terminal nucleotide. A terminal nucleotide, as used herein, is the nucleotide at the end position of the 3'- or 5'-terminus.

[0073] "**Transcription**" means the formation or synthesis of an RNA molecule by an RNA polymerase using a DNA molecule as a template. The invention is not limited with respect to the RNA polymerase that is used for transcription. For example, in some aspects, a T7- type RNA polymerase can be used.

[0074] "**Translation**" means the formation of a polypeptide molecule by a ribosome based upon an RNA template.

[0075] As used herein, the term "**coding sequence**," or "expression sequence" refers to a nucleic acid sequence that encodes a product, e.g., a peptide or polypeptide (*i.e.*, a protein coding sequence), or a regulatory or structural nucleic acid (*i.e.*, a tRNA or a rRNA).

[0076] As used herein, a "**spacer**" refers to a region of a polynucleotide sequence ranging from 1 nucleotide to hundreds or thousands of nucleotides separating two other elements along a polynucleotide sequence. The sequences can be defined or can be random. A spacer is typically non-coding. In some aspects, spacers include duplex forming regions.

[0077] As used herein, an "**internal ribosome entry site**" or "IRES" refers to an RNA sequence or structural element ranging in size from 10 nt to 1000 nt or more, capable of initiating translation of a polypeptide in the absence of a typical RNA cap structure. An IRES is typically about 500 nt to about 700 nt in length.

[0078] As used herein, an "**intron-binding site**" refers to a sequence that is recognized by another portion of an intron (referred to as an "exon binding site") to enable exon splicing.

[0079] As used herein, an "**exon binding site**" refers to a sequence that recognizes an exon and aids in orientating secondary structure to enable exon splicing.

[0080] It is known to the skilled person that a sequence disclosed herein (*e.g.*, a splice site) can be both in a DNA or an RNA form. If a sequence (*e.g.*, a splice site) is in a DNA form, it is known to the skilled person to comprise a "T," and if a sequence (*e.g.*, a splice site) is in an RNA form, it is known to the skilled person to comprise a "U."

II. Pre-circular RNA and DNA Genetic Constructs

[0081] Provided herein are RNA or DNA genetic constructs for the synthesis of circular RNAs (circRNAs). The disclosure also provides for circRNAs produced from such genetic constructs. In some aspects, the genetic constructs for the synthesis of circRNAs are RNA genetic constructs (*i.e.*, pre-circRNAs). In some aspects, the genetic constructs for the synthesis of circRNAs are DNA genetic constructs from which pre-circRNAs can be transcribed. In some aspects, the pre-circRNAs disclosed herein, or the pre-circRNAs transcribed from the DNA genetic constructs disclosed herein, undergo a splicing reaction. In some aspects, the splicing reaction is an auto-catalytic splicing reaction (*i.e.*, self-splicing reaction).

[0082] In some aspects, the genetic constructs disclosed herein comprise one or more intronic sequences (*i.e.*, one or more introns or fragments thereof) that are derived from a Group II intron and one or more exonic sequence (*i.e.*, one or more exon or fragment thereof).

[0083] In some aspects, the genetic constructs disclosed herein are DNA genetic constructs (*e.g.*, comprised in a vector). In some aspects, the DNA genetic constructs disclosed herein are transcribed into RNAs. In some aspects, the RNAs transcribed from the DNA genetic constructs disclosed herein undergo a splicing reaction. In some aspects, the splicing reaction is an auto-

catalytic splicing reaction. In some aspects, the splicing reaction partially removes the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) from the RNAs transcribed from the DNA genetic constructs disclosed herein. In some aspects, the splicing reaction completely removes the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) from the RNAs transcribed from the DNA genetic constructs disclosed herein. In some aspects, the splicing reaction circularizes the one or more exonic sequence (*i.e.*, one or more exon or fragment thereof) comprised in the RNAs transcribed from the DNA genetic constructs disclosed herein.

[0084] In some aspects, the genetic constructs disclosed herein are RNA genetic constructs (*e.g.*, pre-circRNAs). In some aspects, the RNA genetic constructs disclosed herein undergo a splicing reaction. In some aspects, the splicing reaction is an auto-catalytic splicing reaction. In some aspects, the splicing reaction partially removes the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) from the RNA genetic constructs disclosed herein. In some aspects, the splicing reaction completely removes the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) from the RNA genetic constructs disclosed herein. In some aspects, the splicing reaction circularizes the one or more exonic sequence (*i.e.*, one or more exon or fragment thereof) comprised in the RNA genetic constructs disclosed herein.

[0085] In some aspects, the circularized one or more exonic sequence (*i.e.*, one or more exon or fragment thereof) is not a protein-coding exonic sequence. In some aspects, the circularized one or more exonic sequence (*i.e.*, one or more exon or fragment thereof) is a structural RNA. In these aspects, the circularized one or more exonic sequence (*i.e.*, one or more exon or fragment thereof) can be a therapeutic agent (*i.e.*, a therapeutic RNA). In some aspects, the one or more exonic sequence (*i.e.*, one or more exon or fragment thereof) is a protein-coding sequence. In these aspects, the one or more exonic sequence (*i.e.*, one or more exon or fragment thereof) can encode a therapeutic agent (*i.e.*, a therapeutic protein). In these aspects, the one or more exonic sequence (*i.e.*, one or more exon or fragment thereof) are translated into a therapeutic agent (*i.e.*, a therapeutic protein).

[0086] In some aspects, the pre-circRNAs are linear RNAs produced by *in vivo* transcription of DNA genetic constructs disclosed herein. In some aspects, the pre-circRNAs are linear RNAs produced by *in vitro* transcription of DNA genetic constructs disclosed herein. In some aspects, the pre-circRNAs are linear RNAs produced by chemical synthesis. The pre-circRNAs disclosed herein can be unmodified, partially modified or completely modified.

[0087] In some aspects, the genetic constructs (e.g., pre-circRNAs) disclosed herein can circularize to produce a circular RNA (circRNA). In some aspects, the circRNAs disclosed herein comprises an exonic sequence (*i.e.*, one or more exons) comprised in the DNA genetic constructs or in the pre-circRNAs disclosed herein. In some aspects, the circRNAs disclosed herein consists of an exonic sequence (*i.e.*, one or more exons) comprised in the DNA genetic constructs or in the pre-circRNAs disclosed herein. In some aspects, the circRNAs disclosed herein further comprise one or more nucleotides comprised in the DNA genetic constructs or in the pre-circRNAs disclosed herein that are not part of the exonic sequence (*i.e.*, one or more exons). The one or more nucleotides comprised in the DNA genetic constructs or in the pre-circRNAs disclosed herein that are not part of the exonic sequence (*i.e.*, one or more exons) can be, for example comprised in the intronic sequence, in the splice sites, in the homology arms of the DNA genetic constructs or in the pre-circRNAs disclosed herein, or any combination thereof. In these aspects, the circRNAs disclosed herein is described herein as comprising an excision scar. In some aspects, the circular RNA is at least 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000 or 4500 nucleotides in size. The circRNA disclosed herein can be unmodified, partially modified or completely modified.

[0088] In some aspects, the RNA or DNA genetic constructs capable of forming circular RNAs disclosed herein (*i.e.*, pre-circRNAs or DNA genetic constructs from which pre-circRNAs can be transcribed) comprise one or more intronic sequences (*i.e.*, one or more introns or fragments thereof) that are derived from a Group II intron, and/or one or more exonic sequences (*i.e.*, one or more exons or fragments thereof). In some aspects, all intronic sequence are derived from a nucleotide sequence of a Group II intron.

[0089] In some aspects, the DNA or RNA genetic constructs disclosed herein are polynucleotides produced by any one of the molecular cloning techniques described in the published literature. In some aspects, the DNA or RNA genetic constructs disclosed herein are polynucleotides produced by chemical synthesis. In some aspects, the DNA or RNA genetic constructs disclosed herein are comprised in a vector, for example, in a plasmid vector. The DNA or RNA genetic constructs disclosed herein can be unmodified, partially modified or completely modified.

IIA. Genetic Constructs

[0090] The present disclosure provides a genetic construct capable of forming circular RNAs disclosed herein (*i.e.*, pre-circRNAs or DNA genetic constructs from which pre-circRNAs can be transcribed) comprising a formula of **5'-H-A-E-B-H'-3'**, wherein:

H comprises a 5' homology arm;

A comprises a 3' intronic sequence that is optionally derived from a Group II intron;

E comprises an exonic sequence (*i.e.*, one or more exons or fragments thereof);

B comprises a 5' intronic sequence that is optionally derived from a Group II intron; and

H' comprises a 3' homology arm, and

wherein at least one of the 3' intronic sequence and the 5' intronic sequence is derived from a Group II intron. In some aspects, both the 3' intronic sequence and the 5' intronic sequence are derived from a Group II intron. In some aspects, the 3' intronic sequence and the 5' intronic sequence are derived from the same Group II intron. In some aspects, the 3' intronic sequence and the 5' intronic sequence are derived from different Group II introns.

[0091] In some aspects, the construct comprises one or more splice sites, *e.g.*, between A and E and/or between E and B. In some aspects, the 3' terminus of A and 5' terminus of E form a 3' splice site (3S), and the 3' terminus of E and the 5' terminus of B form a 5' splice site (5S). In some aspects, the construct further comprises a promoter, wherein the promoter (P) is to be understood to be comprised in the DNA genetic construct, but not in the RNA genetic construct. In some aspects, the 5' and 3' intronic sequences are reverse compliments of one another.

[0092] In some aspects, the disclosure provides a genetic construct capable of forming circular RNAs disclosed herein (*i.e.*, pre-circRNAs or DNA genetic constructs from which pre-circRNAs can be transcribed) comprise a formula of **5'-P-H-A-3S-E-5S-B-H'-3'**, wherein:

P comprises a promoter (*e.g.*, T7, T3, SP6, etc.);

H comprises a 5' homology arm;

A comprises a 3' intronic sequence that is optionally derived from a Group II intron;

3S comprises a 3' splice site;

E comprises an exonic sequence (*i.e.*, one or more exons or fragments thereof);

5S comprises a 5' splice site;

B comprises a 5' intronic sequence that is optionally derived from a Group II intron;

H' comprises a 3' homology arm;
 wherein the promoter (P) is to be understood to be comprised in the DNA genetic construct, but not in the RNA genetic construct, and
 wherein at least one of the 3' intronic sequence and the 5' intronic sequence is derived from a Group II intron. In some aspects, both the 3' intronic sequence and the 5' intronic sequence are derived from a Group II intron. In some aspects, the 3' intronic sequence and the 5' intronic sequence are derived from the same Group II intron. In some aspects, the 3' intronic sequence and the 5' intronic sequence are derived from different Group II introns.

[0093] In some aspects, the 5' and 3' intronic sequences are reverse compliments of one another. In some aspects, the 3' terminus of A and 5' terminus of E form a 3' splice site (3S), and the 3' terminus of E and the 5' terminus of B form a 5' splice site (5S); wherein at least one of the 3' intronic sequence and the 5' intronic sequence is derived from a Group II intron. In some aspects, both the 3' intronic sequence and the 5' intronic sequence are derived from a Group II intron. In some aspects, the 3' intronic sequence and the 5' intronic sequence are derived from the same Group II intron. In some aspects, the 3' intronic sequence and the 5' intronic sequence are derived from different Group II introns.

[0094] The present disclosure is also directed to a method of producing a circular RNA comprising initiating a genetic construct disclosed herein (e.g., DNA or RNA) to form a circular RNA, wherein the genetic construct comprises a nucleotide sequence having a formula of **5'-H-A-E-B-H'-3'**,

wherein:

H comprises a 5' homology arm;

A comprises a 3' intronic sequence that is optionally derived from a Group II intron;

E comprises an exonic sequence (i.e., one or more exons or fragments thereof);

B comprises a 5' intronic sequence that is optionally derived from a Group II intron; and

H' comprises a 3' homology arm; wherein at least one of the 3' intronic sequence and the 5' intronic sequence is derived from a Group II intron;

or a formula of **5'-P-H-A-3S-E-5S-B-H'-3'**,

wherein:

P comprises a promoter (e.g., T7, T3, SP6, etc.);

H comprises a 5' homology arm;

A comprises a 3' intronic sequence;

3S comprises a 3' splice site;
E comprises an exonic sequence (i.e., one or more exons or fragments thereof);
5S comprises a 5' splice site;
B comprises a 5' intronic sequence;
and H' comprises a 3' homology arm;
or a formula of **5'-P-H-A-3S-E-5S-B-H'-3'**,
wherein:
P comprises a promoter (e.g., T7, T3, SP6, etc.);
H comprises a 5' homology arm;
A comprises a 3' intronic sequence that is optionally derived from a Group II intron;
3S comprises a 3' splice site;
E comprises an exonic sequence (i.e., one or more exons or fragments thereof);
5S comprises a 5' splice site;
B comprises a 5' intronic sequence that is optionally derived from a Group II intron;
and H' comprises a 3' homology arm;
wherein the promoter (P) is to be understood to be comprised in the DNA genetic construct, but not in the RNA genetic construct and wherein at least one of the 3' intronic sequence and the 5' intronic sequence is derived from a Group II intron. In some aspects, the 3' terminus of A and 5' terminus of E form a 3' splice site (3S), and the 3' terminus of E and the 5' terminus of B form a 5' splice site (5S).

[0095] In some aspects, the disclosure comprises a circular RNA produced by the pre-circRNA or the method disclosed herein.

II.B. Components of Genetic Constructs

[0096] Various components of the genetic constructs disclosed herein can be derived from a Group II intron. In some aspects, one or more components of the genetic constructs are derived from the same Group II intron from which the 3' intronic or 5' intronic sequence is derived. In some aspects, one or more components of the genetic constructs are derived from a Group I intron. Non-limiting exemplary components for the genetic constructs are disclosed below.

II.B.1. Splice Sites

[0097] In some aspects, the genetic constructs capable of forming circular RNAs disclosed herein (*i.e.*, pre-circRNAs or DNA genetic constructs from which pre-circRNAs can be transcribed) comprise or form a 5' splice site, a 3' splice site, or a combination thereof.

[0098] In some aspects, the 5' splice site and the 3' splice site are transposed with respect to the position of the same splice sites would they have been in a naturally occurring configuration. In a naturally occurring configuration, a 5' splice site is located upstream of an exon, and a 3' splice site is located downstream of an exonic sequence. In the genetic constructs disclosed herein, a 5' splice site can be located downstream of an exonic sequence, and a 3' splice site can be located upstream of an exonic sequence, *i.e.*, the 5' splice site and the 3' splice site are transposed with respect to the position of the same splice sites would they have been in a naturally occurring configuration.

[0099] A splice site is a nucleotide sequence located at the border between an intronic sequence and an exonic sequence, and comprises a pair of nucleotides linked by a phosphodiester bond that is cleaved during a splicing reaction. Thus, each splice site comprises a nucleotide that is located in the intronic sequence and a nucleotide that is located in the exonic sequence.

[0100] In some aspects, the splice site is a 5' splice site. A 5' splice site is located at the border between the 3' end of an exonic sequence and the 5' end of an intronic sequence. A 5' splice site comprises a pair of nucleotides between which cleavage of the phosphodiester bond occurs during a splicing reaction. The nucleotide at the 5' of the pair of nucleotides (herein also referred to as "nucleotide at the 5' of the 5' splice site" or "5S(5')") is comprised in the exonic sequence, the nucleotide at the 3' of the pair of nucleotides (herein also referred to as "nucleotide at the 3' of the 5' splice site" or "5S(3')") is comprised in the intronic sequence.

[0101] In some aspects, the 5S(5') is a U, and the 5S(3') is a N, wherein N is any one of A, C, G, or U.

[0102] In some aspects, the 5' splice site comprises a sequence of formula 5'-U-N-3', wherein the site of splice is between U and N, and wherein N is any one of A, C, G, or U.

[0103] In some aspects, the 5S(5') is a A, and the 5S(3') is a N, wherein N is any one of A, C, G, T or U. In some aspects, the 5S(5') is a A, and the 5S(3') is a G.

[0104] In some aspects, the 5' splice site comprises a sequence of formula 5'-A-N-3', wherein the site of splice is between A and N, and wherein N is any one of A, C, G, T or U. In some aspects, N is a G.

[0105] In some aspects, the splice site is a 3' splice site. A 3' splice site is located at the border between the 5' end of an exonic sequence and the 3' end of an intronic sequence. A 3' splice site comprises a pair of nucleotides between which cleavage of the phosphodiester bond occurs during a splicing reaction. The nucleotide at the 5' of the pair of nucleotides (herein also referred to as "nucleotide at the 5' of the 3' splice site" or "3S(5')") is comprised in the intronic sequence, the nucleotide at the 3' of the pair of nucleotides (herein also referred to as "nucleotide at the 3' of the 3' splice site" or "3S(3')") is comprised in the exonic sequence.

[0106] In some aspects, the 3S(5') is a G, and the 3S(3') is a N, wherein N is any one of A, C, G, or U.

[0107] In some aspects, the 3' splice site comprises a sequence of formula 5'-G-N-3', wherein the site of splice is between G and N, and wherein N is any one of A, C, G, or U.

[0108] In some aspects, the 3S(5') is a T or U, and the 3S(3') is a N, wherein N is any one of A, C, G, T or U.

[0109] In some aspects, the 3' splice site comprises a sequence of formula 5'-T(orU)-N-3', wherein the site of splice is between T or U and N, and wherein N is any one of A, C, G, T or U. In some aspects, N is a C. In some aspects, N is an A.

[0110] In some aspects, the 3' splice site comprises a nucleotide sequence of Atccaact[^]CA, wherein N is A, U, C, or G, and wherein "[^]" indicates the position of the phosphodiester bond that is cleaved during a splicing reaction. In some aspects, the 3' splice site consists of a nucleotide sequence of Atccaact[^]CA, wherein N is A, U, C, or G, and wherein "[^]" indicates the position of the phosphodiester bond that is cleaved during a splicing reaction.

[0111] In some aspects, the 5' splice site comprises a nucleotide sequence of Nttaaaaa[^]GTGCG, wherein "[^]" indicates the position of the phosphodiester bond that is cleaved during a splicing reaction. In some aspects, the 5' splice site consists of a nucleotide sequence of Nttaaaaa[^]GTGCG, wherein N is A, U, C, or G, and wherein "[^]" indicates the position of the phosphodiester bond that is cleaved during a splicing reaction.

[0112] Non-limiting examples of 5' splice sites and 3' splice sites that are useful for the present genetic constructs are shown in **Table 1**.

Table 1. Exemplary splice sites ("[^]" indicates the position of the phosphodiester bond that is cleaved during a splicing reaction).

DESCRIPTION	SEQUENCE
<i>Pyraliella littoralis</i> 1-2 3' Splice site (SEQ ID NO: 25)	Atccaact [^] CA
<i>Pyraliella littoralis</i> 1-2 5' Splice site (SEQ ID NO: 26)	Nttaaaaa [^] GTGCG

Pyraliella littoralis 1-2 3' Splice site (SEQ ID NO: 106)	Auccaacu^CA
Pyraliella littoralis 1-2 5' Splice site (SEQ ID NO: 107)	Nuuaaaaa^GUGCG

II.B.2. Introns

[0113] Splicing is a process by which a precursor RNA transcript (*i.e.*, primary transcript) is transformed into a mature RNA molecule (*e.g.*, a mature mRNA, tRNA, or rRNA). During splicing, introns are removed from the precursor RNA molecule (*i.e.*, primary transcript) and exons are joined together. For many eukaryotic RNAs, splicing occurs in a series of reactions catalyzed by the spliceosome, a complex of small nuclear ribonucleoproteins (snRNPs), but self-splicing introns can catalyze their own excision from their parent RNA molecule. For example, Group II introns can catalyze their own excision from their parent RNA molecules.

[0114] Group II introns are found in rRNA, tRNA, and mRNA of organelles (chloroplasts and mitochondria) in fungi, plants, and protists, and also in mRNA in bacteria. These introns are self-splicing introns, which can catalyze their own excision from primary transcripts (*e.g.*, pre-mRNA, pre-tRNA and pre-rRNA) in a wide range of organisms. The ability of group II introns to self-splice renders them capable of acting as ribozymes. In contrast to group I introns, intron excision occurs in the absence of GTP and involves the formation of a lariat, with an A-residue branchpoint.

[0115] As depicted in FIG. 4, the secondary structure of group II introns is characterized by six typical stem-loop structures, also called domains I to VI (DI to DVI, or D1 to D6). The domains radiate from a central core that brings the 5' and 3' splice junctions into close proximity. The proximal helix structures of the six domains are connected by a few nucleotides in the central region (linker or joiner sequences). Due to its size, the domain I is divided further into subdomains a, b, c, and d. Due to sequence differences, group II introns are further divided into subgroups IIA, IIB, and IIC.

[0116] Group II introns possess only a few conserved nucleotides, and the catalytic nucleotides are spread over the complete intron structure. The conserved primary sequences are the consensus at the 5' and 3' splicing site (...↓GUGYG&... and ...AY↓..., with the Y representing a pyrimidine), some of the nucleotides of the central core (joiner sequences), nucleotides of DV, and sequence stretches of DI. The unpaired adenosine in DVI (marked by an asterisk in Fig. 4 and located 7 or 8 nt away from the 3' splicing site) is also conserved and plays a central role in the splicing process. The catalytic triad is located within DV and is comprised by a conserved AGC or CGC sequence, which help coordinate metal binding and catalysis. The 2' hydroxyl of the bulged

adenosine attacks the 5' splice site, followed by nucleophilic attack on the 3' splice site by the 3' OH of the upstream exon. This results in a branched intron lariat connected by a 2',5'-phosphodiester linkage at the DVI adenosine.

[0117] Protein machinery is required for splicing in vivo, and long-range intron-intron and intron-exon interactions are important for splice site positioning, as well as a number of tertiary contacts between motifs, including kissing-loop and tetraloop-receptor interactions. During splicing of Group II introns, all reactants are preorganized before the initiation of splicing. The branch site, both exons, the catalytically essential regions of DV and J2/3, and ϵ - ϵ' are in close proximity before the first step of splicing occurs. In addition to the bulge and AGC triad regions of DV, the J2/3 linker region, the ϵ - ϵ' nucleotides and the coordination loop in DI are crucial for the architecture and function of the active-site.

[0118] In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from a single intron. In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from two or more different introns.

[0119] In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is an artificial sequences comprising a nucleotide sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identity with a nucleotide sequence of a naturally occurring intron.

[0120] In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from an intron derived from a bacterial genome, a phage genome, a viral genome, a eukaryotic organellar genome, or from a eukaryotic nuclear genome (e.g., from an organellar or nuclear genome of animal, fungi, plants, protozoan, or algae), or from an RNA transcribed therefrom.

[0121] In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from a multicellular algae (e.g., a brown algae) genome or from an RNA transcribed therefrom. In some aspects, the intronic sequence is derived from a *Pylaiella littoralis* genome, or from an RNA transcribed therefrom.

[0122] In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from a unicellular algae (e.g., a green algae) genome or from an RNA transcribed therefrom. In some aspects, the intronic sequence is derived from a *Mesostigma viridae* genome, or from an RNA transcribed therefrom.

[0123] In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from a eukaryotic genome or from an RNA transcribed therefrom. In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from a protozoan genome or from an RNA transcribed therefrom. In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from an *Amoebidium parasiticum* genome or from an RNA transcribed therefrom.

[0124] In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from a bacterial genome or from an RNA transcribed therefrom. In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from a *Lactococcus lactis* genome or from an RNA transcribed therefrom. In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from an *Oceanobacillus iheyensis* genome or from an RNA transcribed therefrom.

[0125] In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from a genomic region encoding a protein-coding RNA or a structural RNA (e.g., tRNA, rRNA), or from an RNA transcribed therefrom.

In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from an intron of a large ribosomal subunit (*LSU*) from *Pylaiella littoralis*; from an intron of a cytochrome C oxidase subunit 1 (*COX1*) from *Amoebidium parasiticum*; from an intron of a cytochrome C oxidase subunit 2 (*COX2*) from *Mesostigma viridae*; from an intron of a putative relaxase (*LtrB*) from *Lactococcus lactis*; or from any combination thereof. In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from an intron comprised in the database for Bacterial Group II introns (<http://webapps2.ucalgary.ca/~groupii/>).

[0126] In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from an intron of a large ribosomal subunit (*LSU*) from *Pylaiella littoralis*.

[0127] In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from an intron of a cytochrome C oxidase subunit 1 (*COX1*) from *Amoebidium parasiticum*.

[0128] In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from an intron of a cytochrome C oxidase subunit 2 (*COX2*) from *Mesostigma viridae*.

[0129] In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) is derived from an intron of a putative relaxase (*LtrB*) from *Lactococcus lactis*.

[0130] In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) comprises a nucleotide sequence derived from an intron having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to any one of SEQ ID NOs: 1-5 or 6-10. In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) comprises a nucleotide sequence derived from an intron having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity with SEQ ID NOs: 1 or 6. In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) comprises a nucleotide sequence derived from an intron consisting of any one of SEQ ID NOs: 1-5 or 6-10. In some aspects, the one or more intronic sequence (*i.e.*, one or more intron or fragment thereof) comprises a nucleotide sequence derived from an intron consisting of SEQ ID NOs: 1 or 6.

[0131] Non-limiting examples of intron that are useful for the present genetic constructs are shown in **Table 2**.

Table 2. Exemplary Introns

SEQ ID NO	DESCRIPTION	SEQUENCE
1	<i>Pylaiella littoralis</i> 1-2 Full intron (DNA) <i>Pylaiella littoralis</i> 1-2 Full intron (RNA) is depicted in SEQ ID NO: 6	GTGCGACAAGAAGTTCAGGAAGGGATTGAGGTGAAAGTCCTCCTCCCGAATCG TTCATGGGAGAGTCTATCCAGACTTGCCTAGCGAGTAATCGCTAGGTGAGAAG CTCTGGAGACAATGTACCTGCCCTTCAATTGGAGGTGCCAGGGCTAGGCTTTG TTCCTATGGCTAGCGAAAGCGAATACAGGTTATTAGGCGTCGTGAAAACAAAA CTCTTTCGACATAAGGGAGAGGCTCGTAGATGAGTTACCTCCTTTTTATGGG GTAACCTGAACGATCAATATCTACCGCGTAGACTGTGAGCCTAAAGGAAACGA ATGAAAGTGTCCCTTCCTGGGAAGTTGGTAAGCCCAATAGACTCCCTCTGGGA AACCAGAGGAGGAGTAAGAGCAATCTTACTTATCGTCTAGTGGGTAAAAGACA TAATAGGAAGCAAAATGCTTGGTTGTAACGATCGAGATAGAATCTGTTGATTTA AGCTGAAAGGCTGCAGACTTATTAATGGGTGTTCTGCTGTATGGCGTTCGCG CCTAAGCAGCAGAATGCGTGAGCCGTGTGCGATGAAAGTCGCAAGCACGGTTC TGATGGGGGGAAAAACGAGAGATCGTCTACCTATCCAAC
2	<i>Amoebidium parasiticum</i> Full intron (DNA) <i>Amoebidium parasiticum</i> Full intron (RNA) is depicted in SEQ ID NO: 7	GTGCGCCATCTCACTATTAGCATTCGCGGGTACAAATACCCACCACAGTCTT CTCGTTCTTCTATTATATAAGCAAGTCATATGCATGGACCATTTACTCCGACA ACATGTACAGCGTGTGATTATGAGAAGAACCAGGGAATTACGTTTTAGATT GAAATCTTTTACGACATCGAAACACCGAAATAGTATAAGATTAACACCTAGAC TTAGAGATATCTTAGTAGGATTATTTAATACAGACGCATGAAGTCTTTGGAAT GAAAAATGGAAACCCACGTATTGGATTCAAACAGGGTGAAGTAAACTTTCCTTT TTATGGCATGTGTATTTAGAAGTTATATCTTTAACATCTAGTTTCCCTGGCAT TCGACAATCAATAGTAAGAGGAAAGTCTCATAATTCACACTCTTTTGATACAA AAAATCTCCAATGCTTAAATGAAGTAAGCAATATTCTGTACACTAATATAAAT GGTAAATGGTAAATCTATTAAGGAGAACTATTTGATTTATTTAAACTACAG AACATTAGCGTACATGATAATGGGAGATGGAGCGCGCAAAATAAAGGGTTTG TAATATGTCTCGAGAATTTACACTTCAGGAAGTCGTTTTTACTATTAAATATG TTAGCAATCAAATTTAATTTGGAGGACCTAATTTCAAAGTGAAGACTCTAA ACCGTCTCCTTTTAAAAAAGAAAAAGAGGAAATTAAGAAGATTTATAGAATCT ACTTTGGAATAGAGGGAACCAAAACATTATTACCTTTCATTAAACCTTATTTA CTAGACCATTTTGATTATCGCATATCGGGAATGACTAAAGCACAGTATGAACA

		ACGTGGTGTAAGCATAAATCTTAGGTAGTAGGAGCCAATTCTACACTGACAA CAGCATGATTTAAAGGGGAATGAAAGCGTCCCCAAGCTAGAATCCTCATGGT AAGGCAAACGAACCTAAAATTACTTTATACTAATGTGAATTAACAAGTAGC GGGGTTTAATTATGAGAAGATCACATATAAAGTATGGAGTACCTAAAAGGATT TAGAGAACAAGTATGTTACAATGTTCTCACCGAGTGCTAATAGGAATTTGGGA TCACCTGAGATTGGAAGAGTCGGAGGTGACGGAGTTCTCATAGTAAATGGGT AATCAAATACTCTAGATTAGGGGAAACCTTAATTGACTGCTTGGGCAGATTGA AGGGGAACAGGAGAGTGGGGATTGCAAGACCATTCTCTGGAGAGCCGTATGAT GGGAACTATCACGTACGGTTCGGGAAAGGGCTTTTAACATAATGGTCCGGTAA AAGGACTGGGGTTAATCTTCTATTTTAC
3	Mesostigma viridae Full intron (DNA) Mesostigma viridae Full intron (RNA) is depicted in SEQ ID NO: 8	GTGTGCCATAAAAAATTTTTCTAATGTTTTCTTTTTGATTCTCTGCCAGAAAA GGAAAAGCTCAAAAAAACTAAACTTTTTAGTAAAGAAATTTATAAAAGAG AATTCGTCAAGAAAAATATTTTTATGGAATTTATTTGGATCCTTTAGTAAA GCTTTTTATTTAGTTTTTTATAAAAAATTTTAAACCGGTCTTGAATAGTGGAAA GGAAAAGCATTATTGATACGGCAAAAACAAACATAAAAAGATCATTAAATAT TGTTTTATAAACCGCTCGACTTTCTTACTGTCTGGAATGCGAAAACGAAAAAA AAAGGTATCATATAGAAAAAATTTGATGAAAATTTTAATACCTCTTTTTGGATA AATAAGAGGAAAAATTTAATTTCAATTTTTATCTTGGAAATTAAGAAATGCATC ATATATATTTTCATGGTCGTTTTATTTAAGAGGAATCAAGGTCCATACAAAGAAA TATTAGAAAAAGAATATTTCTTTTTTTCGAGAAAATTGGATGCCGCGATTTTTT TGCATAAGTGATCCACATTTTTCCCTAAATCTTCTTTATTTCTTTCCGTGGT CACTGCAATTTCTGCATTATCAGATCCTTTTAGATATTTGGATAGAAAAATCGT TCGGAAATGAAAAATTTTTTATTCAGTTTTTATGGATCAATGTATTGTAGCA TGTTTTTCAGACGCTGATTTTTTAAATTCAAAATTTGAAAAATATCACAAAA ATCTTGTGGAAATTTCTAAATGTTTTTCCACTGTTCTTTTTTGGGTGGCA CGCTAATTCACCTCTTCAACAAGGAAACCCATTGAATCTAATGAGCTAAAAAGT ATTAATAATATTAACAGTTCTTGGAAATGGAAAATTTTCATGGAATGTTCTTAT AAGGTTTTGTCAAAATGAAAGATATTTTCATCAAAATTTCTGCAAAACCTACGG CTGTGCCCTACCTTGTTCACGAACCTCATGAAGAGATTTTGGCTCATTATAAA AAATTAATTTTTCTTTTTTATCTTTATTACAATCCTATGCAAGATCACCAAGC ATTCAATTTGTTTAGTAGATGGTTTAAAGGAATCATGTATATTGACATTGGCCC TTAAATATAAAATGAAAAGTAGAACTAAAATAATTCAAAAATTTTTCTCTCAA AAGCTGTAGGAGTTTTTCTCACATACAGTTTTTTAGGGAAGTAAAAAAAAGAA TTTTTTACTTACCCTCC
4	Lactococcus lactis Full intron (DNA) Lactococcus lactis Full intron (RNA) is depicted in SEQ ID NO: 9	GTGCGCCAGATAGGGTGTTAAGTCAAGTAGTTTAAAGTACTACTCTGTAAAGA TAACACAGAAAACAGCCAACCTAACCGAAAAGCGAAAGCTGATACGGGAACAG AGCACGGTTGGAAGCGATGAGTTACCTAAAGACAATCGGGTACGACTGAGTC GCAATGTTAATCAGATATAAGGTATAAGTTGTGTTTACTGAACGCAAGTTTCT AATTTTCGGTTATGTGTCGATAGAGGAAAGTGTCTGAAACCTCTAGTACAAAGA AAGGTAAGTTATGGTTGTGGACTTATCTGTTATCACCACATTTGTACAATCTG TAGGAGAACCTATGGGAACGAAACGAAAGCGATGCCGAGAATCTGAATTTACC AAGACTTAACACTAACTGGGGATACCCCTAAACAAGAATGCCCTAATAGAAAAGGA GGAAAAAGGCTATAGCACTAGAGCTTGAAAATCTTGCAAGGGTACGGAGTACT CGTAGTAGTCTGAGAAGGGTAACGCCCTTTACATGGCAAAGGGGTACAGTTAT TGTGTACTAAAATTAATAATTTGATTAGGGAGGAAAACCTCAAAATGAAACCAA CAATGGCAATTTTAGAAAAGAAATCAGTAAAAATTCACAAGAAAAATAGACGAA GTTTTTACAAGACTTTATCGTTATCTTTTACGTCCAGATATTTATTACGTGGC GTATCAAAATTTATATTTCCAATAAAGGAGCTTCCACAAAAGGAATATTAGATG ATACAGCGGATGGCTTTAGTGAAGAAAAAATAAAAAAGATTATTCAATCTTTA AAAGACGGAACCTTACTATCCTCAACCTGTACGAAGAATGTATATTGCAAAAAA GAATTTCAAAAAGATGAGACCTTTAGGAATTCCAACTTTTCAGATAAATTTGA TCCAAGAAGCTGTGAGAATAATCTTGAATCTATCTATGAACCGGTATTCGAA GATGTGTCTCACGGTTTTAGACCTCAACGAAGCTGTACACAGCTTTGAAAAC AATCAAAAGAGAGTTTGGCGGCGCAAGATGGTTTGTGGAGGGAGATATAAAAAG GCTGCTTCGATAATATAGACCAGTTACACTCATTGGACTCATCAATCTTAAA ATCAAGATATGAAAATGAGCCAATTGATTTATAAATTTCTAAAAGCAGGTTA TCTGGAAAACCTGGCAGTATCACAAAACCTTACAGCGGAACACCTCAAGGTGGAA TTCTATCTCCTTTTTGGCCAACATCTATCTTCATGAATTTGGATAAGTTTGT TTACAACTCAAAATGAAGTTTGACCGAGAAAGTCCAGAAAGAATAACACCTGA

		ATATCGGGAACCTTCACAATGAGATAAAAAAGAATTTCTCACCGTCTCAAGAAGT TGGAGGGTGAAGAAAAAGCTAAAGTTCTTTTAGAATATCAAGAAAAACGTAAA AGATTACCCACACTCCCCTGTACCTCACAGACAAATAAAGTATTGAAATACGT CCGGTATGCGGACGACTTCATTATCTCTGTTAAAGGAAGCAAAGAGGACTGTC AATGGATAAAAGAACAATTAACCTTTTATTCATAACAAGCTAAAAATGGAA TTGAGTGAAGAAAAACACTCATCACATAGCAGTCAACCCGCTCGTTTTCT GGGATATGATATACGAGTAAGGAGAAGTGGAAACGATAAAACGATCTGGTAAAG TCAAAAAGAGAACACTCAATGGGAGTGTAGAACTCCTTATTCCTCTTCAAGAC AAAATTCGTCAATTTATTTTGGACAAGAAAATAGCTATCCAAAAGAAAGATAG CTCATGGTTTCCAGTTTACAGGAAATATCTTATTCGTTCAACAGACTTAGAAA TCATCACAATTTATAATTCTGAATTAAGAGGGATTTGTAATTACTACGGTCTA GCAAGTAATTTTAACAGCTCAATTAATTTTGCTTATCTTATGGAATACAGCTG TCTAAAAACGATAGCCTCCAAACATAAGGGAACACTTTTCAAAACCATTTCCA TGTTTAAAGATGGAAGTGGTTTCGTGGGGCATCCCGTATGAGATAAAGCAAGGT AAGCAGCGCCGTTATTTTGCAAATTTTAGTGAATGTAAATCCCTTATCAATT TACGGATGAGATAAGTCAAGCTCCTGTATGTATGGCTATGCCCGGAATACTC TTGAAAACAGGTTAAAAGCTAAATGTTGTGAATTATGTGGAACATCTGATGAA AATACTTCTATGAAATTCACCATGTCAATAAGGTCAAAAATCTTAAAGGCAA AGAAAAATGGGAAATGGCAATGATAGCGAAACAACGTAAAACCTCTGTGTAT GCTTTCATTGTCTCGTCACGTGATTCTATAACACAAGTGAATTTTACGAAC GAACAATAACAGAGCCGTATACTCCGAGAGGGGTACGTACGGTTCCCGAAGAG GGTGGTGCAACCAGTCAAGTAATGTGAACAAGGCGGTACCTCCCTACTTCA C
5	<i>Oceanobacillus iheyensis</i> Full intron (DNA) <i>Oceanobacillus iheyensis</i> Full intron (RNA) is depicted in SEQ ID NO: 10	GTGTGCCCGCATGGGTGCAGTCTATAGGGTGAGAGTCCCGAAGTGTGAAGGC AGAAGTAACAGTTAGCCTAACGCAAGGGTGTCCGTGGCGACATGGAATCTGAA GGAAGCGGACGGCAAACCTTCGGTCTGAGGAACACGAACCTCATATGAGGCTA GGTATCAATGGATGAGTTTGCATAACAAAACAAAGTCCCTTCTGCCAAAGTTG GTACAGAGTAAATGAAGCAGATTGATGAAGGGAAAGACTGCATTCTTACCCGG GGAGGTCTGGAAACAGAAGTCAGCAGAAGTCATAGTACCCTGTTTCGACGGGGA AGGACGGAACAAGTATGGCGTTTCGCGCCTAAGCTTGAACCGCGTATACCGAA CGGTACGTACGGTGGTGTGAGAGGAGTTTCGCTCTACTCTAT
6	<i>Pylaiella littoralis</i> 1-2 Full intron (RNA) Wherein "T" is "U"	GTGCGACAAGAAGTTCAGGAAGGGATTGAGGTGAAAGTCCCTCTCCGAATCG TTCATGGGAGAGTCTATCCAGACTTGCCTAGCGAGTAATCGCTAGGTGAGAAG CTCTGGAGACAATGTACCTGCCCTTCAATTGGAGGTGCCAGGGCTAGGCTTTG TTCTTATGGCTAGCGAAAGCGAATACAGGTTATTAGGCGTCTGAAAACAAAA CTCTTTCGACATAAGGGAGAGGCTCGTAGATGAGTTACCCTCCTTTTTATGGG GTAACGTAACGATCAATATCTACCGGCGTAGACTGTGAGCCTAAAGGAAACGA ATGAAAGTGTCCCTTCCTGGGAACCTTGGTAAGCCCAATAGACTCCCTCTGGGA AACCAGAGGAGGAGTAAGAGCAATCTTACTTATCGTCTAGTGGGTAAAAGACA TAATAGGAAGCAAATGCTTGGTTGTAACGATCGAGATAGAATCTGTTGATTTA AGCTGAAAGGCTGCAGACTTATTAAATGGGTGTTCTGCTGTATGGCGTTTCGCG CCTAAGCAGCAGAAATGCGTGAGCCGTGTGCGATGAAAGTCGCAAGCAGGTTTC TGATGGGGGGAAAACGAGAGATCGTCTACCTATCCAAC
7	<i>Amoebidium parasiticum</i> Full intron (RNA) Wherein "T" is "U"	GTGCGCCATCTCACTATTAGCATTCGCGGGTACAAAATACCCACCACAGTCTT CTCGTTCTTCTATTTATAAAGCAAGTCATATGCATGGACCATTACTCCGACA ACATGTACAGCGGTTGTATTATGAGAAGAACCAGGGAATTACGTTTTAGATT GAAATCTTTTACGACATCGAAACACCGAAATAGTATAAGATTAAACACCTAGAC TTAGAGATATCTTAGTAGGATTATTATTAACAGACGCATGAATGTCTTGGAA GAAAATGGAAACCCACGTATTGGATTCAAACAGGGTGAAGTAAACTTTCCCTT TTATGGCATGTGTATTTAGAACTTATATCTTTAACATCTAGTTTCCCTGGCAT TCGACAATCAATAGTAAGAGGAAAGTCTCATAATTCACTCTTTTGATACAA AAAATCTCCAATGCTTAAATGAACTAAGCAATATTCTGTACACTAATATAAAT GGTAAATGGTAAATCTATTAAAGGAGAAGTATTTGATTATTTAAACTACAG AACATTAGCGTACATGATAATGGGAGATGGAGCGCGCAAAAATAAAGGGTTG TAATATGTCTCGAGAAATTCACACTTCAGGAAGTCGTTTTACTATTAAATATG TTAGCAATCAAATTTAATTTGGAGGACCTAATCTTCAACTGAAGACTCTAA

		ACCGTCTCCTTTTAAAAAAGAAAAAGAGGAAATTAAGAAGATTTATAGAATCT ACTTTGGAATAGAGGGAACCAAAACATTATTACCTTTCATTAAACCTTATTTA CTAGACCATTTTGATTATCGCATATCGGGAATGACTAAAGCACAGTATGAACA ACGTGGTGTAAGCATAAATCTTAGGTAGTAGGAGCCAATTCTACACTGACAA CAGCATGATTTAAAGGGGAATGAAAGCGTCCCCAAGCTAGAATCCTCATGGT AAGGCAAACGAACTCAAAATTACTTTATAACTAATGTGAATTAAACAAGTAGC GGGGTTTAATTATGAGAAGATCACATATAAAGTATGGAGTACCTAAAAGGATT TAGAGAACAAGTATGTTACAATGTTCTCACCGAGTGCTAATAGGAATTTGGGA TCACCTGAGATTCGGAAGAGTTCGGAGGTGACGGAGTTCTCATAGTAAATGGGT AATCAAATACTCTAGATTAGGGGAAACCTTAATTGACTGCTTGGGCAGATTGA AGGGGAACAGGAGAGTGGGGATTGCAAGACCATTCTCTGGAGAGCCGTATGAT GGGAACTATCACGTACGGTTTCGGGAAAGGGCTTTTAACTAATGGTCCGGTAA AAGGACTGGGGTTAATCTTCTATTTCAC
8	Mesostigma viridae Full intron (RNA) Wherein "T" is "U"	GTGTGCCATAAAAAATTTTTCTAATGTTTTCTTTTTGATTCTCGCCAGAAAA GGAAAAGCTCAAAAAAACTAAACCTTTTTAGTAAAGAAITTTATAAAAAAGAG AATTCGGTCAAGAAAAATATTTTTATGGAATTATTTGGATCCACTTTAGTAAA GCTTTTTATTAGTTTTTATAAAAAATTTAAACCGGTCTTGAATAGTGGAAA GGAAAAGCATTATTGATACGGCAAAAACAAACATATAAAGAGCATTAATAAT TGTTTATAAACCGCTCGACTTCTTACTGTCTGGAATGCGAAAACGAAAAAAA AAAGGTATCATATAGAAAAAATGATGAAAATTTAATACCTCTTTTTGGATA AATAAGAGGAAAAATTTAATTCAATTTTTATCTTGGAAATTAATAATGCATC ATATATATTTTCATGGTCGTTTTATTTAAGAGGAATCAAGGTCCATACAAAGAAA TATTAGAAAAAGAATTTCTTTTTTCGAGAAAATTTGGATGCCCGGATTTTTT TGCATAAGTGATCCACATTTTTCCCTAAATCTTCTTTATTTCTTTCCGTGGT CACTGCAATTTCTGCATTATCAGATCCTTTAGATATTTGGATAGAAAAATCGT TCGGAAATGAAAAATTTTTTTATTACGTTTTTATGGATCAATGTATTGTAGCA TGTTTTTTCAGACGCTGATTTTTTAAATTCAAAATTTGAAAAAATATCACAAAA ATCTTGTGGAAATTTCTAAATGTTTTTCCACTGTTCTCTTTTTGGGTGGCA CGCTAATTCACCTCTCAACAAGGAAACCCATTGAATCTAATGAGCTAAAAAGT ATTAATAATATTAACAGTTCTTGGAAATGGAATAATTTATGGAATGTTCTTAT AAGGTTTTGTCAAAATGAAAGATATTTTCATCAAAATCTGCAAAAACCTACGG CTGTGCCCTACCTTGTTCACGAACCTCATGAAGAGATTTTGGCTCATTATAAA AAATTAATTTTTCTTTTTTATCTTTATTACAACTCCTATGCAAGATCACCAAGC ATTCATTTGTTTAGTAGATGGTTTAAAGGAATCATGTATATTGACATTGGCCC TTAAATATAAAATGAAAAGTAGAACTAAAAATAATCAAAAACCTTTTTCTCAA AAGCTGTAGGAGTTTTTCTCACATACAGTTTTTTAGGGAAGTAAAAAAAAGAA TTTTTTACTTACCCTCC
9	Lactococcus lactis Full intron (RNA) Wherein "T" is "U"	GTGCGCCAGATAGGGTGTTAAGTCAAGTAGTTTAAGGTACTACTCTGTAAGA TAACACAGAAAACAGCCAACCTAACCGAAAAGCGAAAGCTGATACGGGAACAG AGCACGGTTGGAAGCGATGAGTTACCTAAAGACAATCGGGTACGACTGAGTC GCAATGTTAATCAGATATAAGGTATAAGTTGTGTTTACTGAACGCAAGTTTCT AATTTCGGTTATGTGTGATAGAGGAAAGTGTCTGAAACCTCTAGTACAAAGA AAGGTAAGTTATGGTTGTGGACTTATCTGTTATCACCACATTTGTACAATCTG TAGGAGAACCTATGGGAACGAAACGAAAGCGATGCCGAGAACTGAAATTTACC AAGACTTAACACTAACTGGGGATACCCTAAACAAGAATGCCAATAGAAAAGGA GGAAAAAGGCTATAGCACTAGAGCTTGAAAATCTTGCAAGGGTACGGAGTACT CGTAGTAGTCTGAGAAGGGTAACGCCCTTTACATGGCAAAGGGGTACAGTTAT TGTGTACTAAAAATTAATAATTGATTAGGGAGGAAAACCTCAAAATGAAACCAA CAATGGCAATTTTAGAAAGAATCAGTAAAAATTCACAAGAAAAATAGACGAA GTTTTTACAAGACTTTATCGTTATCTTTTACGTCCAGATATTTATTACGTGGC GTATCAAAATTTATATTCCAATAAAGGAGCTTCCACAAAAGGAATATTAGATG ATACAGCGGATGGCTTTAGTGAAGAAAAATAAAAAAGATTATTCAATCTTTA AAAGACGGAACCTTACTATCCTCAACCTGTACGAAGAATGTATATTGCAAAAAA GAATTCATAAAAGATGAGACCTTTAGGAATTCACACTTTCACAGATAAATGGA TCCAAGAAGCTGTGAGAATAATCTTGAATCTATCTATGAACCGGTATTCGAA GATGTGTCTCACGGTTTTAGACCTCAACGAAGCTGTACACAGCTTTGAAAAC AATCAAAAGAGAGTTTGGCGGCGCAAGATGGTTTGTGGAGGGAGATATAAAAG GCTGCTTCGATAATATAGACCAGTTACACTCATTGGACTCATCAATCTTAAA ATCAAAGATATGAAAATGAGCCAATTGATTTATAAATTTCTAAAAGCAGGTTA

		TCTGGAAAACTGGCAGTATCACAAAACCTTACAGCGGAACACCTCAAGGTGGAA TTCTATCTCCTCTTTTGGCCAACATCTATCTTCATGAATTGGATAAGTTTGT TTACAACCTCAAAATGAAGTTTGACCGAGAAAGTCCAGAAAGAATAACACCTGA ATATCGGGAACCTTCACAATGAGATAAAAAAGAATTTCTCACCCTCTCAAGAAGT TGGAGGGTGAAGAAAAAGCTAAAGTTCTTTTAGAATATCAAGAAAAACGTAAA AGATTACCCACACTCCCCTGTACCTCACAGACAAATAAAGTATTGAAATACGT CCGGTATGCGGACGACTTCATTATCTCTGTTAAAGGAAGCAAAGAGGACTGTC AATGGATAAAAGAACAATTAAAACTTTTATTCATAACAAGCTAAAAATGGAA TTGAGTGAAGAAAAACACTCATCACATAGCAGTCAACCCGCTCGTTTTCT GGGATATGATATACGAGTAAGGAGAAGTGGAAAGATAAAACGATCTGGTAAAG TCAAAAAGAGAACACTCAATGGGAGTGTAGAACTCCTTATTCCTCTTCAAGAC AAAATTCTGTCATTTATTTTGGACAAGAAAATAGCTATCCAAAAGAAAGTAG CTCATGGTTTCCAGTTTCACAGGAAATATCTTATTCGTTCAACAGACTTAGAAA TCATCACAATTTATAATTCTGAATTAAGAGGGATTTGTAATTACTACGGTCTA GCAAGTAATTTTAAACAGCTCAATTATTTTGCTTATCTTATGGAATACAGCTG TCTAAAAACGATAGCCTCCAAACATAAGGGAACACTTTCAAAAACCATTTCCA TGTTTAAAGATGGAAGTGGTTCGTGGGGCATCCCGTATGAGATAAAGCAAGGT AAGCAGCGCCGTTATTTTGCAAAATTTAGTGAATGTAAATCCCTTATCAATT TACGGATGAGATAAGTCAAGCTCCTGTATGTATGGCTATGCCCGGAATACTC TTGAAAACAGGTTAAAAGCTAAATGTTGTGAATTATGTGGAACATCTGATGAA AATACTTCCTATGAAATTCACCATGTCAATAAGGTCAAAAACTTTAAAGGCAA AGAAAAATGGGAAATGGCAATGATAGCGAAACAACGTAAAACTCTTGTGTAT GCTTTCATTGTCTATCGTCACGTGATTTCATAAACACAAGTGAATTTTACGAAC GAACAATAACAGAGCCGTATACCTCCGAGAGGGGTACGTACGGTTCCCGAAGAG GGTGGTGCAAAACAGTCAAGTAATGTGAACAAGGCGGTACCTCCCTACTTCA C
10	<i>Oceanobacillus iheyensis</i> Full intron (RNA) Wherein "T" is "U"	GTGTGCCCGCATGGGTGCAGTCTATAGGGTGAGAGTCCCGAACTGTGAAGGC AGAAGTAACAGTTAGCCTAACGCAAGGGTGTCCGTGGCGACATGGAATCTGAA GGAAGCGGACGGCAAAACCTTCGGTCTGAGGAACACGAACTTCATATGAGGCTA GGTATCAATGGATGAGTTTGCATAACAAAACAAAGTCCTTTCTGCCAAAGTTG GTACAGAGTAAATGAAGCAGATTGATGAAGGGAAAGACTGCATTCTTACCCGG GGAGGTCTGGAAACAGAAGTCAGCAGAAGTCATAGTACCCTGTTTCGACGGGGA AGGACGGAACAAGTATGGCGTTTCGCGCCTAAGCTTGAACCGCGGTATACCGAA CGGTACGTACGGTGGTGTGAGAGGAGTTTCGCTCTACTCTAT

[0132] When referring to pre-circRNAs "derived from an intron of" means comprising an intronic sequence derived from the RNA encoded by a certain gene. When referring to a DNA genetic construct (from which pre-circRNAs can be transcribed) "derived from an intron of" means comprising an intronic sequence derived from the DNA sequence of a certain gene.

[0133] In some aspects, the intronic sequence (*i.e.*, one or more intron or fragment thereof) comprised in the genetic constructs capable of forming circular RNAs disclosed herein (*i.e.*, pre-circRNAs or DNA genetic constructs from which pre-circRNAs can be transcribed) comprises a nucleotide sequence of a full length intron. In some aspects, the intronic sequence (*i.e.*, one or more intron or fragment thereof) comprised in the genetic constructs capable of forming circular RNAs disclosed herein (*i.e.*, pre-circRNAs or DNA genetic constructs from which pre-circRNAs can be transcribed) does not comprise a nucleotide sequence of a full length intron.

[0134] In some aspects, the intronic sequence comprises less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than

55%, less than 50%, less than 45%, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1% of a sequence of a full length intron.

[0135] In some aspects, the intronic sequence comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to any one of SEQ ID NOs: 1-5 or 6-10. In some aspects, the intronic sequence comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity with any one of SEQ ID NOs: 1 or 6. In some aspects, the intronic sequence consists of a nucleotide sequence of any one of SEQ ID NOs: 1-5 or 6-10. In some aspects, the intronic sequence consists of a nucleotide sequence of SEQ ID NOs: 1 or 6.

[0136] In some aspects, the intronic sequence (*i.e.*, one or more intron or fragment thereof) comprised in the genetic constructs capable of forming circular RNAs disclosed herein (*i.e.*, pre-circRNAs or DNA genetic constructs from which pre-circRNAs can be transcribed) is split in two segments.

[0137] In some aspects, the intronic sequence is split in a 5' segment and a 3' segment. The 5' segment of the intronic sequence is herein also referred to as "5' intronic sequence," and the 3' segment of the intronic sequence is herein also referred to as "3' intronic sequence."

[0138] It is to be understood, that the 5' and 3' descriptors of the intron segments refer solely to the position of the 2 intron segments with respect to the full length sequence of the intron, and that both the 5' intronic sequence and the 3' intronic sequence can comprise any portion of the intronic sequence, so long as the intronic sequences required for circularization (e.g., the IGS, or the catalytic core (triad)) are present in the intronic sequence. For example, an intronic sequence comprised in a genetic constructs disclosed herein (*i.e.*, pre-circRNAs or DNA genetic constructs from which pre-circRNAs can be transcribed) can comprise a sequence comprising the 80% of the sequence of a full length intron (*i.e.*, an intron fragment comprising 80% of the sequence of the full length intron), wherein the full length intron has a length of 1000 nucleotides (from nucleotide 1 at the 5' to nucleotide 1000 at the 3'). Such exemplary intronic sequence comprises 800 nucleotide. The 800 nucleotides can correspond, for example, to nucleotides 1-300 and 501-1000 of the 1000 nucleotides of the full length intron, *i.e.*, from nucleotide 1 to nucleotide 300 and from nucleotide 501 to nucleotide 1000 of the full length intron. Such exemplary intronic sequence does not comprise nucleotides 301-500 of the full length intron. In this exemplary a case, the 5' intronic sequence may comprise, for example, 300 nucleotides, those nucleotides correspond to nucleotides

1-300 of the 1000 nucleotides of the full length intron, and the 3' intronic sequence may comprise, for example, 500 nucleotides corresponding to nucleotides 501-1000 of the 1000 nucleotides of the full length intron.

[0139] Additionally, both the 5' intronic sequence and the 3' intronic sequence can comprise a nucleotide sequence comprising nucleotides that are not contiguous in the sequence of the full length intron. For example, an exemplary intronic sequence comprising the 80% of the sequence of a full length intron (*i.e.*, an intron fragment comprising 80% of the sequence of the full length intron), wherein the full length intron has a length of 1000 nucleotides (from nucleotide 1 at the 5' to nucleotide 1000 at the 3'), comprises 800 nucleotide. In this exemplary case, the 5' intronic sequence may comprise, for example, 200 nucleotides, those nucleotides may correspond to nucleotides 1-50 and 100-250 of the 1000 nucleotides of the full length intron, *i.e.*, from nucleotide 1 to nucleotide 50 and from nucleotide 100 to nucleotide 250 of the full length intron, and the 3' intronic sequence may comprise, for example, 600 nucleotides corresponding to nucleotides 401-1000 of the 1000 nucleotides of the full length intron.

In some aspects, the 5' intronic sequence and the 3' intronic sequence are derived from a same intron. In some aspects, the 5' intronic sequence and the 3' intronic sequence are derived from at least two different introns. In some aspects a 5' intronic sequence can be a chimeric 5' intronic sequence, *i.e.*, a 5' intronic sequence comprising portions derived from one, two, or more introns. In some aspects a 3' intronic sequence can be a chimeric 3' intronic sequence, *i.e.*, a 3' intronic sequence comprising portions derived from one, two, or more introns. For example, an intronic sequence can comprise domain I-IV from a first intron, a catalytic core (triad) domain V from a second intron, and domain VI from a third intron.

[0140] In some aspects, the 5' intronic sequence and the 3' intronic sequence together correspond to a full length intron. In some aspects, the 5' intronic sequence and the 3' intronic sequence together are less than a full length intron. In some aspects, the 5' intronic sequence comprises less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1% of a sequence of a full length intron.

[0141] In some aspects, the 3' intronic sequence comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity

with any one of SEQ ID NOs: 17-18 or 19-20. In some aspects, the 3' intronic sequence consists of a nucleotide sequence of any one of SEQ ID NOs: 17-18 or 19-20.

[0142] In some aspects, the 5' intronic sequence comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with any one of SEQ ID NOs: 21-22 or 23-24. In some aspects, the 5' intronic sequence consists of a nucleotide sequence of any one of SEQ ID NOs: 21-22 or 23-24.

[0143] In some aspects, the 3' intronic sequence comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 17 or 19. In some aspects, the 3' intronic sequence consists of a nucleotide sequence of SEQ ID NO: 17 or 19. In some aspects, the 5' intronic sequence comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 21 or 23. In some aspects, the 5' intronic sequence consists of a nucleotide sequence of SEQ ID NO: 21 or 23. In some aspects, the 3' intronic sequence comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 17 or 19, and the 5' intronic sequence comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 21 or 23. In some aspects, the 3' intronic sequence consists of a nucleotide sequence of SEQ ID NO: 17 or 19, and the 5' intronic sequence consists of a nucleotide sequence of SEQ ID NO: 21 or 23.

[0144] In some aspects, the 3' intronic sequence comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 18 or 20. In some aspects, the 3' intronic consists of a nucleotide sequence of SEQ ID NO: 18 or 20. In some aspects, the 5' intronic sequence comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 22 or 24. In some aspects, the 5' intronic sequence consists of a nucleotide sequence of SEQ ID NO: 22 or 24. In some aspects, the 3' intronic sequence comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 18 or 20, and the 5' intronic sequence comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 22 or 24. In some aspects, the 3' intronic sequence consists of a nucleotide sequence of SEQ ID NO: 18 or 20, and the 5' intronic sequence consists of a nucleotide sequence of SEQ ID NO: 22 or 24.

[0145] Non-limiting examples of 5' intronic sequences and 3' intronic sequences that are useful for the present genetic constructs are shown in **Table 3**.

Table 3. Exemplary 5' intronic sequences and 3' intronic sequences.

SEQ ID NO	DESCRIPTION	SEQUENCE
3' Intronic sequence		
17	<i>Pyraliella littoralis</i> 1 3' intronic sequence (DNA) The corresponding RNA sequence is depicted as SEQ ID NO: 19.	gtcccttcctgggaacttggttaagcccaatagactccctct gggaaaccagaggaggagtaagagcaatcttacttatcgctc tagtgggtaaaagacataataggaagcaaatgcttggttgt aacgatcgagatagaatctgttgatttaagctgaaaggctg cagacttattaaatgggtgttctgctgtatggcggttcgcgc ctaagcagcagaatgcgtgagccgtgtgcatgaaagtcgc aagcacggttctgatggggggaaaacgagagatcgtctacc tAtccaact
18	<i>Pyraliella littoralis</i> 2 3' intronic sequence (DNA) The corresponding RNA sequence is depicted as SEQ ID NO: 20.	cgcgctaagcagcagaatgcgtgagccgtgtgcatgaaa gtcgcaagcacggttctgatggggggaaaacgagagatcgt ctacctAtccaact
19	<i>Pyraliella littoralis</i> 1 3' intronic sequence (RNA) Wherein "T" is "U"	gtcccttcctgggaacttggttaagcccaatagactccctct gggaaaccagaggaggagtaagagcaatcttacttatcgctc tagtgggtaaaagacataataggaagcaaatgcttggttgt aacgatcgagatagaatctgttgatttaagctgaaaggctg cagacttattaaatgggtgttctgctgtatggcggttcgcgc ctaagcagcagaatgcgtgagccgtgtgcatgaaagtcgc aagcacggttctgatggggggaaaacgagagatcgtctacc tAtccaact
20	<i>Pyraliella littoralis</i> 2 3' intronic sequence (RNA) Wherein "T" is "U"	cgcgctaagcagcagaatgcgtgagccgtgtgcatgaaa gtcgcaagcacggttctgatggggggaaaacgagagatcgt ctacctAtccaact
5' intronic sequence		
21	<i>Pyraliella littoralis</i> 1 5' intronic sequence (DNA) The corresponding RNA sequence is depicted as SEQ ID NO: 23.	GTGCGACAAGAAGTTCAGGAAGGGATTGAGGTGAAAGTCCT CCTCCCGAATCGTTCATGGGAGAGTCTATCCAGACTTGCGT AGCGAGTAATCGCTAGGTGAGAAGCTCTGGAGACAATGTAC CTGCCCTTCAATTGGAGGTGCCAGGGCTAGGCTTTGTTCCCT ATGGCTAGCGAAAGCGAATACAGGTTATTAGGCGTCGTGAA AACAAAActcttttcgacataagggagaggctcgtagatgag ttacctcctttttatgggtaactgaacgatcaatatcta ccggcgtagactgtgagcctaaaggaaacgaatgaaagt
22	<i>Pyraliella littoralis</i> 2 5' intronic sequence (DNA) The corresponding RNA sequence is depicted as SEQ ID NO: 24.	GTGCGACAAGAAGTTCAGGAAGGGATTGAGGTGAAAGTCCT CCTCCCGAATCGTTCATGGGAGAGTCTATCCAGACTTGCGT AGCGAGTAATCGCTAGGTGAGAAGCTCTGGAGACAATGTAC CTGCCCTTCAATTGGAGGTGCCAGGGCTAGGCTTTGTTCCCT ATGGCTAGCGAAAGCGAATACAGGTTATTAGGCGTCGTGAA AACAAAActcttttcgacataagggagaggctcgtagatgag ttacctcctttttatgggtaactgaacgatcaatatcta ccggcgtagactgtgagcctaaaggaaacgaatgaaagtgt cccttcctgggaacttggttaagcccaatagactccctctgg gaaaccagaggaggagtaagagcaatcttacttatcgctcta gtgggtaaaagacataataggaagcaaatgcttggttgtaa cgatcgagatagaatctgttgatttaagctgaaaggctgca gacttattaaatgggtgttctgctgtatggcggtt

23	<i>Pyraliella littoralis</i> 1 5' intronic sequence (RNA) Wherein "T" is "U"	GTGCGACAAGAAGTTCAGGAAGGGATTGAGGTGAAAGTCCT CCTCCCGAATCGTTCATGGGAGAGTCTATCCAGACTTGCGT AGCGAGTAATCGCTAGGTGAGAAGCTCTGGAGACAATGTAC CTGCCCTTCAATTGGAGGTGCCAGGGCTAGGCTTTGTTTCCT ATGGCTAGCGAAAGCGAATACAGGTTATTAGGCGTCGTGAA AACAAAActcttttcgacataagggagaggctcgtagatgag ttaccctcctttttatggggtaactgaacgatcaatatcta ccggcgtagactgtgagcctaaaggaaacgaatgaaagt
24	<i>Pyraliella littoralis</i> 2 5' intronic sequence (RNA) Wherein "T" is "U"	GTGCGACAAGAAGTTCAGGAAGGGATTGAGGTGAAAGTCCT CCTCCCGAATCGTTCATGGGAGAGTCTATCCAGACTTGCGT AGCGAGTAATCGCTAGGTGAGAAGCTCTGGAGACAATGTAC CTGCCCTTCAATTGGAGGTGCCAGGGCTAGGCTTTGTTTCCT ATGGCTAGCGAAAGCGAATACAGGTTATTAGGCGTCGTGAA AACAAAActcttttcgacataagggagaggctcgtagatgag ttaccctcctttttatggggtaactgaacgatcaatatcta ccggcgtagactgtgagcctaaaggaaacgaatgaaagtgt cccttcctgggaacttggttaagcccaatagactccctctgg gaaaccagaggaggagtaagagcaatcttacttatcgtcta gtgggtaaaagacataataggaagcaaatgcttggttgtaa cgatcgagatagaatctgttgatttaagctgaaaggctgca gacttattaaatgggtgttctgctgtatggcggt

[0146] In some aspects, the 5' intronic sequence comprises an intron-binding site. In some aspects, the 5' intronic sequence consists of an intron-binding site flanked by any number of additional intronic nucleotides at the 5' (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, or 50, etc.), and/or any number of additional intronic nucleotides at the 3' (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, or 50, etc.).

[0147] In some aspects, the 5' intronic sequence consists of an intron-binding site flanked by any number of additional intronic nucleotides at the 5' (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, or 50, etc.), and/or any number of additional intronic nucleotides at the 3' (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, or 50, etc.). In some aspects, the 5' intronic sequence consists of an intron-binding site.

[0148] As use herein, the term "intronic nucleotides" refers to nucleotides that are part of the intron from which the intronic sequences are derived.

[0149] In some aspects, the 3' intronic sequence comprises less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1% of a sequence of a full length intron.

[0150] In some aspects, the 3' intronic sequence comprises a catalytic core (triad) sequence. In some aspects, the 3' intronic sequence consists of a catalytic core (triad) sequence flanked by any number of additional intronic nucleotides at the 5' (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, or 50, etc.), and/or any number of additional intronic nucleotides at the 3' (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, or 50, etc.). In some aspects, the 3' intronic sequence consists of a catalytic core (triad) sequence flanked by any number of additional intronic nucleotides at the 5' (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, or 50, etc.), and/ any number of additional intronic nucleotides at the 3' (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, or 50, etc.). In some aspects, the 3' the intronic sequence consists of a catalytic core (triad) sequence.

II.B.3. Catalytic Core (Triad)

[0151] In some aspects, the intronic sequence comprises a catalytic core (triad). As used herein, the term "**catalytic core (triad)**" refers to a nucleotide sequence comprising sequences and structures indispensable for catalytic function. The catalytic core (triad) interacts with the substrates used for the steps of the self-splicing reaction. The substrates used for the steps of the self-splicing reaction comprise a 5'-splice site and a 3' splice site or a combination thereof.

[0152] In some aspects, the catalytic core (triad) comprises a sequence of GAG and CC, wherein the GAG and the CC are separated by (N)_n, wherein N is any one of A, C, G, T, or U, and n is an integer. In some aspects, the catalytic core (triad) comprises a sequence of ACG and CT(or U), wherein the ACG and the CT(or U) are separated by (N)_n, wherein N is any one of A, C, G, T, or U, and n is an integer. In some aspects, the catalytic core (triad) comprises a nucleotide sequence of any one of AGC or CGC.

[0153] In some aspects, the catalytic core (triad) comprises a nucleotide sequence of any one of AGC or CGC. In some aspects, the catalytic core (triad) consists of a nucleotide sequence any one of AGC or CGC.

[0154] Non-limiting examples of catalytic core (triad) that are useful for the present genetic constructs are shown in **Table 4**.

Table 4. Exemplary catalytic core (triad)

DESCRIPTION	SEQUENCE
Group IIA and B introns (DNA or RNA)	AGC

Group IIC introns (DNA or RNA)	CGC
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II.B.4. Intron-Binding Site and Exon-Binding Site

[0155] In some aspects, the intronic sequence comprises an intron-binding site. As used herein, the term "**intron-binding site**" or "IBS" refers to a nucleic acid sequence comprised in the intronic sequence that pairs with the exon located at its 5' during the splicing reaction. In some aspects, the intron-binding site pairs with a nucleotide comprised in the exon. In some aspects, the intron-binding site is fully or substantially complementary to a nucleotide sequence comprised in the exon.

[0156] In some aspects, the intron-binding site comprises a nucleotide sequence of any one of TAAAAA (or UAAAAA), TGTTT (or UGUUU), or C. In some aspects, the intron-binding site consists of a nucleotide sequence of any one of TAAAAA (or UAAAAA), TGTTT (or UGUUU), or C.

[0157] In some aspects, the exon-binding site comprises a nucleotide sequence of any one of TTTTTA (or UUUUUA), AAAACAA, or G. In some aspects, the intron-binding site consists of a nucleotide sequence of any one of TTTTTA (or UUUUUA), AAAACAA, or G.

[0158] Non-limiting examples of intron-binding site and exon-binding sites that are useful for the present genetic constructs are shown in **Table 5**.

Table 5. Exemplary intron- and exon-binding sites

DESCRIPTION	SEQUENCE
IBS1 (DNA)	taaaaa
IBS2 (DNA)	tgttt
IBS3 (DNA)	C
EBS1 (DNA)	ttttta
EBS2 (DNA)	AAAACAA
EBS3 (DNA)	g
Gamma/Gamma ' (DNA)	A/T
IBS1 (RNA)	uaaaaa
IBS2 (RNA)	uguuu
IBS3 (RNA)	C
EBS1 (RNA)	UUUUUA
EBS2 (RNA)	AAAACAA
EBS3 (RNA)	g
Gamma/Gamma ' (RNA)	A/U

II.B.5. Homology Arms

[0159] In some aspects, the genetic constructs capable of forming circular RNAs disclosed herein (i.e., pre-circRNAs or DNA genetic constructs from which pre-circRNAs can be transcribed) further comprise one or more homology arms. As used herein, the term "homology arm" refers to a sequence that is capable of pairing (i.e., hybridizing) with another sequence.

[0160] In some aspects, the homology arms are at the 5' and/or at the 3' end of the genetic constructs disclosed herein. For example, the first homology arm can be located at the 5' end of the genetic construct, and a second homology arm can be located at the 3' end of the genetic construct. In some aspects, the homology arms are interposed between any two other sequences comprised in the genetic constructs disclosed herein. For example, a first homology arm can be between the 3' splice site and the exonic sequence, and a second homology arm can be between the 5' splice site and the exonic sequence. In some aspects, the homology arms are comprised within any other sequence comprised in the genetic constructs disclosed herein. For example, the homology arms can be comprised in the intronic sequences, in the exonic sequences, in the splice sites, or any combination thereof. In some aspects, the homology arms can be comprised in a spacer sequence. As used herein, a spacer sequence is any sequence that is designed to be located between the 5'/3' splice sites and/or functional regions of the exonic sequence (e.g., IRES, payload, etc.). In some aspects, the homology arms are excised during circularization and are not comprised in the circRNAs. In some aspects, the homology arms are not excised during circularization and are comprised in the circRNAs.

[0161] The first and the second homology arms are the reverse complement of the other and are capable of pairing with one another. In some aspect, the pairing of the first and the second homology arms changes the spatial conformation of the genetic construct. For example, the pairing of the first and the second homology arms can bring the 3' splice site and the 5' splice site in proximity.

[0162] In some aspects, the homology arms comprise a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with a nucleotide sequence of any one of SEQ ID NOs: 27-28 or 29-30. In some aspects, the homology arms consist of a nucleotide sequence of any one of SEQ ID NOs: 27-28 or 29-30.

[0163] Non-limiting examples of homology arms that are useful for the present genetic constructs are shown in **Table 6**.

Table 6. Exemplary homology arms

SEQ ID NO	DESCRIPTION	SEQUENCE
27	5' Homology (DNA)	GGTTGTTGGAGATCTCTGGC
28	3' Homology (DNA)	GCCAGAGATCTCCAACAACC
29	5' Homology (RNA)	GGUUGUUGGAGAUUCUCUGGC
30	3' Homology (RNA)	GCCAGAGAUCCUCCAACAACC

II.B.6. Exons

[0164] In some aspects, the exonic sequences (*i.e.*, one or more exon or fragment thereof) comprised in the RNA or DNA genetic constructs capable of forming circular RNAs disclosed herein (*i.e.*, pre-circRNAs or DNA genetic constructs from which pre-circRNAs can be transcribed) comprise one exon, or two or more exons.

[0165] In some aspects, the exonic sequence is (*i.e.*, in the pre-circRNA construct) or encodes for (*i.e.*, in the DNA genetic constructs from which the pre-circRNAs can be transcribed) a functional RNA. In some aspects, the functional RNA is not a protein-coding RNA. In some aspects, the functional RNA is a therapeutic RNA. In some aspects, the functional RNA is a protein-coding RNA. In some aspects, the protein-coding RNA is translated into a protein. In some aspects, the protein is a therapeutic protein.

[0166] In some aspects, an RNA or DNA genetic construct capable of forming circular RNAs disclosed herein (*i.e.*, a pre-circRNAs or a DNA genetic construct from which the pre-circRNAs can be transcribed) encodes one protein. In some aspects, an RNA or DNA genetic construct capable of forming circular RNAs disclosed herein (*i.e.*, a pre-circRNAs or a DNA genetic construct from which the pre-circRNAs can be transcribed) encodes two or more proteins. In some aspects, the sequences encoding the two or more proteins can be separated by a ribosomal skipping element or a nucleotide sequence encoding a protease cleavage site. Non limiting examples of self-cleaving 2A peptides are: thosea-assigna virus 2 A peptide (T2A), porcine teschovirus-1 2 A peptide (P2A), foot-and-mouth disease virus 2 A peptide (F2A), equine rhinitis A vims 2A peptide (E2A), cytoplasmic polyhedrosis vims 2A peptide (BmCPV 2A), or flacherie vims of B. mori 2A peptide (BmIFV 2A).

[0167] Non-limiting examples of proteins that can be encoded by the RNA or DNA genetic constructs capable of forming circular RNAs disclosed herein (*i.e.*, pre-circRNAs or DNA genetic constructs from which pre-circRNAs can be transcribed) are chimeric antigen receptor (CAR), T-cell receptor (TCR), B-cell receptor (BCR), immune cell activation or inhibitory receptor, recombinant fusion protein, chimeric mutant protein, or fusion protein, or a combination thereof.

In some aspects, the therapeutic protein comprises an antibody, nanobody, non-antibody protein, immune modulatory ligand, receptor, structural protein, growth factor ligand or receptor, hormone or hormone receptor, transcription factor, checkpoint inhibitor or agonist, Fc fusion protein, anticoagulant, blood clotting factor, chaperone protein, antimicrobial protein, structural protein, biochemical enzyme, tight junction protein, mitochondrial stress response, cytoskeletal protein, metal-binding protein, or small molecule. In some aspects, the immune modulatory ligand comprises an interferon, cytokine, chemokine, or interleukin. In some aspects, the structural protein is a channel protein or nuclear pore protein, a cytokine, an immune checkpoint inhibitor, an agonist, an antagonist, a chimeric antigen receptor, one or more TCR chains, a secreted T cell or immune cell engager, a transcription factor, an immunosuppressive enzyme.

[0168] In some aspects, the protein is a cytokine. Non limiting examples of cytokines are, but are not limited to, IL-2, IL-4, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21, IL-35, IL-12p70, IL-27beta, IFN- α , IFN- β , IFN γ , TGFbeta, or a functional fragment thereof.

[0169] In some aspects, the protein is an antigen binding protein, or a functional fragments thereof. In some aspects, the antigen binding protein is a trispecific antigen-binding protein (TRITEs), or bispecific antigen-binding protein (BITEs), or a functional fragments thereof. In some aspects, the antigen binding protein is an antibody, or a functional fragments thereof. Non limiting examples of antigens are, but are not limited to, Ebola virus glycoprotein Ebola virus glycoprotein, α 4 integrin, Amyloid beta, Amyloid beta protofibrils, Amyloid beta, N3pG (N-terminal truncated), Angiopoietin-like 3, B. anthrax PA, B7-H3, B-cell maturation antigen, BLyS, C1s, C5, CCR4, CD11a, CD19, CD20, CD22, CD3, CD30, CD33, CD38, CD4, CD52, CD62 (aka P-selectin), CD79b, CGRP, CGRP receptor, Clostridium difficile enterotoxin B, Complement 5, CTLA-4, Dabigatran, Ebola virus, EGFR, EGFR, cMET, Endotoxin, EpCAM, EPCAM/CD3, Factor Ixa, X, FcRn, FGF23, Folate receptor alpha, G protein-coupled receptor 5D, CD3, GD2, gp100, CD3, GPIIb/IIIa, HER2, IFNAR1, IFN γ , IgE, IGF-1R, IL-12/23, IL-13, IL-17a, IL-17A, F, IL-17R, IL-1 β , IL-23 p19, IL-23p19, IL-2R, IL-36 receptor, IL-4R α , IL-5, IL-5R α , IL-6, IL-6R, LAG-3, MASP-2, Nectin-4, PCSK9, PD-1, PDGFR α , PD-L1, Plasma kallikrein, RANK-L, RSV, SARS-CoV-2, Sclerostin, SLAMF7, Thymic stromal lymphopoietin, Tissue factor, Tissue factor pathway inhibitor, TNF, TROP-2, VEGF, VEGF-A, VEGF-A, Ang-2, VEGFR2, von Willebrand factor, and α 4 β 7 integrin. Non limiting examples of antibodies are, but are not limited to, Muromonab-CD3, Efalizumab, Tositumomab-I131, Nebacumab, Edrecolomab, Catumaxomab, Daclizumab, Olaratumab, Abciximab, Rituximab, Basiliximab, Palivizumab, Infliximab,

Trastuzumab, Adalimumab, Ibritumomab tiuxetan, Omalizumab, Cetuximab, Bevacizumab, Natalizumab, Panitumumab, Ranibizumab, Eculizumab, Certolizumab pegol, Ustekinumab, Canakinumab, Golimumab, Ofatumumab, Tocilizumab, Denosumab, Belimumab, Ipilimumab, Brentuximab vedotin, Pertuzumab, Ado-trastuzumab emtansine, Raxibacumab, Obinutuzumab, Siltuximab, Ramucirumab, Vedolizumab, Nivolumab, Pembrolizumab, Blinatumomab, Alemtuzumab, Evolocumab, Idarucizumab, Necitumumab, Dinutuximab, Secukinumab, Mepolizumab, Alirocumab, Daratumumab, Elotuzumab, Ixekizumab, Reslizumab, Bezlotoxumab, Atezolizumab, Obiltoxaximab, Brodalumab, Dupilumab, Inotuzumab ozogamicin, Guselkumab, Sarilumab, Avelumab, Emicizumab, Ocrelizumab, Benralizumab, Durvalumab, Gemtuzumab ozogamicin, Erenumab, erenumab-aooe, Galcanezumab, galcanezumab-gnlm, Burosumab, burosumab-twza, Lanadelumab, lanadelumab-flyo, Mogamulizumab, mogamulizumab-kpkc, Tildrakizumab, tildrakizumab-asmn, Fremanezumab, fremanezumab-vfrm, Ravulizumab, ravulizumab-cwvz, Cemiplimab, cemiplimab-rwlc, Ibalizumab, ibalizumab-uiyk, Emapalumab, emapalumab-lzsg, Moxetumomab pasudotox, moxetumomab pasudotox-tdfk, Caplacizumab, caplacizumab-yhdp, Risankizumab, risankizumab-rzaa, Polatuzumab vedotin, polatuzumab vedotin-piiq, Romosozumab, romosozumab-aqqg, Brolucizumab, brolucizumab-dbll, Crizanlizumab, crizanlizumab-tmca, Enfortumab vedotin, enfortumab vedotin-ejfv, [fam-]trastuzumab deruxtecan, fam-trastuzumab deruxtecan-nxki, Isatuximab, isatuximab-irfc, Belantamab mafodotin, belantamab mafodotin-blmf, Sacituzumab govitecan, sacituzumab govitecan-hziy, Tafasitamab, tafasitamab-cxix, Satralizumab, satralizumab-mwge, Eptinezumab, eptinezumab-jjmr, Inebilizumab, inebilizumab-cdon, Teprotumumab, teprotumumab-trbw, Evinacumab, Dostarlimab, dostarlimab-gxly, Amivantamab, amivantamab-vmjw, Tralokinumab, tralokinumab-ldrm, Anifrolumab, anifrolumab-fnia, Loncastuximab tesirine, loncastuximab tesirine-lpyl, Atoltivimab, maftivimab, odesivimab-ebgn, Naxitamab-gqgk, Margetuximab-cmkb, Ansuvimab-zykl, Aducanumab, aducanumab-avwa, Regdanvimab, Sotrovimab, Tisotumab vedotin, tisotumab vedotin-tftv, Bimekizumab, Casirivimab + imdevimab, Tezepelumab, tezepelumab-ekko, Faricimab, faricimab-svoa, Sutimlimab, sutimlimab-jome, Tixagevimab, cilgavimab, Spesolimab, Nirsevimab, Teplizumab, teplizumab-mzwv, Ublituximab, Tebentafusp, tebentafusp-tebn, Relatlimab, Mosunetuzumab, Teclistamab, Tremelimumab, Mirvetuximab soravtansine, mirvetuximab soravtansine-gynx, Lecanemab, Toripalimab, Trastuzumab duocarmazine, Epcoritamab, Mirikizumab, Glofitamab, Pozelimab, Lebrikizumab, Talquetamab,

Rozanolixizumab, Cosibelimab, Concizumab, Elranatamab, Sugemalimab, Penpulimab, Donanemab, Sintilimab, Tislelizumab, Retifanlimab, Narsoplimab, and Omburtamab.

[0170] In some aspects, the protein is a chimeric antigen receptors (CARs or CAR-Ts). Non limiting examples of CARs are, but are not limited to, CAR comprising an antigen binding domain specific for an antigen selected from the group consisting of CD19, CD123, CD22, CD30, CD171, CS-1, C-type lectin-like molecule- 1, CD33, epidermal growth factor receptor variant III (EGFRvIII), ganglioside G2 (GD2), ganglioside GD3, TNF receptor family member B cell maturation (BCMA), Tn antigen ((Tn Ag) or (GalNAc-Ser/Thr)), prostate-specific membrane antigen (PSMA), Receptor tyrosine kinase-like orphan receptor 1 (ROR1), Fms-Like Tyrosine Kinase 3 (FLT3), Tumor-associated glycoprotein 72 (TAG72), CD38, CD44v6, Carcinoembryonic antigen (CEA), Epithelial cell adhesion molecule (EPCAM), B7H3 (CD276), KIT (CD117), Interleukin- 13 receptor subunit alpha-2, mesothelin, Interleukin 11 receptor alpha (IL-11Ra), prostate stem cell antigen (PSCA), Protease Serine 21, vascular endothelial growth factor receptor 2 (VEGFR2), Lewis(Y) antigen, CD24, Platelet-derived growth factor receptor beta (PDGFR-beta), Stage-specific embryonic antigen-4 (SSEA-4), CD20, Folate receptor alpha, HER2, HER3, Mucin 1, cell surface associated (MUC1), epidermal growth factor receptor (EGFR), neural cell adhesion molecule (NCAM), Prostase, prostatic acid phosphatase (PAP), elongation factor 2 mutated (ELF2M), Ephrin B2, fibroblast activation protein alpha (FAP), insulin-like growth factor 1 receptor (IGF -I receptor), carbonic anhydrase IX (CAIX), Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2), glycoprotein 100 (gp100), oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl), tyrosinase, ephrin type-A receptor 2 (EphA2), Fucosyl GM1, sialyl Lewis adhesion molecule (sLe), ganglioside GM3, transglutaminase 5 (TGS5), high molecular weight-melanoma-associated antigen (HMWMAA), o-acetyl-GD2 ganglioside (OAcGD2), Folate receptor beta, tumor endothelial marker 1 (TEM1/CD248), tumor endothelial marker 7-related (TEM7R), claudin 6 (CLDN6), thyroid stimulating hormone receptor (TSHR), G protein-coupled receptor class C group 5, member D (GPRC5D), chromosome X open reading frame 61 (CXORF61), CD97, CD179a, anaplastic lymphoma kinase (ALK), Polysialic acid, placenta-specific 1 (PLAC1), hexasaccharide portion of globoH glycosphingolipid (GloboH), mammary gland differentiation antigen (NY-BR-1), uroplakin 2 (UPK2), Hepatitis A virus cellular receptor 1 (HAVCR1), adrenoceptor beta 3 (ADRB3), pannexin 3 (PANX3), G protein-coupled receptor 20 (GPR20), lymphocyte antigen 6 complex, locus K 9 (LY6K), Olfactory receptor 51E2 (OR51E2), TCR

Gamma Alternate Reading Frame Protein (TARP), Wilms tumor protein (WT1), Cancer/testis antigen 1 (NY-ESO-1), Cancer/testis antigen 2 (LAGE-1a), MAGE family members (including MAGE-A1, MAGE-A3 and MAGE-A4), ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML), sperm protein 17 (SPA17), X Antigen Family, Member 1 A (XAGE1), angiopoietin-binding cell surface receptor 2 (Tie 2), melanoma cancer testis antigen-1 (MAD-CT-1), melanoma cancer testis antigen-2 (MAD-CT-2), Fos-related antigen 1, tumor protein p53 (p53), p53 mutant, prostatein, surviving, telomerase, prostate carcinoma tumor antigen-1, melanoma antigen recognized by T cells 1, Rat sarcoma (Ras) mutant, human Telomerase reverse transcriptase (hTERT), sarcoma translocation breakpoints, melanoma inhibitor of apoptosis (ML-IAP), ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene), N-Acetyl glucosaminyl-transferase V (NA17), paired box protein Pax-3 (PAX3), Androgen receptor, Cyclin B1, v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN), Ras Homolog Family Member C (RhoC), Tyrosinase-related protein 2 (TRP-2), Cytochrome P450 1B1 (CYP1B1), CCCTC-Binding Factor (Zinc Finger Protein)-Like, Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3), Paired box protein Pax-5 (PAX5), proacrosin binding protein sp32 (OY-TES1), lymphocyte-specific protein tyrosine kinase (LCK), A kinase anchor protein 4 (AKAP-4), synovial sarcoma, X breakpoint 2 (SSX2), Receptor for Advanced Glycation Endproducts (RAGE-1), renal ubiquitous 1 (RU1), renal ubiquitous 2 (RU2), legumain, human papilloma virus E6 (HPV E6), human papilloma virus E7 (HPV E7), intestinal carboxyl esterase, heat shock protein 70-2 mutated (mut hsp70-2), CD79a, CD79b, CD72, Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1), Fc fragment of IgA receptor (FCAR or CD89), Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2), CD300 molecule-like family member f (CD300LF), C-type lectin domain family 12 member A (CLEC12A), bone marrow stromal cell antigen 2 (BST2), EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2), lymphocyte antigen 75 (LY75), Glypican-3 (GPC3), Fc receptor-like 5 (FCRL5), MUC16, 5T4, 8H9, α nbq integrin, α nb6 integrin, α fetoprotein (AFP), B7-H6, ca-125, CA9, CD44, CD44v7/8, CD52, E-cadherin, EMA (epithelial membrane antigen), epithelial glycoprotein-2 (EGP-2), epithelial glycoprotein-40 (EGP-40), ErbB4, epithelial tumor antigen (ETA), folate binding protein (FBP), kinase insert domain receptor (KDR), κ -light chain, L1 cell adhesion molecule, MFJC18, NKG2D, oncofetal antigen (h5T4), tumor/testis-antigen IB, GAGE, GAGE-1, BAGE, SCP-1, CTZ9, SAGE, CAGE, CT10, MART-1, immunoglobulin lambda-like polypeptide 1 (IGLL1), Hepatitis B Surface Antigen Binding Protein (HBsAg), viral

capsid antigen (VC A), early antigen (EA), EBV nuclear antigen (EBNA), HHV-6 p41 early antigen, HHV-6B U94 latent antigen, HHV-6B p98 late antigen, cytomegalovirus (CMV) antigen, large T antigen, small T antigen, adenovirus antigen, respiratory syncytial virus (RSV) antigen, haemagglutinin (HA), neuraminidase (NA), parainfluenza type 1 antigen, parainfluenza type 2 antigen, parainfluenza type 3 antigen, parainfluenza type 4 antigen, Human Metapneumovirus (HMPV) antigen, hepatitis C virus (HCV) core antigen, HIV p24 antigen, human T-cell lymphotropic virus (HTLV-1) antigen, Merkel cell polyoma virus small T antigen, Merkel cell polyoma virus large T antigen, Kaposi sarcoma-associated herpesvirus (KSHV) lytic nuclear antigen and KSHV latent nuclear antigen.

[0171] In some aspects, the protein is a transcription factor. Non limiting examples of transcription factors are, but are not limited to, Forkhead box P3 (Foxp3), and signal transducer and activator of transcription (STAT, e.g., STAT1, STAT2, STAT3, STAT4, STAT 5 (including STAT5 A and STAT5B), and STATE).

II.B.7. IRES

[0172] In some aspects, the RNA or DNA constructs disclosed herein further comprise an internal ribosome entry site (IRES). In some aspects, the IRES is comprised in an exon.

[0173] First identified as a feature Picorna virus RNA, IRES plays an important role in initiating protein synthesis in absence of the 5' cap structure. An IRES can act as the sole ribosome binding site, or can serve as one of multiple ribosome binding sites of an mRNA. Nucleic acids or mRNA containing more than one functional ribosome binding site can encode several peptides or polypeptides that are translated independently by the ribosomes ("multicistronic nucleic acid molecules"). When nucleic acids or mRNA are provided with an IRES, further optionally provided is a second translatable region.

[0174] Examples of IRES sequences that can be used according to the disclosure include without limitation, those from picornaviruses (e.g., FMDV), pest viruses (CFFV), polio viruses (PV), encephalomyocarditis viruses (ECMV), foot-and-mouth disease viruses (FMDV), hepatitis C viruses (HCV), classical swine fever viruses (CSFV), murine leukemia virus (MLV), simian immune deficiency viruses (SIV) or cricket paralysis viruses (CrPV). In some aspects, the genetic constructs disclosed herein comprise an IRES. In some aspects, the genetic constructs disclosed herein comprises an IRES having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity with a nucleotide sequence of SEQ ID NOs: 44-67 or 68-91. Modifications of IRES and accessory sequences are disclosed herein to increase or reduce IRES

activities, for example, by truncating the 5' and/or 3' ends of the IRES, adding a spacer 5' to the IRES, modifying the 6 nucleotides 5' to the translation initiation site (Kozak sequence), modification of alternative translation initiation sites, and creating chimeric/hybrid IRES sequences. In some aspects, the IRES sequence in the genetic constructs disclosed herein comprises one or more of these modifications relative to a native IRES (e.g., a native IRES disclosed in Table 7 (SEQ ID NOs: 44-67 or 68-91).

Table 7. Exemplary IRES

SEQ ID NO	DESCRIPTION	SEQUENCE
	IRES (DNA)	
44	CVB3 (DNA) CVB3 (RNA) is depicted as SEQ ID NO: 68.	TTAAACAGCCTGTGGGTTGATCCCACCCACAGGCCCATTTGGGCGCTAGCACTCTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCTCCCCAACTGTAACCTTAGAAGTAACACACACCCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTGTATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCGTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGTAAACCCGTGGAAGTTGCAGAGTGTTCGCTCAGCACTACCCAGTGTTAGATCAGGTTCGATGAGTCACCGCATTTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCCTGAATGCGGCTAATCCTAAC TGCGGAGCACACACCTCAAGCCAGAGGGCAGTGTGTGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCA TCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTTGAAAGAGGTTAAACATTACAATTCAATTGTTAAGTTGAATACAGCAAA
45	EV71 (DNA) EV71 (RNA) is depicted as SEQ ID NO: 69.	TTAAACAGCTGTGGGTTGTCAACCCACCCACAGGGTCCACTGGGCGCTAGTACACTGGTATCTCGGTACCTTTGTACGCCTGTTTTATACCCCTCCCTGATTTGC AACTTAGAAGCAACGCAAACAGATCAATAGTAGGTGTGACATACCAGTCGCA TCTTGATCAAGCACTTCTGTATCCCGGACCGAGTATCAATAGACTGTGCACA CGGTTGAAGGAGAAAACGTCCGTTACCCGGCTAACTACTTCGAGAAGCCTAGT AACGCCATTGAAGTTGCAGAGTGTTCGCTCAGCACTCCCCCGTGTAGATCA GGTTCGATGAGTCACCGCATTTCCCCACGGGCGACCGTGGCGGTGGCTGGTGG CGGCCTGCCTATGGGGTAACCCATAGGACGCTCTAATACGGACATGGCGTGAA GAGTCTATTGAGCTAGTTAGTAGTCCTCCGGCCCCCTGAATGCGGCTAATCCTA ACTGCGGAGCACATACCCCTTAATCCAAAGGGCAGTGTGTGTAACGGGCAACT CTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTATTCTTGTATT GGCTGCTTATGGTGACAATTAAAGAATTGTTACCATATAGCTATTGGATTGGC CATCCAGTGTCAAACAGAGCTATTGTATATCTCTTTGTTGGATTACACCTCT CACTCTTGAAACGTTACACACCCCTCAATTACATTATACTGCTGAACACGAAGC G
46	CVB5 (DNA) CVB5 (RNA) is depicted as SEQ ID NO: 70.	TTAAACAGCCTGTGGGTTGTTCCACCCGACAGGGCCCACTGGGCGCCAGCAC TCTGGTATCACGGTACCTTTGTGCGCCTGTTTTAAACCCCTCTCCCCAATTTG AAACCTTAGAAGCAATACACCTCGATCAATAGTAGGCATGACACGCCAGCCATG TCTTGATCAAGCACTTCTGTTTCCCGGACTGAGTATCAATAAACTGCTTTCGCG CGGTGGAAGGAGAAAACGTCCGTTACCCGACTAACTACTTCGAGAAACCCAGT AACACCATGGAAATTGCGGAGTGTTCCTCACTCAGCACATTCCAGTGTTAGATCA GGTTCGATGAGTCACCGCATTTCCCCACGGGTGACCGTGGCGGTGGCTGCGCTGG CGGCCTGCCCATGGGGCAACCCATGGGACGCTTCAATATGGACATGGTGTGAA GAGTCTATTGAGCTAGTTAGTAGTCCTCCGGCCCCCTGAATGCGGCTAATCCTA ACTGCGGAGCACGTGCCTCCATTCCAGGGGGTGGCGTGTGTAACGGGCAACT CTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTAAATTTTATACTG GCTGCTTATGGTGACAATTGAAAGATTGTTGCCATATAGCTATTGGATTGGCC ATCCGGTATCCAACAGAGCAATTGTGTACCTTTTGTGGATTGTGTAACCACTT ACCAGAACAAGTTTTCATACACTGTGTTACATTATTAGACTAAACACAGAAAA

47	EchoV11 (DNA) EchoV11 (RNA) is depicted as SEQ ID NO: 71.	TTAAACAGCCTGTGGGTGTACCCACCCACAGGCCCACTGGGCGCTAGCAC ACTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCTTCCCGCAACCG CAAATTTAGAAGCAAAGCTAACCCGATCGATAGCGGATGCGCATGCCAGCCGC ATTTTGATCAAGTACTTCTGTTTTCCCGGACCGAGTATCAATAGACTGCTCAC GCGGTTGAAGGAGAAAACGTCCGTTACCCGACCACTACTTCGAGAAACCTAG TAACATCATGAATGTTGCAGGGCGTTTCGATCAGCAGACCCCTGGTGTAGATC AGGCTGATGAGTCACCGCATTTCCACGCGGTGACCGTGGCGGTGGCTGCGTTG GCGGCTGCCTATGGGGTGACCCATAGGACGCTCTAATACGGACATGGTGCGA AGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCTGAATGCGGTAAATCCT AACTGCGGACGACATACCCCTAATCCAAGGGGAGTGTGTGTAACGGGCAAC TCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTATTTTATAC TGGCTGCTTATGGTGACAATCTCAGAGTTGTTACCATATAGCTATTGGATTGG CCATCCGGTGAGCAACAGAGCTGTCAATTTATCAGTTTGTGGCTTTATACCTC TAAATCACACGGTTTTTTTTTTTTTGGAAACGCTTGATTATCATCTAACCTCAA TAAGGCAAA
48	EVB107 (DNA) EVB107 (RNA) is depicted as SEQ ID NO: 72.	TTAAACAGCCTGTGGGTGTTCACCCACCGCAGGCCCACTGGGCGCTAGCAC ACTGGTATCCCGGTACCTTTGTGCGCCTGTTTTATATACCTCCCCCTTATGT AACTTAGAAGTATGATTCAAACGTCGACAGGCGGCTCAGTGCACCAACTGAG TCATGACCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAAGCTGTTTACA CGGCTGAAGGAGAAAACGTTTCGTTACCCGGCCAATTACTTCGAGAAACCTAGT ACCACCATGAAGGTTGCGCAGTGTTCGCTCCACACAACCCAGTGTAGATCA GGTCGATGAGTCACCGCATTTCCACGCGGCGACCGTGGCGGTGGCTGCGTTGG CGGCTGCCCATGGGGCAACCCATGGGACGCTTCAATACTGACATGGTGTGAA GAGTCTATTGAGCTAATTGGTAGTCCTCCGGCCCTGAATGCGGCTAATCCCA ACTGTGGAGCAGATACTCACAACCAAGTGAGCGGTCTGTGTAACGGGCAACT CCGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTATTCTTACATT GGCTGCTTATGGTGACAATTGACAAATTGTTACCATATAGCTATTGGATTGGC CATCCGGTGACAAACAGAGCTATTGTTTACTTGTGTTGTTGGTTTCATACCATT AAATTACAAGGCTCTAGAACTCTCAACTTTATTTTACACTCAATACAGCAA A
49	PV3 (DNA) PV3 (RNA) is depicted as SEQ ID NO: 73.	TTAAACAGCTCTGGGGTTGCTCCCAACCCAGAGGCCACGTGGCGGCCAGTA CTCTGGTATTGCGGTACCTTTGTACGCCTGTTTTATACTCCCTCCCCCGTGC AACTTTAGAAGAAATCCACAAAGTTCAATAGAGGGGGTGCAAAACAGCACCAC CACGAACAAGCACTTCTGTTTCCCGGTGATGTCGTATAAGCTGTACCCACGG CTGAAGGCGACGGATCCGTTATCCGCTTGAGTACTTCGAGAAGCCCTAGTATTA CCTTGGAATCTTTCGACGCGTTGCGCTCAGCACTCTACCCCGAGTGTAGCTTAG GTCGATGAGTCTGGGACGCCCCACCGCGACCGTGGGCCAGGCTGCGTTGGC GGCTACCCATGGCTATCACCATGGGACGCTAGTTGTGAACAAGGTGTGAAGA GCCTATTGAGCTACCAAGAGTCTCCGGCCCTGAATGCGGCTAATCCTAAC CACGGAGCAAGTGTCTCAACCCAGGGGATGGCTTGTGTAACGCGAAAGTCT GTGGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTATTTTATGTATG GCTGCTTATGGTGACAATCAAAGGTTGTTACCATAAAGCAATTTGGATTGGCC ATCCGGTGAGAATCAAACATATTATCTACCTGTTTGTGGGTTTTCTTCTTTT ACTTGAACAATACCTCTAATAATAACTGCTATATTGTCAATAAGACATTATCA TCACA
50	CVB1 (DNA) CVB1 (RNA) is depicted as SEQ ID NO: 74.	TTAAACAGCCTGTGGGTGTTCACCCACCCACAGGCCCATTTGGGCGCTAGCACT CTGGTATCACGGTACCTTTGTGCGCCTGTTTTACATCCCTCCCCAAATTGTA ATTTAGAAGTTTCACACACCGATCATTAGCAAGCGTGCGACACACGACCATGTT TTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACCGCTAACGCG GTTGAAGGAGAAAACGTTTCGTTACCCGGCCAATACTTCGAAAAACCTAGTAA CACCATGGAAGTTGCGGAGTGTTCGCTCAGCACTACCCAGTGTAGATCAGG TCGATGAGTCACCGCGTTCCCAACGGGCGACCGTGGCGGTGGCTGCGTTGGCG GCCTGCCTACGGGGAACCCGTAGGACGCTCTAATACAGACATGGTGCGAAGA GTCTATTGAGCTAGTTGGTAATCTCCGGCCCTGAATGCGGCTAATCCTAAC TGCGGAGCACATACCTCAAACAGGGGGCAGTGTGTGTAACGGGCAACTCT GCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCAATTTTATCTTACTGG CTGCTTATGGTGACAATTGACAGGTTGTTACCATATAGTTATTGGATTGGCCA TCCGGTGACTAACAGAGCAATTATATATCTCTTGTGGGTTTATACCACTTA GCTTGAAAGAGGTTAAACACTACATCTCATCTAACTAAATACAACAAA

51	EchoVE15 (DNA) EchoVE15 (RNA) is depicted as SEQ ID NO: 75.	TTAAAACAGCCTGTGGGTTGTACCCACCCACAGGGCCCACTGGGCGCTAGCAC TCTGGTATTACGGTACCCCTTGTGCGCCTGTTTATATACCCCGCCCCAAGCAA ACGCTAGATGTAACGCACTTATGATCAATAGCAGGCGTGGCACTCCAGCCACG TTATGATCAAGCACTTCTGTCTCCCCGGACCGAGTATCAATAGACTGCTCAGC CGGTCAAGGAGAAAAACGTTTCGTTACCCGACCAGCTACTTCGAGAAACCTAGT AACTCCATGGAGGTTGCAGAGTGTTCGTTTCAGCACTTCCCCCGTGTAGATCA GGCTGATGAGTCACCGCGTTCCCTCAGGGCGACCGTGGCGGTGGCTGCGTTGG CGGCCTGCCTATGGGTATACCCATAGGACGCTCTAATACTGACATGGTGCAGAA GAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCTGAATGCGGCTAATCCTA ACTGCGGAGCACATAACCCCAACCCAGGGGGCAGTGTGTCGTAACGGGTAACT CTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTATTCTTACATT GGCTGCTTATGGTGACAATTGAGAGATTGTTACCATATAGCTATTGGATTGGC CATCCAGTGACTAACAGAGCTATTATTTACCTTTTGTGGCTTCGTATCACT TGTTTAAAAGAGGTTAGTACTTTATATTGCATTATATTACTAAACACAGAGAA A
52	EchoV1 (DNA) EchoV1 (RNA) is depicted as SEQ ID NO: 76.	TTAAAACAGCCTGTGGGTTGTTCCACCCACAGGGCCCACTGGGCGTTAGCAC ACTGGTATCACGGTACCTTTGTGCGCCTGTTTATACTCCCCCCCCCTAAGGAA ACTTTAGAAGCAAAGCAATTGTGATCAATAGTGGGTATGGCACACCACTGCATA TCTTGATCAAGCACTTCTGTTCCTCCCGGACTTAGTACCAATAGACTGCTCAAG CGGTTGAAGGGGAAAAACGTTTCGTTATCCGGCCAACTACTTCGAGAAACCTAGT AGCACCATGAAAGTTGCGGAGTGTTCGCTCAGCACTTCCCCCGTGTAGATCA GGCTGATGAGTCACCGTATTCCTCCACGGGCGACCGTGACGGTGGCTGCGTTGG CGGCCTGCCCATGGGGTAACCCATGGGACGCTCTAAAACAGACACGGTGCAGAA GAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCTGAATGCGGCCAATCCTA ACTGCGGAGCACATACTCCCAATCCAGGGAGCAGTGTGTCGTAATGGGTAACT CTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTATTCTCACATT GACTGCTTATGGTGACAATTGAAAGATTGTTACCATATAGCTATTGGATTGGT CATCCGGTGAGCAATAGAGCTATTGTTTATCAATTTGTTGGATTGTACCCT CAACTTTTCTGTTTTGAGAACACTCAACTACATCTTACTGCTAAACACATCAA A
53	EVD94 (DNA) EVD94 (RNA) is depicted as SEQ ID NO: 77.	TTAAAACAGCCTGTGGGTTGTTCCACCCACAGGGCCCACTGGGCGGCCAGTA CTCTGGTATCACGGTACCTTTGTACGCCTGTTTATATCCCTTCCCCCGCAA CTTAGAAGAAAAACAATCAAGTTCACTAGGAGGGGTACAAACCAGTACCACC ACGAACAAGCACTTCTGTTTCCCGGTGATGTCGTATAGACTGTAACCACGGT TGAAAACGATTGATCCGTTATCCGCTCTTGTACTTCGAAAAGCCCAGTATCAC CTTGGAATCTTCGATGCGTTGCGCTCAGCACTCAACCCAGAGTGATAGCTTAG GTCGATGAGTCTGGACACTCCTCACC GGCGACGGTGGTCCAGGCTGCGTTGGC GGCCTACCTGTGGTCCAAAGCCACAGGACGCTAGTTGTGAACAAGGTGTGAAG AGCCTATTGAGCTACAAGAGAATCCTCCGGCCCTGAATGCGGCTAATCCTAA CCACGGAGCAAGGGTACACAAACCAGTGTATATCTTGTGCGTAACGCGCAAGTC TGTGGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTGTGTTTTATCATG GCTGCTTATGGTGACAATCTAAGATTGTTATCATATAGCTGTGGATTGGCCA TCCGGTAATTTATTGAGATTGAGCATTGCTTGTGTTCTTCAACAATTTCAAC TATTCATTGCATTTCAAGCAGTCAAA
54	PV1 (DNA) PV1 (RNA) is depicted as SEQ ID NO: 78.	TTAAAACAGCTCTGGGTTGTACCCACCCACAGGGCCCACTGGGCGGCTAGTA CTCCGGTATTGCGGTACCCCTTGTACGCCTGTTTATACTCCCTTCCCGTAACT TAGACGCACAAAACCAAGTTCAATAGAAGGGGTACAAACCAGTACCACCACG AACAAGCACTTCTGTTTCCCGGTGATGTCGTATAGACTGCTTGCGTGGTTGA AAGCGACGGATCCGTTATCCGCTTATGTACTTCGAGAAGCCAGTACCACCTC GGAATCTTCGATGCGTTGCGCTCAGCACTCAACCCAGAGTGTAGCTTAGGCT GATGAGTCTGGACATCCCTCACC GGTGACGGTGGTCCAGGCTGCGTTGGCGGC CTACCTATGGCTAACGCCATGGGACGCTAGTTGTGAACAAGGTGTGAAGAGCC TATTGAGCTACATAAGAATCCTCCGGCCCTGAATGCGGCTAATCCCAACCTC GGAGCAGGTGGTCACAAACCAGTATTGGCCTGTCGTAACGCGCAAGTCCGTG GCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTATTGTTGTTGCTGCTGC TTATGGTGACAATCACAGATTGTTATCATAAAGCGAATTGGATTGGCCATCCG GTGAAAGTGAGACTCATTATCTATCTGTTTGTGGATCCGCTCCATTGAGTGT GTTTACTCTAAGTACAATTTCAACAGTTATTTCAATCAGACAATTGTATCACC

55	PV2 (DNA) PV2 (RNA) is depicted as SEQ ID NO: 79.	TTAAAACAGCTCTGGGGTTGTTCCACCCCAGAGGCCACGTTGGCGGCCAGTACACTGGTATCGCGGTACCTTTGTACGCCTGTTTTATACTCCCTTCCCCGTAACTTAGAAGCACAACTCCAAGTTCAATAGAAGGGGTACAAACAGTACCACCACGAACAAGCACTTCTGTTCCCCCGGTGAGGCTGTATAGGCTGTTTCCACGGCTAAAAGCGGCTGATCCGTTATCCGCTCATGTACTTCGAGAAGCCTAGTATCACCTTGGGAATCTTCGATGCGTTGCGCTCAACACTCAACCCAGAGTGTAGCTTAGGTCGATGAGTCTGGACGTTCTCACCAGGCGACGGTGGTCCAGGCTGCGTTGGCGGCTACCTGTGGCCCAAAGCCACAGGACGCTAGTTGTGAACAAGGTGTGAAGAGCCTATTGAGCTACTTGAGAGTCTCCGGCCCTGAATGCGGCTAATCCTAACACGGAGCAGGCACTGGCAATCCAGCGACCAGCCTGTCGTAACGCGCAAGTTCGTGGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTATTTTACAATGCTGCTTATGGTGACAATTATTGATAGTTATCATAAGCAAAATGAGCAATGCGATCCGGTGAGAATTGATTATTAAATTACTCTCTTGTGGGATTGCTCCTTTGAAATCCTGTGCACTCACACCTATTGGAATTACCTCATTGTTGAGATATTATTA CCACT
56	CVA24 (DNA) CVA24 (RNA) is depicted as SEQ ID NO: 80.	ttaaaacagctctggggttggttccccaccccagagggccacgtggcgccagta ctccggtattgcggtaccccttgtaacgctgttttacactcccttccccggtaa cttagaagtaaaacaaacaaagttcaatagaaggggtacaaacagtagtaccacc acgaacaagcacttctgttcccccggtgacgttgtagactgtacccacggt tgaaaacaactgatccggttatccgctcttgtagtctcgagaagcccagtagcac cttggaatcttcgatgctgttgctcagcactcaaccccagagtgtagcttag gtcgatgagtcctggacaatcctcaccggcgacggtgggtccaggtgctgcttggc ggctacctgtggcccaaagccacaggacgctagttgtgaacaaggtgtgaag agcctattgagctacgagagagtcctccggccctgaatgcggctaatcccaa ccacggagcaggtgatcacaagccagtgattagcctgtcgtaacgcgcaagtc tgtggcggaaccgactactttgggtgtccgtgtttcctttattttattatggc tgcttatggtgacaatcacagattgttatcataaagcgacttggaattggccat ccagtgaaaaataaaacacatcatttacctgattgttggaattactccgctgac tatctactcaccaggtcttattaaggttattctgttgattaggtcacaaatttc aca
57	CVB6 (DNA) CVB6 (RNA) is depicted as SEQ ID NO: 81.	ttaaaacagcctgtggggtgtaccaccccacagggccactgggcgctagcac tctgggtattgcggtacctttgtgcgctgttttataccacccctcccccaacgta acttagaagcctgacatatacgggtcacaagccagcccagtagcgaactgggt accgaccaagcacttctgttaccgccgactgagtagtcaatagctgccaaagc ggctgaaggagaaaaacgttcgttaccgggccaattacttcgagaaaccagta acaccatgaaaagttgcgtagcgtttcattcagcacaatcccagtgtagtcag gtcgatgagtcaccgcattccccacaggcgactgtggcggtggctgctgttggc ggctgccccatggggcaaacccatgggacgcttcaatactgacatgggtgcgaa gagtctattgagctaattggtagtcctccggccctgaatgcggctaataccta actgcggagcaggcactcgcagaccagcgagcagcttgtcgtaatgggcaact ccgcagcggaaccgactactttgggtgtccgtgtttcctatttctctttacact ggctgcttatggtgacaattgagaaattgttaccatatagctattggagtggc catccggtgactaacagagcaataatataatttgtttgttggcttgtgccact taatttgaaggttgttaagacgctcaatctgatactacttctaataaagcaa a
58	EV89 (DNA) EV89 (RNA) is depicted as SEQ ID NO: 82.	ttaaaacagcctctggggtgacccaccccacagggccactgggcgctagcac actgggtatcacggtacctttgtgcgctgttttataccacccctcccccttgta acttagaagaaacaaacaaacgatcaatagtaggcttagcactocagctaagt ctcgatcaagcacttctgttaccgccgacgagtagtcaataggtgtaccacac ggctgaaagagaaaaacgttcgttaccggctatctacttcgggaaaccagta ccatcatgaagattgcaaagcgtttcgctcagcacaaccccagtgtagtcag gtcgatgagtcactgcattccccacaggcgactgtggcagtggtgctgcttggc ggctgcctatggggaaacccataggacgctttaatattgacatgggtgcgaag agtctactgagctaggtagtagtcctccggccctgaatgcggctaatacccaa ccacggagcaggcgctcacactccagtgagtagcttgtcgtaatgcgaaagtc tgtggcggaaccgactactttgggtgtccgtgtttcctttattttattcatg gctgcttatggtgacaatctaagattgttatcatatagctattggattggcca tccggtgaattctagagatattatttacttgtttgttgggttttaccacattaa

		acattaccaattacagaactctcctctacatcctgttattgaataatagaaaag agaaa
59	EV91 (DNA) EV91 (RNA) is depicted as SEQ ID NO: 83.	ttaaacagctctaggggtgttcccaccctagagggccacgtggcggttagcac tctgggtatcacggtagcctttgtgcgcctgttttatatccctttcccatgttaa cttagaagttattaaacaaagttcaatagaaggggtacaaaccagtgccacc acgaacaagcacttctgtttcccgggtgaagctgtatagactgtctccacgggt tgaaaatggcagatccgttaccgcctttggtagcttcgagaaacctagatcac cttggaatcttcgatgcgttgcgctcagcactcaacccagagtgtagcttag gtcgatgagtcgtggacgatcctcactgggtgacagtggtccaggtgcgttggc ggcctacctgtgttgaaaaccacaggacgctagttgtgaaacaaggtgtgaaga gtctattgagctaccaaagagtcctccggccctgaatgcggctaatacctaac cacggagcaagtgcttcaaaccagtaggtagcttgtcgtaacgcgcgaagtc gtggcggaaaccgactactttgggtgtccgtgttctcttttattttatcatggc tgcttatggtgacaatctaagattgttatcatatagctattggattggccatc cgggtgactaacagagatctcatatacctttttgttgggtttactaaattaaat attaccacgttcaagacacttctccttatcatacaactaaatagtgagaagag aaa
60	EVJ108 (DNA) EVJ108 (RNA) is depicted as SEQ ID NO: 84.	ttaaaatagcctcaggggtgttcccaccctgagggccacgtggcgtagtact ctgggtattacggtagcctttgtacgcctattttaccccttcccttgttaactt agaagcaaagcaaaccagttcaatagtaagcaacacacccagtggtgtgacg aacaagtaacttctgtttcccgggaggggtctgacggtaagctgtacccacggc tgaagtatgacctaccgttaaccggctacctacttcgagaagctctagtaatac cattgaagttttgttggcggttacgctcaacacactaccccggtgtgtagttttg gctgatgagtcacggcattccccacgggcgaccgtggccgtggctgcgttgcg gccaaccaaggggcgcaagctccttggacgtcacttaacagacatgggtgtgaa gaacctattgagctaggtagtagtctcctccggccctgaatgcggctaataccta actccggagcacatcagtgcaaccacagcatttgggtgtgtgttaatacgcgaagt ctggagcggaaaccgactactttgggtgtccgtgttctctgtttaccttattt ggctgcttatgggtgacaatttgatatgtttaccatatagtctgttgattggcc atccggatttttgaagagaccacaaaactttctctctactcagattcaagt gcgaagttttctctttcatatattacttactaatttgaagtaccaaag
61	EV87 (DNA) EV87 (RNA) is depicted as SEQ ID NO: 85.	ttaaaacagcctgtgggtgttcccaccacagggccactgggcgctagcac actgggtatcacggtagcctttgtgcgcctgttttatacaccttccctactgt gacttagaagcaaaacaatgatgaccaatagcaagcgcagcactccagctgtg tttcgggtcaagcacttctgtttcccggactgagtagtcaataggccactcacg tggccgaaggagaaaaacgtccgttaccggccaattacttcgagaaccacagt aacaccatagaggttgcgcagcgtttcgctccgcactccccagtgtagatca gggtcgatgagtcaccgcgttccccacgggcgaccgtggcggtggctgcgttgg cggcctgcccatgggtaaccatgggacgcttcaatactgacatgggtgcgaa gagtcattatgagctaattggtagtcctccggccctgaatgcggctaataccta actgcggagcagatacccacgctccagtgggcagtcgtcgtaacgggtaact ctgcagcggaaaccgactactttgggtgtccgtgttctcttttattcttatatt ggctgcttatgggtgacaattgaaagattgttaccatatagtctattggattggc catccggtgtctaataagagcaattatattcctgtttgttgggtttataccact gaatatcacagtttacaagaccctcaattacatcctacggttaacaagacaa a
62	EchoV31 (DNA) EchoV31 (RNA) is depicted as SEQ ID NO: 86.	ttaaaacagcctgtgggtgttcccaccacagggcccatgggcgctagcaca ctgggtatcggtgtacctttgtgcgcctgttttatacaccttcccatgttaa cttagaagtaagcaatattgatcaatagcaagcatggcacaccagcgtgtt cagatcaagcacttctgtaaccccgactgagtagtcaataggctgccaaagcg gctgaaggagaaaaacgttcgttaaccggccaactacttcgagaagccagtat caccatgaaagttgcagagcgtttcgctcagcactcccccggtgtagattagg tcgatgagtcaccgcactccccacaggcgactgtggcggtggctgcgttggcg gcctacctacggggcaaccgcgtaggacgctctaatacacagacatgggtgagaaga gcctattgagctagttggtagtcctccggccctgaatgcggataatacctaac tgccgagcatgtgctcgcaatccagcgagaggcatgtcgtaacgggcaactct gcagcggaaaccgactactttgggtgtccgtgttctcttttattcttgtttgg ctgcttatgggtgacaattgaaagattgttgccatatagtctattgggtcggcca

		cctgggtatctaacagagcaattatttacttgttgggttttgacccttaa atctcaagggttcttgaaacccttaactacacattgaaactcaattcattgaa
63	EV68 (DNA) EV68 (RNA) is depicted as SEQ ID NO: 87.	ttaaaacagctctggggtgttcccactcaagggccacgtggcggttagtac tctgggtatctcggtacctttgtacgcctgttttaattccctccccaacgtaac ttagaagcttttaaaccaaagctcaataggtggagcgcaaacaccagcgctctta tgagcaagcacttctgtctccccgggtgtggttgtatagactgtccccacgggt gaaaacaacttatccgttaaccgctatagtagtctcgagaaacctagtttgcc ttcggagtgttgatgcgttgcgctcagcacactaaccgctgtgtagcttgggt cgatgagtctggacgtacccactggcgacagtgggtccaggtgcgttggcgg cctactcatggtgaaaaccatgagacgctagacatgaacaagggtgtgaagagt ctattgagctgctatagagtcctccggccctgaatgcggtcaatcctaacca tggagcaagtgtcacaaccagtgagttacttgcgtaacgcgcaagtcctgt ggcggaaccgactactttgggtgtccgtgtttcactttttacttttatgactg ctaattggtgacaatttaatatgtttaccatttggcttgcgaattgatcacat aagatctatagttttgttctactgatttgccttgaaataatctcacctcaaac ctccagtagcataacatttaagaggtttaaacttatttataaca
64	EV92 (DNA) EV92 (RNA) is depicted as SEQ ID NO: 88.	tttaaacagcctgtgggtgtgacccacccacagggccacgcggcggttagta ctctgggtatcacggtacctttgtatgectgttttatctccctcccccgtaac gttagaagaatgaacaaaagacaattagcaaatccaccaaccaggtgggttt tcgtcaagcacttctgtttccccggacctagtaccaataagctgtgccacgg ctgaaggggaaaacgttcgttatccggctacctaacttcgagaagcctagtacc accaatgattgtctcaagtgttacgctcagcacaaacccagtgtagttcaggt cgatgagtcaccgaattccccacggggcagccgtgtcggtggctgcgttggcgg cctgcctgcgagtttcaactcgcaggacgcttgattcatgacatggtgcgaaga gcctattgagctaggcagtagtcctccggccctgaatgcggctaatcctaacc cacggagccatgcgtcacaaccagtggtgcgtcatggtcgtaacgagcaattct gtggcggaaccgactactttgggtgtccgtgtttcctgttttatttaccttgg ctgcttatggtgacaatctagagttgtcatcatatagcgattggattggccat ccggatattactttgtgtgatttatctcttgcgtcgtgaagcaattttaaatagt ttatagcatcatt
65	EVG2 (DNA) EVG2 (RNA) is depicted as SEQ ID NO: 89.	ctaaaacagcctgcgggtgtgacccgccgcagggccactgggcgctagtag tctgggtacgctagtagactagcacgttagtgctttttagtccagtagcctttg tacgctgtttttattttcccttccccacatgtaactttagaagtttagaaaca aacaatgaccaataggagtcacagcatccagttggattgcggtcaagtagtctct gtttccccggacctagtagtgataggtgtacccacggccgaagatgaacccg tccgttatccggctacctaacttcgggaagcctagtaacattctgaagtcctg aggcgtttcgcctcagcacgaccccggtgtagatcgggtgaggtgtcgcga taccacacgggtgaccgtggcggagggcgcgttggcgcccccgcctatggcgaa agccataggacgcctcttagatgacaggggtgtgaagagcctactgagctgggt agtagtcctccggccctgaatgcggctaatcctaaccacggagcgtccaccg gcaaaccgggtcgggagggcgtcgtaacgggttaactctgtggcggaaccgacta ctttgggtgtccgtgtttccttttgatcctattttggctgcttatggtgacaa cgataagttgttatcataaagcttttgggttggccacctggaaaaagttatca gtgtttgatattgttcggctctcacgccttccaaacaaaacaggccttacttct tttaattgcattttatccatcggaggttattacagatttccatctgaagcttc ttctcacagtagcagtaaa
66	CVA2 (DNA) CVA2 (RNA) is depicted as SEQ ID NO: 90.	ttaaaacagcctgtgggtgtgacccacccacagggccactgggcgctagcac tctgggtatcacgatacctttgtgcgcctgttttatatccccaccccgagttaa cgttagaagttacgcaaccccgatcaatagtaggtgtagcactccagctgcat cgagatcaagcacttctgtctccccggaccgagtatcaatagactgctaacgc ggttgaaggagaaaacgttcgttacccggccaattacttcgagaagcccagta gtgccgtgaaagttgcggagtgttctgcctcagcactcccccggtgtagatcag gctgatgagtcaccgcgatccccacaggtgactgtggcggtggtgcgttggc ggcctgcctatggggcaaccataggacgctctaatacagacatggtgcgaag agcctattgagctaatggtagtcctccggccctgaatgcggctaatcctaa ctgcggagcacatgccctcaaaccagggggtggtgtgtcgtaacgggttaactc tgcagcggaaccgactactttgggtgtccgtgtttctttttattcttataatg gctgcttatggtgacaattaaagaattgttaccatatagctattggattggcc

		atccggtgactaacaatatcgctcatataaccagtttggtggttttgttccctta tcacatacagctcataaacaccctcttatatttactacaattgaatagcaagaa
67	CVA6 (DNA) CVA6 (RNA) is depicted as SEQ ID NO: 91.	ttaaaacagcctgtgggttgacccactcacagggccactgggcgctagcac actgatttcccgaatccttgtgcgcctgttttatatcccctccccatgcgc aacttagaagcaatctacaccttcgatcaatagcaggcgtggcgcgccagcca tgtctagatcaagcacttctgtttccccggactgagtatcaataaactgtc cgcggttgaaggagaaaatggttcgttaccgggctaactacttcgagaaacta gtagcaccatgaaaattgcagagcgtttcgctcagcgcttccccgcgtagat caggctgatgagtcaatcctcacgggacgcgtggcagtggtgcgtt ggcggtgcccattgggttaacccatgggacgctctaataatggacatggtgtg aagagtctattgagctagttagtagtccctccggccccctgaatgcggctaacc taactgcggagcacatacccccaaacaggggggtggtgcgtaacgggcaa ctctgcagcggaaccgactacttgggtgtccgtgttcccttttattcttata ttggctgcttatggtgacaattgaaagattgttaccatatagctattggattg gcccacgggtgaataacagagccttgatatacctttttagagggtttatacca cttactcttcgcgttggtgagactctaaagtacattctaattctgaacactag aaa
	IRES (RNA)	
68	CVB3 (RNA) Wherein "T" is "U"	TTAAACAGCCTGTGGGTTGATCCACCCACAGGCCCATTTGGGCGCTAGCACT CTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCTCCCCAACTGTA ACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTT TTGATCAAGCACTTCTGTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACCTACTTCGAAAAACCTAGTAA CACCGTGGAAAGTTGAGAGTGTTCGCTCAGCACTACCCAGTGTAGATCAGG TCGATGAGTCACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCG GCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGACATGGTGCGAAGA GTCTATTGAGCTAGTTGGTAGTCTCCGGCCCCCTGAATGCGGCTAATCCTAAC TGCGGAGCACACACCTCAAGCCAGAGGGCAGTGTGTCTGAACGGGCAACTCT GCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTATTCCTATACCTGG CTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATTGGATTGGCCA TCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTTA GCTTGAAAGAGGTTAAACATTACAATTCAATTGTTAAGTTGAATACAGCAA
69	EV71 (RNA) Wherein "T" is "U"	TTAAACAGCTGTGGGTTGTACCCACCCACAGGGTCCACTGGGCGCTAGTAC ACTGGTATCTCGGTACCTTTGTACGCCTGTTTTATACCCCTCCCTGATTGTC AACTTAGAAGCAACGCAAAACAGATCAATAGTAGGTGTGACATACCAGTCGCA TCTTGATCAAGCACTTCTGTATCCCGGACCGAGTATCAATAGACTGTGCACA CGGTTGAAGGAGAAAACGTCCGTTACCCGGCTAACTACTTCGAGAAGCCTAGT AACGCCATTGAAGTTGCAGAGTGTTCGCTCAGCACTCCCCCGGTGATGATCA GGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGG CGGCCTGCCTATGGGGTAACCCATAGGACGCTCTAATACGGACATGGCGTGAA GAGTCTATTGAGCTAGTTAGTAGTCCTCCGGCCCCCTGAATGCGGCTAATCCTA ACTGCGGAGCACATACCTTAATCCAAAGGGCAGTGTGTCTGAACGGGCAACT CTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTATTCTTGTATT GGCTGCTTATGGTGACAATTAAAGAATTGTTACCATATAGCTATTGGATTGGC CATCCAGTGTCAAACAGAGCTATTGTATATCTCTTTGTTGGATTACACCTCT CACTCTTGAAACGTTACACACCTCAATTACATTATACTGCTGAACACGAAGC G
70	CVB5 (RNA) Wherein "T" is "U"	TTAAACAGCCTGTGGGTTGTTCCACCCCGCAGGGCCCACTGGGCGCCAGCAC TCTGGTATCACGGTACCTTTGTGCGCCTGTTTTAAACCCCTCTCCCCAATTG AACTTAGAAGCAATACACCTCGATCAATAGTAGGCATGACACGCCAGCCATG TCTTGATCAAGCACTTCTGTTCCTCCCGGACTGAGTATCAATAAACTGCTTGCG CGGTCGAAGGAGAAAACGTCCGTTACCCGACTAACTACTTCGAGAAACCCAGT AACACCATGGAAATTGCGGAGTGTTCACCTCAGCACATTCCAGTGTAGATCA GGTCGATGAGTCACCGCATTCCCCACGGGTGACCGTGGCGGTGGCTGCGCTGG CGGCCTGCCCATGGGGCAACCCATGGGACGCTTCAATATGGACATGGGTGAA GAGTCTATTGAGCTAGTTAGTAGTCCTCCGGCCCCCTGAATGCGGCTAATCCTA ACTGCGGAGCACGTGCCTCCATTCCAGGGGTGGCGTGTCTGAACGGGCAACT CTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTAAATTTTATACTG GCTGCTTATGGTGACAATTGAAAGATTGTTGCCATATAGCTATTGGATTGGCC

		ATCCGGTATCCAACAGAGCAATTGTGTACCTTTTGTGGATTGTACCACTT ACCAGAACAAGTTTTTCATACACTGTGTACATTATTAGACTAAACACAGAAAA
71	EchoV11 (RNA) Wherein "T" is "U"	TTAAACAGCCTGTGGGTGTGTACCCACCCACAGGGCCCACTGGGCGCTAGCAC ACTGGTATCACGGTACCTTTGTGCGCCTGTTTTATACCCCTTCCCGCAACCG CAAATTTAGAAGCAAAGCTAACCCGATCGATAGCGGATGCGCATGCCAGCCGC ATTTTGATCAAGTACTTCTGTTTCCCGGACCGAGTATCAATAGACTGCTCAC GCGGTTGAAGGAGAAAACGTCCGTACCCGACCAACTACTTCGAGAAACCTAG TAACATCATGAATGTTGCAGGGCGTTTCGATCAGCACGACCCTGGTGTAGATC AGGCTGATGAGTCACCGCATTCCCCACGGGTGACCGTGGCGGTGGCTGCGTTG GCGGCCTGCCTATGGGGTGACCCATAGGACGCTCTAATACGACATGGTGCGA AGAGTCTATTGAGCTAGTTGGTAGTCTCTCCGGCCCCCTGAATGCGGTTAATCCT AACTGCGGACGACATACCCCTAATCCAAGGGGAGTGTGTCTGTAACGGGCAAC TCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTATTTTATAC TGGCTGCTTATGGTGACAATCTCAGAGTTGTTACCATATAGCTATTGGATTGG CCATCCGGTGAGCAACAGAGCTGTCAATTTATCAGTTTGTGGCTTTATACCTC TAAATCACACGGTTTTTTTTTTTTTGGAACGCTGTATTATCATCTTAACCTCAA TAAGGCAAA
72	EVBl07 (RNA) Wherein "T" is "U"	TTAAACAGCCTGTGGGTGTGTCCACCCGACAGGGCCCACTGGGCGCTAGCAC ACTGGTATCCCGGTACCCCTTGTGCGCCTGTTTTATATACCTTCCCTTATGT AACTTAGAAGTATGATTCAAACGGTCGACAGGCGCTCAGTGCACCAACTGAG TCATGACCAAGCACTTCTGTTACCCCGACTGAGTATCAATAAGCTGTTTACA CGGCTGAAGGAGAAAACGTTTCGTACCCGGCCAATTACTTCGAGAAACCTAGT ACCACCATGAAGTTTGCAGTGTTCGCTCCACACAACCCAGTGTAGATCA GGTCGATGAGTCACGCAATTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGG CGGCCTGCCCATGGGGCAACCCATGGGACGCTTCAATACTGACATGGTGTGAA GAGTCTATTGAGCTAATTGGTAGTCTCTCCGGCCCCCTGAATGCGGCTAATCCCA ACTGTGGAGCAGATACTCACAAACAGTGAGCGGTCTGTCTAACGGGCAACT CCGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTATTCTTACATT GGCTGCTTATGGTGACAATTGACAAATTGTTACCATATAGCTATTGGATTGGC CATCCGGTGACAAACAGAGCTATTGTTTACTTGTGTTGGTTTCATACCACT AAATTACAAGGTCTTAGAAACTCTCAACTTTATTTTGACACTCAATACAGCAA A
73	PV3 (RNA) Wherein "T" is "U"	TTAAACAGCTCTGGGGTTGCTCCACCCAGAGGCCACGTGGCGGCCAGTA CTCTGGTATTGCGGTACCTTTGTACGCCTGTTTTATACCTCCCTCCCCCGTGC AACTTTAGAAGAAATCCACAAAGTTCAATAGAGGGGGTGCAAAACAGCACCAC CACGAACAAGCACTTCTGTTTCCCGGTGATGTCTGATAAGCTGTACCCACGG CTGAAGGCGACGGATCCGTTATCCGCTTGAGTACTTCGAGAAGCCTAGTATTA CCTTGGAATCTTCGACGCGTTGCGCTCAGCACTCTACCCCGAGTGTAGCTTAG GTCGATGAGTCTGGGCACGCCCCACCGGCGACGGTGGCCCAGGCTGCGTTGGC GGCCTACCCATGGCTATCACCATGGGACGCTAGTTGTGAACAAGGTGTGAAGA GCCTATTGAGCTACCCAAGAGTCTCTCCGGCCCCCTGAATGCGGCTAATCCTAAC CACGGAGCAAGTGTCTCAACCCAGGGGATGGCTTGTCTGAACGCGAAAAGTCT GTGGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTATTTTATGTATG GCTGCTTATGGTGACAATCAAAGGTTGTTACCATAAAGCAATTGGATTGGCC ATCCGGTGAGAATCAAACATATTATCTACCTGTTTGTGGGTTTTCTTCTTTT ACTTGAACAATACCTCTAATAATAACTGCTATATTGTCAATAAGACATTATCA TCACA
74	CVB1 (RNA) Wherein "T" is "U"	TTAAACAGCCTGTGGGTGTGTCCACCCACAGGCCCATTTGGGCGCTAGCACT CTGGTATCACGGTACCTTTGTGCGCCTGTTTTACATCCCTTCCCAAATTGTA ATTTAGAAGTTTACACACCGATCATTAGCAAGCGTGGCACACCAGCCATGTT TTGATCAAGCACTTCTGTTACCCGGACTGAGTATCAATAGACCGCTAACCGG GTTGAAGGAGAAAACGTTTCGTACCCGGCCAATACTTCGAAAAACCTAGTAA CACCATGGAAGTTGCGGAGTGTTCGCTCAGCACTACCCAGTGTAGATCAGG TCGATGAGTCACCGCGTTCCCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCG GCCTGCCTACGGGGAAACCCGTAGGACGCTCTAATACAGACATGGTGCGAAGA GTCTATTGAGCTAGTTGGTAATCTCTCCGGCCCCCTGAATGCGGCTAATCCTAAC TGCGGAGCACATACCCTCAAACAGGGGGGAGTGTGTCTGTAACGGGCAACTCT GCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCAATTTATTCCTATACTGG CTGCTTATGGTGACAATTGACAGGTTGTTACCATATAGTTATTGGATTGGCCA

		TCCGGTGACTAACAGAGCAATTATATATCTCTTGTGGGTTTATACCACTTAGCTTGAAAGAGGTTAAACACTACATCTCATCATTAACATAACAACAA
75	EchoVE15 (RNA) Wherein "T" is "U"	TTAAACAGCCTGTGGGTTGTACCCACCCACAGGGCCCACTGGGCGCTAGCAC TCTGGTATTACGGTACCCTTGTGCGCCTGTTTTATATACCCCGCCCCAAGCAA ACGCTAGATGTAACGCACTTATGATCAATAGCAGGCGTGGCACTCCAGCCACG TTATGATCAAGCACTTCTGTCTCCCCGACCGAGTATCAATAGACTGCTCAGC CGGTCTGAAGGAGAAAACGTTTCGTTACCCGACCAGCTACTTCGAGAAACCTAGT AACTCCATGGAGGTTGCAGAGTGTTCGTTTCAGCACTTCCCCCGTGTAGATCA GGCTGATGAGTCACCGCGTTCCTCACGGGCGACCGTGGCGGTGGCTGCGTTGG CGGCCTGCCTATGGGTATACCCATAGGACGCTCTAATACTGACATGGTGCAGAA GAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCCTGAATGCGGCTAATCCTA ACTGCGGAGCACATAACCCCAACCCAGGGGCGAGTGTGTCGTAACGGGTAACT CTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCCTTTTATTCTTACATT GGCTGCTTATGGTGACAATTGAGAGATTGTTACCATATAGCTATTGGATTGGC CATCCAGTGACTAACAGAGCTATTATTTACCTTTTGTGGCTTCGTATCACT TGGTTTAAAAGAGGTTAGTACTTTATATTGCATTATATTACTAAACACGAGAA A
76	EchoV1 (RNA) Wherein "T" is "U"	TTAAACAGCCTGTGGGTTGTTCCACCCACAGGGCCCACTGGGCGTTAGCAC ACTGGTATCACGGTACCCTTGTGCGCCTGTTTTATACTCCCCCCCCCTAAGGAA ACTTTAGAAGCAAAGCAATTGTGATCAATAGTGGGTATGGCACACCAGTCATA TCTTGATCAAGCACTTCTGTTCCTCCCGACTTAGTACCAATAGACTGCTCAAG CGGTTGAAGGGGAAAACGTTTCGTTATCCGGCCAACTACTTCGAGAAACCTAGT AGCACCATGAAAGTTGCGGAGTGTTCGCTCAGCACTTCCCCCGTGTAGATCA GGCTGATGAGTCACCGTATTTCCTCACGGGCGACCGTGACGGTGGCTGCGTTGG CGGCCTGCCCATGGGGTAACCCATGGGACGCTCTAAACAGACACGGTGCAGAA GAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCCTGAATGCGGCCAATCCTA ACTGCGGAGCACATACTCCCAATCCAGGGAGCAGTGTGTCGTAATGGGTAACT CTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCCTTTTATTCTCACATT GACTGCTTATGGTGACAATTGAAAGATTGTTACCATATAGCTATTGGATTGGT CATCCGGTGAGCAATAGAGCTATTGTTTATCAATTGTGGATTGTGACCCT CAACTTTTCTGTTTGTGAGAACACTCAACTACATCTTACTGCTAAACACATCAA A
77	EVD94 (RNA) Wherein "T" is "U"	TTAAACAGCCTGTGGGTTGTTCCACCCACAGGGCCCACTGGGCGGCCAGTA CTCTGGTATCACGGTACCCTTGTACGCCTGTTTTATATCCCTTCCCCCGCAA CTTAGAAGAAAACAAATCAAGTTCAGTAGGAGGGGTACAAACAGTACCACC ACGAACAAGCACTTCTGTTTCCCGGTGATGTCGTATAGCTGTAAACACGGT TGAAAACGATTGATCCGTTATCCGCTCTTGTACTTCGAAAAGCCCAGTATCAC CTTGGAATCTTCGATGCGTTGCGCTCAGCACTCAACCCAGAGTGTAGCTTAG GTCGATGAGTCTGGACACTCCTCACCGGCGACGGTGGTCCAGGCTGCGTTGGC GGCCTACCTGTGGTCCAAAGCCACAGGACGCTAGTTGTGAACAAGGTGTGAAG AGCCTATTGAGCTACAAGAGAATCCTCCGGCCCCCTGAATGCGGCTAATCCTAA CCACGGAGCAAGGGTACACAAACAGTGTATATCTTGTGCTAACGCGCAAGTC TGTGGCGGAACCGACTACTTTGGGTGTCCGTGTTTCCTTTTGTTTTATCATG GCTGCTTATGGTGACAATCTAAGATTGTTATCATATAGCTGTTGGATTGGCCA TCCGGTAATTTATTGAGATTGAGCATTTGCTTGTTCCTTCAACAATTTACC TATTCATTGCATTTTCAGCAGTCAAA
78	PV1 (RNA) Wherein "T" is "U"	TTAAACAGCTCTGGGTTGTACCCACCCACAGGGCCCACTGGGCGGCTAGTA CTCCGGTATTGCGGTACCCTTGTACGCCTGTTTTATACTCCCTTCCCGTAACT TAGACGCACAAAACCAAGTTCATAGAAAGGGGTACAAACAGTACCACCACG AACAAGCACTTCTGTTTCCCGGTGATGTCGTATAGACTGCTTGCCTGGTTGA AAGCGACGGATCCGTTATCCGCTTATGTACTTCGAGAAGCCAGTACCACCTC GGAATCTTCGATGCGTTGCGCTCAGCACTCAACCCAGAGTGTAGCTTAGGCT GATGAGTCTGGACATCCCTCACCGGTGACGGTGGTCCAGGCTGCGTTGGCGGC CTACCTATGGCTAACGCCATGGGACGCTAGTTGTGAACAAGGTGTGAAGAGCC TATTGAGCTACATAAGAATCCTCCGGCCCCCTGAATGCGGCTAATCCCAACCTC GGAGCAGGTGGTCACAAACAGTGAATTGGCCTGTGTAACGCGCAAGTCCGTG GCGGAACCGACTACTTTGGGTGTCCGTGTTTCCTTTTATTTTATTGTGGCTGC TTATGGTGACAATCACAGATTGTTATCATAAAGCGAATTGGATTGGCCATCCG

		GTGAAAGTGAGACTCATTATCTATCTGTTTGCTGGATCCGCTCCATTGAGTGT GTTTACTCTAAGTACAATTTCAACAGTTATTTCAATCAGACAATTGTATCACC
79	PV2 (RNA) Wherein "T" is "U"	TTAAACAGCTCTGGGGTTGTTCCACCCAGAGGCCACGTGGCGGCCAGTA CACTGGTATCGCGGTACCTTTGTACGCCTGTTTATACTCCCTTCCCCGTAA CTTAGAAGCACAACGTCCAAGTTCAATAGAAGGGGTACAAACAGTACCACC ACGAACAAGCACTTCTGTTCCCCCGGTGAGGCTGTATAGGCTGTTTCCACGGC TAAAAGCGGCTGATCCGTTATCCGCTCATGTACTTCGAGAAGCCTAGTATCAC CTTGGAAATCTTCGATGCGTTGCGCTCAACACTCAACCCAGAGTGTAGCTTAG GTCGATGAGTCTGGACGTTCCCTACCGGCGACGGTGGTCCAGGCTGCGTTGGC GGCCTACCTGTGGCCCAAAGCCACAGGACGCTAGTTGTGAACAAGGTGTGAAG AGCCTATTGAGCTACCTGAGAGTCTCCGGCCCTGAATGCGGCTAATCTCAA CCACGGAGCAGGCACTGGCAATCCAGCGACCAGCCTGTCGTAACGCGCAAGTT CGTGGCGGAACCGACTACTTTGGGTGTCCGTGTTTCTTTTATTTTACAAATG GCTGCTTATGGTGACAATTATTGATAGTTATCATAAAGCAAATTGGATTGGCC ATCCGGTGAGAATTTGATTATTAAATTACTCTCTTGTGGGATTGCTCCTTTG AAATCCTGTGCACTCACACCTATTGGAATTACCTCATTGTTGAGATATTATTA CCACT
80	CVA24 (RNA) Wherein "T" is "U"	ttaaaacagctctggggttggtccacccacagagggccacgtggcgccagta ctccggtattgcggtacccttgtagcctgttttacactccctcccccggttaa cttagaagtaaaacaaccaagttcaatagaaggggtacaaaccagtaccacc acgaacaagcacttctgtttcccggtgacgttgtagactgtaccacgggt tgaaaacaactgatccggttatccgctcttgtagcttcgagaagccagtatcac cttggaatcttcgatgcggttgcgctcagcactcaacccagagtgtagcttag gtcgatgagtcctggacaatcctcaccggcgacgggtgggtccaggtgctgcttggc ggcctacctgtggcccaaagccacaggacgctagtgtgtaacaaggtgtgaag agcctattgagctacgagagagtctccggccctgaatgcggtcaatcccaa ccacggagcaggtgatcacaagccagtgattagcctgtcgtaacgcgcaagtc tgtggcggaaccgactactttgggtgtccgtgtttcctttattttattatggc tgcttatggtgacaatcacagattgttatcataaagcgacttgattggccat ccagtgaaaataaaacacatcattacctgattgtgtggattactccgctgac tatctactcaccaggtcttattaaaggttattctgttgattaggtcacaaatttc aca
81	CVB6 (RNA) Wherein "T" is "U"	ttaaaacagcctgtgggttgtagccacccacagggccactggcgctagcac tctggtattgcggtacctttgtgcgctgttttacacccctcccccaagcta acttagaagcctgacatatacgggtcacaagccagccagtagcgcgaactgggt accgaccaagcacttctgttaccgggactgagtagtcaataagctgccaaagc ggctgaaggagaaaaagcttcgttaccgggccaattacttcgagaaacccagta acaccatgaaagttgcgtagcgtttcattcagcacaatccagtgtagatcag gtcgatgagtcaccgcattccccacaggcgactgtggcggtgggtgcttggc ggcctgcccatggggcaaacccatgggacgcttcaatactgacatgggtgcgaa gagtcattgagctaattggtagtcctccggccctgaatgcggtcaatccta actgcgagcagggcactcgcagaccagcgagcagcttgcgtcaatgggcaact ccgcagcggaaccgactactttgggtgtccgtgtttcctatttccctttacact ggctgcttatggtgacaattgagaaattgttaccatatagctattggagtggc catccggtgactaacagagcaataatatattgtttgttggttggcttggcact taattgaaggttgtaagacgctcaatctgatactacttctaataaagcaa a
82	EV89 (RNA) Wherein "T" is "U"	ttaaaacagcctctgggttgtagccacccacagggccactggcgctagcac actggtatcacggtacctttgtgcgctgtttatacacccctcccccttgta acttagaagaaacaacaaacgatcaatagtaggcttagcactccagctaagt ctcgatcaagcacttctgttaccgggaccgagtagtcaataggctgtaccac ggctgaaagagaaaaagcttcgttaccgggctatctacttcgggaaaccagta ccatcatgaagattgcaaagcgtttcgctcagcacaacccagtgtagatcag gtcgatgagtcactgcattccccacaggcgactgtggcagtggtgctgcttggc ggcctgcctatggggaaacccataggacgctttaataattgacatgggtgcgaag agtctactgagctaggtagtagtcctccggccctgaatgcggtcaatcccaa ccacggagcagggcgtcacactccagtgagtagcttgcgtcaatgcgaaagtc tgtggcggaaccgactactttgggtgtccgtgtttcctttattttatcatg gctgcttatggtgacaatctaagattgttatcatatagctattggattggcca

		tccggtgaattctagagatattatttacttggttggtgtttaccacattaa acattaccaattacagaactctcctctacatcctgttattgaataatagaaa agaaa
83	EV91 (RNA) Wherein "T" is "U"	ttaaacagctctaggggtgttcccaccctagaggccacgtggcggtagcac tctgggtatcacggtaacctttgtgcgcctgttttatatccctttcccatgtaa cttagaagttattaaacaaagtccaatagaaggggtacaaaccagtgccacc acgaacaagcacttctgtttcccgggtgaagctgtatagactgtctccacggg tgaaaatggcagatccgttaccgcgtttggtagtctcgagaaacctagatcac cttgggaatcttcgatgcgttgcgctcagcactcaacccagagtgtagcttag gtcgatgagtcctggacgatcctcactggtagacagtggtccaggtgcgttggc ggcctacctgtggtgaaaaccacaggacgctagtgtgaaacaaggtgtgaaga gtctattgagctaccaaagagtcctccggcccctgaatgcggctaataccta cacggagcaagtgccttcaaaccagtaggtagcttgcgtaacgcgcaagtct gtggcggaaccgactactttgggtgtccgtgtttcccttttattttatcatggc tgcttatggtgacaatctaagattgttatcatatagctattggattggccatc cggtagctaacagagatctcatatacctttttgttggttttactaaattaaat attaccacggttcaagacacttctccttatcatacactaaatagtgagaagag aaa
84	EVJ108 (RNA) Wherein "T" is "U"	ttaaaatagcctcagggttggttcccaccctgagggccacgtggcgtagtact ctgggtattacggtaacctttgtacgcctattttaccccccttccccttgtaactt agaagcaaagcaaaccagttcaatagtaagcaacacacacccagtggtgtgacg aacaagtaacttctgtttcccgggaggggtctgacggtaagctgtacccacggc tgaagtatgacctaccgttaaccggctacctacttcgagaagtctagtaatac cattgaagttttgttggcggttacgctcaacacactaccccggtgtgtagtttg gctgatgagtcacggcattccccacggggcgaccgtggccgtggtgcgttgcg gccaaccaagggggcgcaagctccttggacgtcacttaacagacatgggtgtgaa gaacctattgagctaggtagtagtctcctccggcccctgaatgcggctaataccta actccggagcacatcagtgcaaccacagcatttgggtgtgtgttaatacgaagt ctggagcggaaccgactactttgggtgtccgtgtttccctgttttaccttattt ggctgcttatggtgacaatttgatattgttaccatatagctgttggtattggcc atccggatttttgaaagagacccaaaacttctctctacttcagattcaagt gcgaagttttcccttttcatataattacttactaatttgaagtaccaaag
85	EV87 (RNA) Wherein "T" is "U"	ttaaaacagcctgtgggttggttcccaccacagggcccactggcgctagcac actggtatcacggtaacctttgtgcgcctgttttatacaccttcccctactgt gacttagaagcaaaacaatgatgaccaatagcaagcgcagcactccagctgtg tttcgggtcaagcacttctgtttcccggactgagtagtcaataggccactcacg tggccgaaggagaaaaacgctccgttaccgggccaattacttcgagaaaccag aacaccatagaggttgcgcagcgttttcgctccgcactccccagtgtagatca ggtcgatgagtcacgcggttccccacggggcgaccgtggcggtggctgcgttgg cggcctgcccattggggttaaccatgggacgcttcaatactgacatgggtgcgaa gagtcattgagctaattggtagtcctccggcccctgaatgcggctaataccta actgcggagcagataccacacgctccagtgggcagtcgtcgttaacgggtaact ctgcagcggaaccgactactttgggtgtccgtgtttcccttttattcttattt ggctgcttatggtgacaattgaaagattgttaccatatagctattggattggc catccgggtgtctaataagagcaattatattcctgtttgttggttttataccact gaatatcacagtttacaagaccctcaattacatcctacgtttaaacaagcaaa a
86	EchoV31 (RNA) Wherein "T" is "U"	ttaaaacagcctgtgggttggttcccaccacagggcccactggcgctagcaca ctgggtatcggtggtacctttgtgcgcctgttttatacaccttcccatttgtaa cttagaagtaagcaatattgatcaatagcaagcatggcacaccagccgtgtt cagatcaagcacttctgtaaccccgactgagtagtcaataggctgccaaagcg gctgaaggagaaaaacgcttcgttaaccgggccaactacttcgagaagccagtat caccatgaaagttgcagagcgtttcgtcagcactcccccggtgtagattagg tcgatgagtcacgcacatccccacaggcgactgtggcggtggctgcgttggcg gcctacctacggggcaaccgtaggacgctctaatacagacatgggtgagaaga gcctattgagctagttggtagtcctccggcccctgaatgcggataataccta tgccgagcatgtgctcgcaatccagcgagaggcatgtcgtaacgggcaactct gcagcggaaccgactactttgggtgtccgtgtttcccttttattccctgtttgg ctgcttatggtgacaattgaaagattgttgccatatagctattgggtcggcca

		cctgggtatctaacagagcaattatttacttgttgggttttgtacccttaa atctcaagggttcttgaaacccttaactacacattgaaactcaattcattgaa
87	EV68 (RNA) Wherein "T" is "U"	ttaaaacagctctggggttgttcccactcaagggccacgtggcggttagtac tctgggtatctcggtacctttgtacgcctgttttaattccctccccaacgtaac ttagaagcttttaaaccaaagctcaataggtggagcgcaaacaccagcgctctta tgagcaagcacttctgtctccccgggtgtggttgtatagactgtccccacgggt gaaaacaacttatccgttaaccgctatagtagtctcgagaaacctagattgcc ttcggagtgttgatgcgttgcgctcagcacactaaccgctgtgtagcttgggt cgatgagtctggacgtacccactggcgacagtgggtccaggtgcgttggcgg cctactcatggtgaaaaccatgagacgctagacatgaacaagggtgtgaagagt ctattgagctgctatagagtcctccggccctgaatgcggttaatcctaacca tggagcaagtgtcacaaccagtgagttacttgtcgtaacgcgcaagtcctgt ggcggaaaccgactactttgggtgtccgtgtttcactttttacttttatgactg ctaattggtgacaatttaatatgtttaccatttggcttgcgaattgatcacat aagatctatagttttgttccactgatttgccttgaaataatctcacctcaaac ctccagtacataaacatttaaagagtttaaacttatttataaca
88	EV92 (RNA) Wherein "T" is "U"	tttaaacagcctgtgggttgtacccacccacagggccacgcggcggttagta ctctgggtatcacggtacctttgtatgectgttttatctccctcccccgtaac gttagaagaatgaacaaaagacaattagcaaatccaccaaccaggtgggttt tcgtcaagcacttctgtttccccggacctagtaccaataagctgtgccacgg ctgaaggggaaaacgttcgttatccggctacctaacttcgagaagcctagtacc accaatgattgtctcaagtgttacgctcagcacaaacccagtgtagttcaggt cgatgagtcaccgaattccccacggggcagccgtgtcggtggctgcgttggcgg cctgcctgcgagtttcaactcgcaggacgcttgattcatgacatggtgcgaaga gcctattgagctaggcagtagtcctccggccctgaatgcggctaatcctaacc cacggagccatgcgtcacaaccagtggtgcgtcatggtcgtaacgagcaattct gtggcgaaccgactactttgggtgtccgtgtttcctgttttatttaccttgg ctgcttatggtgacaatctagagttgtcatcatatagcgattggattggccat ccgatattactttgtgtgatttatctcttgcctcgtaagcaattttaaatagt ttatagcatcatt
89	EVG2 (RNA) Wherein "T" is "U"	ctaaaacagcctgcgggttgtacccgccgcagggccactgggcgctagtac tctgggtacgctagtagactagcacgttagtgcttttgtacgccagtacctttg tacgcctgttttattttcccttccccacatgtaactttagaagtttagaaaca aacaatgaccaataggagtcacagcatccagttggattgcggtcaagtagtctt gtttccccggacctagtagtgataggtgtacccacggccgaagatgaacccg tccgttatccggctacctaacttcgggaagcctagtaaccttctgaagtcctg aggcgtttcgcctcagcacgaccccggtgtagatcgggtgagtggttctcgca taccacacgggtgaccgtggcggaggccgcgttggcggcccgctatggcgaa agccataggacgcctcttagatgacaggggtgtgaagagcctactgagctgggt agtagtcctccggccctgaatgcggctaatcctaaccacggagcgtccaccg gcaaaccggtcgggaggggcgtcgtaacgggttaactctgtggcgaaccgacta ctttgggtgtccgtgtttccttttgatcctattttggctgcttatggtgacaa cgataagttgttatcataaagcttttgggttggccacctggaaaaagttatca gtgtttgatattgttcggctctcacgccttccaacaaaacaggccttacttct tttaattgcattttatccatcggaggttattacagatttccatctgaagcttc ttctcacagtagcagtaaa
90	CVA2 (RNA) Wherein "T" is "U"	ttaaaacagcctgtgggttgcacccacccacagggccactgggcgctagcac tctgggtatcacgatacctttgtgcgcctgttttatatccccaccccgagttaa cgttagaagttacgcaaccgcgacatagtaggtgtagcactccagctgcat cgagatcaagcacttctgtctccccggaccgagtagtcaatagactgctaacgc ggttgaaggagaaaacgttcgttacccggccaattacttcgagaagcccagta gtgccgtgaaagttgcggagtgttctgctcagcactcccccggttagatcag gctgatgagtcaccgcgatccccacaggtgactgtggcgggtggtgcgttggc ggcctgcctatggggcaaccataggacgctctaatacagacatggtgcgaag agcctattgagctaatggtagtcctccggccctgaatgcggctaatcctaa ctgcggagcacatgccctcaaaccagggggtggtgtgtcgtaacgggttaactc tgcagcggaaaccgactactttgggtgtccgtgtttctttttattcttataatg gctgcttatggtgacaattaaagaattgttaccatatagctattggattggcc

		atccggtgactaacaatatcgctcatataaccagtttggtggtttgttccctta tcacatacagctcataacaccctcttatatttactacaattgaatagcaagaa
91	CVA6 (RNA) Wherein "T" is "U"	ttaaaacagcctgtgggttgacccactcacagggcccactgggcgctagcac actgatttcccgaatccttggtgcgctgttttatatccccccccatgcgc aacttagaagcaatctacaccttcgatcaatagcaggcgtggcgcgccagcca tgtctagatcaagcacttctgtttccccggactgagtatcaataaactgctca cgcggttgaaggagaaaatgttcgttaccgggctaactacttcgagaaacct gtagcaccatgaaaattgcagagcgtttcgtcagcgcttccccgcgtagat caggctgatgagtcactgcattcctcacgggacgcgtggcagtggtgcgtt ggcggtgccccatggggttaacccatgggacgctctaataatggacatggtgtg aagagtctattgagctagttagtagtcctccggccccctgaatgcggctaacc taactgcggagcacataaccccaaccagggggcggtgtgcgtaacgggcaa ctctgcagcggaaccgactactttgggtgtccgtgttcccttttattcttata ttggctgcttatggtgacaattgaaagattgttaccatatagctattggattg gccaatccggtgaataacagagccttgatataacctttttaggggtttatacca cttactcttcgcgttggtgagactctaaagtacattctaatttgaacactag aaa

[0175] In some aspects, inclusion of an IRES permits the translation of one or more open reading frames from a circular RNA (e.g., open reading frames comprised in the exonic sequences). The IRES element attracts a eukaryotic ribosomal translation initiation complex and promotes translation initiation. See, e.g., Kaufman et al., Nuc. Acids Res. (1991) 19:4485-4490; Gurtu et al., Biochem. Biophys. Res. Comm. (1996) 229:295-298; Rees et al., BioTechniques (1996) 20: 102-110; Kobayashi et al., BioTechniques (1996) 21:399-402; and Mosser et al., BioTechniques (1997) 22:150-161). A multitude of IRES sequences are available and include sequences derived from a wide variety of viruses, such as from leader sequences of picornaviruses such as the encephalomyocarditis virus (EMCV) UTR (Jang et al., J. Virol. (1989) 63: 1651-1660), the polio leader sequence, the hepatitis A virus leader, the hepatitis C virus IRES, human rhinovirus type 2 IRES (Dobrikova et al., Proc. Natl. Acad. Sci. (2003) 100(25): 15125- 15130), an IRES element from the foot and mouth disease virus (Ramesh et al., Nucl. Acid Res. (1996) 24:2697-2700), a giardiavirus IRES (Garlapati et al., J. Biol. Chem. (2004) 279(5):3389-3397), and the like.

[0176] In some aspects, the IRES is an IRES sequence of Taura syndrome virus, Triatoma virus, Theiler's encephalomyelitis virus, Simian Virus 40, Solenopsis invicta virus 1, Rhopalosiphum padi virus, Reticuloendotheliosis virus, Human poliovirus 1, Plautia stali intestine virus, Kashmir bee virus, Human rhinovirus 2, Homalodisca coagulata virus- 1, Human Immunodeficiency Virus type 1, , Himetobi P virus, Hepatitis C virus, Hepatitis A virus, Hepatitis GB virus , Foot and mouth disease virus, Human enterovirus 71, Equine rhinitis virus, Ectropis obliqua picorna-like virus, Encephalomyocarditis virus, Drosophila C Virus, Human coxsackievirus B3, Crucifer tobamovirus, Cricket paralysis virus, Bovine viral diarrhea virus 1, Black Queen Cell Virus, Aphid lethal paralysis virus, Avian encephalomyelitis virus, Acute bee

paralysis virus, Hibiscus chlorotic ringspot virus, Classical swine fever virus, Human FGF2, Human SFTPA1, Human AML1/RUNX1, Drosophila antennapedia, Human AQP4, Human AT1R, Human BAG-1, Human BCL2, Human BiP, Human c-IAP1, Human c-myc, Human eIF4G, Mouse NDST4L, Human LEF1, Mouse HIF1 alpha, Human n.myc, Mouse Gtx, Human p27kipl, Human PDGF2/c-sis, Human p53, Human Pim-1, Mouse Rbm3, Drosophila reaper, Canine Scamper, Drosophila Ubx, Human UNR, Mouse UtrA, Human VEGF-A, Human XIAP, Drosophila hairless, S. cerevisiae TFIID, S. cerevisiae YAP1, tobacco etch virus, turnip crinkle virus, EMCV-A, EMCV-B, EMCV-Bf, EMCV-Cf, EMCV pEC9, Picobirnavirus, HCV QC64, Human Cosavirus E/D, Human Cosavirus F, Human Cosavirus JMY, Rhinovirus NAT001, HRV14, HRV89, HRVC-02, HRV-A21, Salivirus A SHI, Salivirus FHB, Salivirus NG-J1, Human Parechovirus 1, Crohivirus B, Yc-3, Rosavirus M-7, Shanbavirus A, Pasivirus A, Pasivirus A2, Echovirus E14, Human Parechovirus 5, Aichi Virus, Hepatitis A Virus HA16, Phopivirus, CVA10, Enterovirus C, Enterovirus D, Enterovirus J, Human Pegivirus 2, GBV-C GT110, GBV-C K1737, GBV-C Iowa, Pegivirus A 1220, Pasivirus A 3, Sapelovirus, Rosavirus B, Bakunsa Virus, Tremovirus A, Swine Pasivirus 1, PLV-CHN, Pasivirus A, Sicinivirus, Hepacivirus K, Hepacivirus A, BVDV1, Border Disease Virus, BVDV2, CSFV-PK15C, SF573 Dicistrovirus, Hubei Picorna-like Virus, CRPV, Salivirus A BN5, Salivirus A BN2, Salivirus A 02394, Salivirus A GUT, Salivirus A CH, Salivirus A SZ1, Salivirus FHB, CVB3, CVB1, Echovirus 7, CVB5, EVA71, CVA3, CVA12, EV24 or an aptamer to eIF4G.

II.B.8. UTRs and Kozak Sequence

[0177] In some aspects, genetic constructs disclosed herein further comprises a UTR. For example, in some aspects, a pre-circRNA or circRNA produced as disclosed herein comprises a payload sequence, wherein the payload sequence comprises a UTR. In some aspects, the UTR is a 5'-UTR. In some aspects, the UTR is a 3'-UTR. In some aspects, the UTR comprises both a 5'-UTR and a 3'-UTR.

[0178] Untranslated regions (UTRs) of a gene are transcribed but not translated. The 5'-UTR starts at the transcription start site and continues to the start codon but does not include the start codon; whereas, the 3'-UTR starts immediately following the stop codon and continues until the transcriptional termination signal. There is growing body of evidence about the regulatory roles played by the UTRs in terms of stability of the nucleic acid molecule and translation. Accordingly, where a pre-circRNA or circRNA produced as disclosed herein comprises a UTR, the stability of the payload sequence is increased, *e.g.*, as compared to a sequence without the UTR. As described

herein, in some aspects, increased stability results in increased expression of the encoded protein. Natural 5'-UTRs bear features which play roles in translation initiation. They harbor signatures like Kozak sequences which are commonly known to be involved in the process by which the ribosome initiates translation of many genes. Kozak sequences have the consensus CCR(A/G)CCAUGG, where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another 'G'. 5'-UTR also have been known to form secondary structures which are involved in elongation factor binding.

[0179] 5'-UTR secondary structures involved in elongation factor binding can interact with other RNA binding molecules in the 5'-UTR or 3'-UTR to regulate gene expression. For example, the elongation factor EIF4A2 binding to a secondarily structured element in the 5'-UTR is necessary for microRNA mediated repression (Meijer H A et al., Science, 2013, 340, 82-85, herein incorporated by reference in its entirety). The different secondary structures in the 5'-UTR can be incorporated into the flanking region to either stabilize or selectively destabilize mRNAs in specific tissues or cells.

[0180] By engineering the features typically found in abundantly expressed genes of specific target organs, one can enhance the stability and protein production of a nucleic acid sequence (*e.g.*, payload sequence of a synthetic circuit provided herein). For example, introduction of 5'-UTR of liver-expressed mRNA, such as albumin, serum amyloid A, Apolipoprotein A/B/E, transferrin, alpha fetoprotein, erythropoietin, or Factor VIII, could be used to enhance expression of a nucleic acid molecule, such as a mRNA, in hepatic cell lines or liver. Likewise, use of 5'-UTR from other tissue-specific mRNA to improve expression in that tissue is possible—for muscle (MyoD, Myosin, Myoglobin, Myogenin, Herculin), for endothelial cells (Tie-1, CD36), for myeloid cells (C/EBP, AML1, G-CSF, GM-CSF, CD11b, MSR, Fr-1, i-NOS), for leukocytes (CD45, CD18), for adipose tissue (CD36, GLUT4, ACRP30, adiponectin) and for lung epithelial cells (SP-A/B/C/D).

[0181] Other non-UTR sequences can also be incorporated into the UTRs (*e.g.*, 5'-UTR and/or 3'-UTR). For example, introns or portions of introns sequences can be incorporated into the flanking regions of a nucleic acid sequence (*e.g.*, payload sequence of a synthetic circuit provided herein).

[0182] In some aspects, one or more nucleotides within a UTR (*e.g.*, 5'-UTR and/or 3'-UTR) can be mutated, replaced and/or removed. For example, one or more nucleotides upstream of the start codon can be replaced with another nucleotide. The nucleotide or nucleotides to be

replaced can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60 or more than 60 nucleotides upstream of the start codon. As another example, one or more nucleotides upstream of the start codon can be removed from the UTR. In some aspects, the genetic constructs disclosed herein comprise a Kozak sequence.

[0183] 3'-UTRs are known to have stretches of adenosines and uridines embedded in them. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995): Class I AREs contain several dispersed copies of an AUUUA motif within U-rich regions. C-Myc and MyoD contain class I AREs. Class II AREs possess two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Molecules containing this type of AREs include GM-CSF and TNF- α . Class III AREs are less well defined. These U rich regions do not contain an AUUUA motif. c-Jun and Myogenin are two well-studied examples of this class. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3'-UTR of nucleic acid molecules can lead to HuR binding and thus, stabilization of the message *in vivo*.

[0184] In some aspects, introduction, removal, or modification of 3'-UTR AU rich elements (AREs) can be used to modulate the stability of a nucleic acid sequence. When engineering specific genetic constructs disclosed herein, one or more copies of an ARE can be introduced to make the nucleic acid sequence less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein.

[0185] In some aspects, the genetic constructs disclosed herein comprise a 3' UTR. In some aspects, the 3' UTR is from human beta globin, human alpha globin *Xenopus* beta globin, *Xenopus* alpha globin, human prolactin, human GAP-43, human eEF1 α , human Tau, human TNF α , dengue virus, hantavirus small mRNA, bunyavirus small mRNA, turnip yellow mosaic virus, hepatitis C virus, rubella virus, tobacco mosaic virus, human IL-8, human actin, human GAPDH, human tubulin, hibiscus chlorotic ringspot virus, woodchuck hepatitis virus post translationally regulated element, sindbis virus, turnip crinkle virus, tobacco etch virus, or Venezuelan equine encephalitis virus.

[0186] In some aspects, the genetic constructs disclosed herein comprise a 5' UTR. In some aspects, the 5' UTR is from human beta globin, *Xenopus laevis* beta globin, human alpha globin, *Xenopus laevis* alpha globin, rubella virus, tobacco mosaic virus, mouse Gtx, dengue virus, heat shock protein 70kDa protein 1 A, tobacco alcohol dehydrogenase, tobacco etch virus, turnip crinkle virus, or the adenovirus tripartite leader

II.B.9. Spacers

[0187] In some aspects, the genetic constructs disclosed herein comprise a spacer sequence. In some aspects, the genetic constructs disclosed herein comprise a spacer sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity with a nucleotide sequence of SEQ ID NOs: 31-32 or 33-34. In some aspects, the genetic constructs disclosed herein comprise a spacer consisting of a nucleotide sequence of SEQ ID NOs: 31-32 or 33-34.

[0188] Non-limiting examples of spacer sequences that are useful for the present genetic constructs are shown in **Table 8**.

Table 8. Exemplary spacers

SEQ ID NO	DESCRIPTION	SEQUENCE
31	5' spacer (all) (DNA)	AGCTGAGACGGCGCCGATGGATAGCGGACTTTCGGTCAACCACAATTCCC CACGAGACAGGTCCTGCCGT
32	3' spacer (all) (DNA)	CTTCTCATGACTGAACTCGCGAGGGTCGTGATGTCGGTTCCTTCAAAGGT TAAAGAACAAAGGCTTACTG
33	5' spacer (all) (RNA)	AGCUGAGACGGCGCCGAUGGAUAGCGGACUUUCGGUCAACCACAAUCCCC CACGAGACAGGUCCUGCCGU
34	3' spacer (all) (RNA)	CUUCUCAUGACUGAACUCGCGAGGGUCGUGAUGUCGGUCCUCAAAGGU UAAAGAACAAAGGCUUACUG

[0189] In some aspects, the genetic constructs disclosed herein comprise a first (5') and/or a second (3') spacer. In some aspects, including a spacer between the 3' intron fragment and the IRES may conserve secondary structures in those regions by preventing them from interacting, thus increasing splicing efficiency. In some aspects, the first (between 3' group fragment and IRES) and second (between the expression sequence and 5' intron fragment) spacers comprise additional base pairing regions that are predicted to base pair with each other and not to the first and second duplex forming regions. In some aspects, such spacer base pairing brings the intron fragments in close proximity to each other, further increasing splicing efficiency. In some aspects, the combination of base pairing between the first and second duplex forming regions, and separately, base pairing between the first and second spacers, promotes the formation of a splicing bubble

containing the intron fragments flanked by adjacent regions of base pairing. In some aspects, a spacer is a contiguous sequence with one or more of the following qualities: 1) predicted to avoid interfering with proximal structures, for example, the IRES, expression sequence, or intron; 2) is at least 7 nt long and no longer than 100 nt; 3) is located after and adjacent to the 3' intron fragment and/or before and adjacent to the 5' intron fragment; and 4) contains one or more of the following: a) an unstructured region at least 5 nt long, b) a region of base pairing at least 5 nt long to a distal sequence, including another spacer, and c) a structured region at least 7 nt long limited in scope to the sequence of the spacer. Spacers may have several regions, including an unstructured region, a base pairing region, a hairpin/structured region, and combinations thereof. In some aspects, the spacer has a structured region with high GC content. In some aspects, a region within a spacer base pairs with another region within the same spacer. In some aspects, a region within a spacer base pairs with a region within another spacer. In some aspects, a spacer comprises one or more hairpin structures. In some aspects, a spacer comprises one or more hairpin structures with a stem of 4 to 12 nucleotides and a loop of 2 to 10 nucleotides. In some aspects, there is an additional spacer between the 3' intron fragment and the IRES. In some aspects, this additional spacer prevents the structured regions of the IRES from interfering with the folding of the 3' intron fragment or reduces the extent to which this occurs. In some aspects, the 5' spacer sequence is at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25 or 30 nucleotides in length. In some aspects, the 5' spacer sequence is no more than 100, 90, 80, 70, 60, 50, 45, 40, 35 or 30 nucleotides in length. In some aspects the 5' spacer sequence is between 5 and 50, 10 and 50, 20 and 50, 20 and 40, and/or 25 and 35 nucleotides in length. In some aspects, the 5' spacer sequence is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 nucleotides in length. In some aspects, the 5' spacer sequence is a polyA sequence. In some aspects, the 5' spacer sequence is a poly AC sequence. In some aspects, a spacer comprises about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% poly AC content. In some aspects, a spacer comprises about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% polypyrimidine (C/T or C/U) content.

II.B.9. Poly(A)

[0190] In some aspects, the genetic constructs disclosed herein further comprises a polyA region. In some aspects, the polyA region is at least 15, 30, or 60 nucleotides long. In some aspects, the polyA sequence is removed upon circularization. Thus, an oligonucleotide hybridizing with the polyA sequence, such as a deoxythymine oligonucleotide (oligo(dT)) conjugated to a solid surface

(e.g., a resin), can be used to separate circular RNA from its precursor RNA. Other sequences can also be disposed 5' to the 3' intron fragment or 3' to the 5' intron fragment and a complementary sequence can similarly be used for circular RNA purification. In some aspects, the polyA region comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NOs: 42 or 43. In some aspects, the polyA region consists of SEQ ID NOs: 42 or 43.

[0191] Non-limiting examples of polyA region that are useful for the present genetic constructs are shown in **Table 9**.

Table 9. Exemplary polyA regions

SEQ ID NO	DESCRIPTION	SEQUENCE
42	5' PolyAC (DNA/RNA)	AAAAACAAAAACAAAAAACAAAAAACCAAAAAACAAAA CACA
43	3' PolyAC (DNA/RNA)	AAAAAACAAAAACAAAC

II.B.10. Excision Scars

[0192] In some aspects, the RNA or DNA genetic constructs disclosed herein further comprise one or more nucleotide sequences derived from the organism from which the intronic or the exonic sequences are derived. In some aspects, the one or more nucleotide sequences derived from the organism from which the intronic or the exonic sequences are derived are intronic sequences, exonic sequences, or any combination thereof. In some aspects, the one or more nucleotide sequences derived from the organism from which the intronic or the exonic sequences are derived are located in proximity of the splice sites. In some aspects, the one or more nucleotide sequences derived from the organism from which the intronic or the exonic sequences are derived are adjacent to the splice sites. In some aspects, the one or more nucleotide sequences derived from the organism from which the intronic or the exonic sequences are derived are not excised from the pre-circRNA during splicing. In these aspects, the one or more nucleotide sequences derived from the organism from which the intronic or the exonic sequences are derived are also herein referred to as pre-excision scars, when referring to those sequences as comprised in the pre-circRNAs or in the DNA constructs from which the pre-circRNAs can be transcribed, and as excision scars when referring to those sequences as comprised in the circRNAs.

II.B.11. Promoter

[0193] In some aspects, the RNA or DNA genetic constructs disclosed herein further comprises a promoter. In some aspects, the promoter is removed upon circularization. In some

aspects the promoter is T7, T3, or SP6. In some aspects, the promoter comprises a nucleotide sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to any one of SEQ ID NOs: 35-37 or 38-39. In some aspects, the promoter consists of a nucleotide sequence of SEQ ID NOs: 35-37, or 38-39 or 40.

II.C. Exemplary Constructs

[0194] Exemplary aspects of the genetic constructs disclosed herein are described in detail below. For simplicity, the exemplary genetic constructs are preferentially described with reference to the RNA genetic constructs (*i.e.*, pre-circRNAs). It is to be understood that the DNA genetic constructs, from which such pre-circRNAs can be transcribed, are also to be considered as encompassed by the following description. As such, for example, any RNA nucleotide sequence reciting uracil (U) it is to be understood as encompassing a corresponding DNA nucleotide sequence reciting thymine (T).

[0195] In some aspects, the genetic constructs capable of forming circular RNAs disclosed herein (*i.e.*, pre-circRNAs or DNA genetic constructs from which pre-circRNAs can be transcribed) comprise a formula of: 5'-P-H-A-3S-E-5S-B-H'-3', wherein: P comprises a promoter (e.g., T7, T3, SP6, etc.), H comprises a 5' homology arm, A comprises a 3' intronic sequence, 3S comprises a 3' splice site, E comprises an exonic sequence (*i.e.*, one or more exons or fragments thereof), 5S comprises a 5' splice site, B comprises a 5' intronic sequence, H' comprises a 3' homology arm, and wherein the promoter (P) is to be understood to be comprised in the DNA genetic construct, but not in the RNA genetic construct. In some aspects, the 5' and 3' intronic sequences are reverse complements of one another.

[0196] In some aspects, the intronic sequence comprises a nucleotide sequence derived from a group II intron. In some aspects, the group II intron comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NOs: 1-5 or 6-10. In some aspects, the group I intron consists of SEQ ID NOs: 1-5 or 6-10.

[0197] In some aspects, the 5' intronic sequence comprises a nucleotide sequence derived from a group II intron. In some aspects, the 5' intronic sequence comprises an intron-binding site (IBS). In some aspects, the 5' intronic sequence consists of an intron-binding site (IBS). In some aspects, the IBS comprises a nucleotide sequence of any one of TAAAAA (or UAAAAA), TGTTT (or UGUUU, or C. In some aspects, the IBS consists of any one of TAAAAA (or UAAAAA), TGTTT (or UGUUU, or C. In some aspects, the 5' intronic sequence comprises a nucleotide

sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NOs: 21-22 or 23-24. In some aspects, the 5' intronic sequence consists of SEQ ID NOs: 21-22 or 23-24.

[0198] In some aspects, the 3' intronic sequence comprises a nucleotide sequence derived from a group II intron. In some aspects, the 3' intronic sequence comprises a catalytic core (triad). In some aspects, the catalytic core (triad) comprises a sequence of AGC or CGC. In some aspects, the catalytic core (triad) consists of a sequence of AGC or CGC. In some aspects, the 3' intronic sequence comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 17-18 or 19-20. In some aspects, the 3' intronic sequence consists of SEQ ID NO: 17-18 or 19-20.

[0199] In some aspects, the 5' intronic sequence and the 3' intronic sequence together are less than a full intron.

[0200] In some aspects, the 5' intronic sequence comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NOs: 21 or 23. In some aspects, the 3' intronic sequence comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NOs: 17 or 19. In some aspects, the 5' intronic sequence comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NOs: 21 or 23, and the 3' intronic sequence comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NOs: 17 or 19.

[0201] In some aspects, the 5' intronic sequence comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NOs: 18 or 20. In some aspects, the 3' intronic sequence comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NOs: 22 or 24. In some

aspects, the 5' intronic sequence comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NOs: 18 or 20, and the 3' intronic sequence comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NOs: 22 or 24.

[0202] In some aspects, the 3' splice site comprises 2 nucleotides, wherein one nucleotide is upstream of the site of splice and one nucleotide is downstream of the site of splice. In some aspects, the nucleotide upstream of the site of splice is a G, and the nucleotide downstream of the site of splice is a N, wherein N is any one of A, C, G, T, or U. In some aspects, N is C. In some aspects, the 3' splice site comprises a sequence of formula 5'-G-N-3', wherein the site of splice is between G and N, and wherein N is any one of A, C, G, T, or U. In some aspects, the 3' splice site comprises a sequence of formula 5'-G-C-3'.

[0203] In some aspects, the 3' splice site comprises 2 nucleotides, wherein one nucleotide is upstream of the site of splice and one nucleotide is downstream of the site of splice. In some aspects, the nucleotide upstream of the site of splice is a T or U, and the nucleotide downstream of the site of splice is a N, wherein N is any one of A, C, G, T, or U. In some aspects, N is C. In some aspects, N is A. In some aspects, the 3' splice site comprises a sequence of formula 5'-T(or U)-N-3', wherein the site of splice is between T or U and N, and wherein N is any one of A, C, G, T, or U. In some aspects, the 3' splice site comprises a sequence of formula 5'-T-C-3'. In some aspects, the 3' splice site comprises a sequence of formula 5'-T-A-3'.

[0204] In some aspects, the 5' splice site comprises 2 nucleotides, wherein one nucleotide is upstream of the site of splice and one nucleotide is downstream of the site of splice. In some aspects the nucleotide upstream of the site of splice is a U or a T, and the nucleotide downstream of the site of splice is a N, wherein N is any one of A, C, G, T, or U. In some aspects, N is A. In some aspects, the 5' splice site comprises a sequence of formula 5'-U-N-3', wherein the site of splice is between U and N, and wherein N is any one of A, C, G, T, or U. In some aspects, the 5' splice site comprises a sequence of formula 5'-U-A-3'.

[0205] In some aspects, the 5' splice site comprises 2 nucleotides, wherein one nucleotide is upstream of the site of splice and one nucleotide is downstream of the site of splice. In some aspects the nucleotide upstream of the site of splice is a A, and the nucleotide downstream of the site of splice is a N, wherein N is any one of A, C, G, T, or U. In some aspects, N is G. In some

aspects, the 5' splice site comprises a sequence of formula 5'-A-N-3', wherein the site of splice is between A and N, and wherein N is any one of A, C, G, T, or U. In some aspects, the 5' splice site comprises a sequence of formula 5'-A-G-3'.

[0206] In some aspects, the 3' splice site comprises a nucleotide sequence of Atccaact[^]CA. In some aspects, the 5' splice site comprises a nucleotide sequence of Nttaaaaa[^]GTGCG. In some aspects, the 3' splice site comprises a nucleotide sequence of Atccaact[^]CA, and the 5' splice site comprises a nucleotide sequence of Nttaaaaa[^]GTGCG.

[0207] In some aspects, the 5' homology arm comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NO: 27 or 29. In some aspects, the 3' homology arm comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NO: 28 or 30.

[0208] In some aspects, the 5' splice site is at the border between the 5' intronic sequence and the exonic sequence. In some aspects, the 3' splice site is at the border between the 3' intronic sequence and the exonic sequence. In some aspects, the intronic sequences are fully excised from the RNA genetic constructs (*i.e.*, pre-circRNAs). In some aspects, the resulting circRNAs do not comprise any intronic sequence. In some aspects, the resulting circRNAs do not comprise one or more intronic sequence residue. In some aspects, the resulting circRNAs do not comprise one or more nucleotide sequences derived from the organism from which the 5' intronic sequence and the 3' intronic sequence are derived, *i.e.*, the resulting circRNAs does not comprise "excision scars."

[0209] In some aspects, the pre-circRNA is capable of forming a circular RNA. In some aspects, the pre-circRNA is capable of forming a circular RNA in the presence of an exogenous G. In some aspects, the pre-circRNA is capable of forming a circular RNA, wherein the circular RNA does not comprise any sequence derived from the organism from which the 5' intronic sequence and the 3' intronic sequence are derived ("excision scar").

[0210] In some aspects, the genetic constructs comprises from 5' to 3' a 5' homology arm, a 3' intronic sequence, a 3' splice site, a polyA, an IRES, one or more exons, a polyA, a 5' intronic sequence comprising an intron-binding site (IBS), a 5' splice site, and H' comprises a 3' homology arm, wherein the 3' intronic sequence comprises a catalytic core (triad), and wherein the RNA construct is capable of forming a circular RNA that does not contain any sequence derived from the genome from which the 5' intronic sequence and the 3' intronic sequence are derived ("excision

scar”). In some aspects, the genetic constructs disclosed herein comprise a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NOs: 102 or 103, wherein "N" represents any one of A, T (or U), C, or G; and wherein "n" represents any integer. In some aspects, "(N)n" comprises a nucleotide sequence of an exonic sequences. In some aspects, "(N)n" comprises a nucleotide sequence of an exonic sequences and a nucleotide sequence of an IRES. Non-limiting examples of constructs of the present disclosure are shown in Table 10.

Table 10. Exemplary constructs

SEQ ID NO	DESCRIPTION	SEQUENCE
102	Pylaiella littoralis 1 Plasmid (DNA)	CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTCTGCGCGTAATCTGCTGC TTGCAAACAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCA ACTCTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTA GTGTAGCCGTAGTTAGGCCACCACCTCAAGAACTCTGTAGCACCGCCTACATACCTCGCT CTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTG GACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTTCGGGCTGAACGGGGGGTTCGTGC ACACAGCCAGCTTGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTA TGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGG GTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGT CCTGTGCGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTGTGATGCTCGTCAGGGGGG CGGAGCCTATGGAAAAACGCCAGCAACGCGGCCCTTTTACGGTTCCTGGCCTTTTGCTGG CCTTTTGCTCACATGTTCTTCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACC GCCTTTGAGTGAGCTGATACCGCTCGCCGAGCCGAAACGACCGAGCGCAGCGAGTCAGTG AGCGAGGAAGCGGAGGAGCGCCCAATACGCAAACCGCCTCTCCCGCGCGTTCGGCCGATT CATTAATGCAGCTGGCAGCACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCA ATTAATACGCGTACCGCTAGCCAGGAAGAGTTTGTAGAAACGCAAAAAGGCCATCCGTCA GGATGGCCTTCTGCTTAGTTTGTATGCCTGGCAGTTTATGGCGGGCGTCTGCCGCCACC CTCCGGGCGGTTGCTTCACAACGTTCAAATCCGCTCCCGGCGGATTTGTCTACTCAGGA GAGCGTTCAACGACAAACAACAGATAAAACGAAAGGCCAGTCTTCCGACTGAGCCTTTC GTTTTATTTGATGCCTGGCAGTTCCTACTCTCGCGTTAACGCTAGCATGGATGTTTTCC CAGTCAGACGTTGTAAACGACGGCCAGTCTTAAGCTCGGCTCTTCGCACTAATACGAC TCACTATAGggAGACCTCGGGTGTGTGGAGATCTCTGGCgtcccttcctgggaacttgg taagcccaatagactccctctgggaaccagaggaggagtaagagcaatcttacttatcgt tctagtgggtaaaagacataataggaagcaaatgcttgggtgtaacgatcgagatagaat ctggttgatttaagctgaaaggctgcagacttattaaatgggtgttctgctgtatggcggt cgcgccctaagcagcagaatgcgtgagccgtgtgcgatgaaagtgcgaagcacggttctga tggggggaaaacgagagatcgctctacctAtccaactCacaAGCTGAGACGGCGCCGATGG ATAGCGGACTTTCGGTCAACCACAATTCCCCACGAGACAGGTCCTGCCGTAAAAACAAAA AACAAAAAACAACAAAAAACCACAAAAACAAAAACACA (N) nAAAAAACAAAAACAA AACCTTCTCATGACTGAACTCGCGAGGGTCGTGATGTCGGTTCCTTCAAAGGTTAAAGAA CAAAGGCTTACTGactgtttattaaaaaGTGCGACAAGAAGTTCAGGAAGGGATTGAGGT GAAAGTCCCTCCCGAATCGTTCATGGGAGAGTCTATCCAGACTTGCGTAGCGAGTAAT CGCTAGGTGAGAAGCTCTGGAGACAAATGTACCTGCCCTTCAATTGGAGGTGCCAGGGCTA GGCTTTGTTCCTATGGCTAGCGAAAGCGAATACAGGTTATTAGGCGTCGTGAAAAACAAAA ctctttcgacataagggagaggctcgtagatgagttaccctccttttatggggtaactg aacgatcaatatctaccggcgtagactgtgagcctaaaggaacgaatgaaagtGCCAGA GATCTCCAACAACCATAGAAGAGCATCCCATGGTCATAGCTGTTTCCTGGCAGCTCTGG CCCGTGTCTCAAATCTCTGATGTTACATTGCACAAGATAAAAAATATCATCATGAACA ATAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGG GAAACGTCGAGGCCGCGATTAAATTTCCAACATGGATGCTGATTTATATGGGTATAAATGG

		GCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGAT GCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAG ATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATC CGTACTCCTGATGATGCATGGTTACTCACCCTGCGATCCCCGGAAAAACAGCATTCAG GTATTAGAAGAATATCCTGATTAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTTCTG CGCCGGTTGCATTTCGATTCTGTGTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGT CTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGAC GAGCGTAATGGCTGGCTGTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTC TCACCGGATTTCAGTCGTCATCATGGTGATTTCTCACTTGATAACCTTATTTTGGACGAG GGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGAT CTTGCCATCCTATGGAACCTGCCTCGGTGAGTTTCTCCTTCATTACAGAAACGGCTTTTT CAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGCT GAGTTTTTCTAATCAGAAATTGGTTAATTGGTTGTAACACTGGCAGAGCATTTACGCTGACT TGACGGGACGGCGCAAGCTCATGACCAAAATCCCTTAACGTGAGTTACGCGTCGTTCAC TGAGCGTCAGAC
103	Pylaiell a littoral is 2 Plasmid (DNA)	CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGC TTGCAAAACAAAAAACACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCA ACTCTTTTTCCGAAGGTAACCTGGCTTACGAGAGCGCAGATACCAAATACGTTCTTCTA GTGTAGCCGTAGTTAGGCCACCCTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCT CTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTG GACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGGCTGAACGGGGGGTTTCGTGC ACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTA TGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGG GTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAACGCCCTGGTATCTTTATAGT CCTGTGCGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGG CGGAGCCTATGGAACACGCCAGCAACGCGGCTTTTTACGGTTCTGGCCTTTTGCTGG CCTTTTGCTCACATGTTCTTCTGCGTTATCCCCGATTCTGTGGATAACCGTATTACC GCCTTTGAGTGAGCTGATACCGCTCGCCGACCGCAACGACCGAGCGCAGCGAGTCAGTG AGCGAGGAAGCGGAGGAGCGCCCAATACGCAAAACCGCTCTCCCGCGCGTTGGCCGATG CATTAATGCAGCTGGCAGCAGAGGTTTCCCGACTGGAAGCGGGCAGTGAGCGCAACGCA ATTAATACCGGTACCGCTAGCCAGGAAGAGTTTGTAGAAACGCAAAAAGGCCATCCGTCA GGATGGCCTTCTGCTTAGTTTGTATGCCTGGCAGTTTATGGCGGGCGTCTGCCCGCCACC CTCCGGGCGGTTGCTTCAACAGTTCAAATCCGCTCCCGCGGATTTGTCTACTCAGGA GAGCGTTCACCGACAAACAACAGATAAAACGAAAGGCCAGTCTTCCGACTGAGCCTTTC GTTTTATTTGATGCCTGGCAGTTCCTACTCTCGCGTTAACGCTAGCATGGATGTTTTCC CAGTCACGACGTTGTAAAACGACGGCCAGTCTTAAGCTCGGCTCTTCGCATTAATACGAC TCACTATAGggAGACCTCGGGTTGTTGGAGATCTCTGGCcgcgccaaagcagcagaatg cgtgagccgtgtgcatgaaagtcgcaagcacggttctgatgggggaaaaacgagagatc gtctacctAtccaactCacaAGCTGAGACGGCGCCGATGGATAGCGGACTTTCCGTCAAC CACAATTTCCACGAGACAGGTCTGCGGTAAAAACAAAAACAAAAACAAAAACAAAAA AACCAAAAAACAAAACACA (N) nAAAAACAAAAACAAAACCTTCTCATGACTGAACT CGCGAGGGTCGTGATGTCGGTTCTTCAAAGGTTAAAGAACAAAGGCTTACTGactgttt attaaaaaGTGCGACAAGAAGTTCAGGAAGGGATTGAGGTGAAAGTCTCTCTCCCGAATC GTTTCATGGGAGAGTCTATCCAGACTTGCCTAGCGAGTAATCGCTAGGTGAGAAAGCTCTGG AGACAATGTACCTGCCCTTCAATTGGAGGTGCCAGGGCTAGGCTTTGTTCCATAGGCTAG CGAAAGCGAATACAGGTTATTAGGCGTCGTGAAAACAAAActctttcgacataagggaga ggctcgtagatgagttaccctccttttatggggtaactgaacgatcaatatctaccggc gtagactgtgagcctaagggaacgaatgaaagtgtcccttcctgggaacttggttaagcc caatagactccctctgggaaaccagaggaggagtaagagcaatcttacttatcgtctagt gggtaaaagacataaataggaagcaaatgcttggttgtaacgatcgagatagaatctgttg atttaagctgaaaggctgcagacttattaaatgggtgttctgctgtatggcgcttGCCAGA GATCTCCAACAACCATAAGAAGAGCATCCCATGGTCATAGCTGTTTCTTGGCAGCTCTGG CCCGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAAATATATCATCATGAACA ATAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGG GAAACGTCGAGGCCGCGATTAAATTTCAACATGGATGCTGATTTATATGGGTATAAATGG GCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGAT GCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAG ATGGTCAGACTAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATC CGTACTCCTGATGATGCATGGTTACTCACCCTGCGATCCCCGGAAAAACAGCATTCAG

		GTATTAGAAGAATATCCTGATTCAGGTGAAAAATATTGTTGATGCGCTGGCAGTGTTCCTG CGCCGGTTGCATTCGATTCCTGTTTGTAAATTGTCCTTTTAAACAGCGATCGCGTATTTTCGT CTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGTTGATGCGAGTGATTTTGATGAC GAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCT TCACCGGATTCAAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTGACGAG GGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGAT CTTGCCATCCTATGGAAGTCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTT CAAAAATATGGTATTGATAATCCTGATATGAATAAATGCAGTTTCAGTTGATGCTCGAT GAGTTTTTCTAATCAGAAATTGGTTAATTGGTTGTAACACTGGCAGCATTTACGCTGACT TGACGGGACGGCGCAAGCTCATGACCAAAATCCCTTAACGTGAGTTACGCGTCGTTCCAC TGAGCGTCAGAC
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[0211] In some aspects, the genetic constructs disclosed herein comprise a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NOs: 104 or 105, wherein "N" represents any one of A, T (or U), C, or G; and wherein "n" represents any integer. In some aspects, "(N)n" comprises a nucleotide sequence of an exonic sequences. In some aspects, "(N)n" comprises a nucleotide sequence of an exonic sequences and a nucleotide sequence of an IRES.

[0212] In some aspects, an RNA transcript comprising a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least about 99%, or 100% sequence identity with SEQ ID NOs: 104 or 105, wherein "N" represents any one of A, T (or U), C, or G; and wherein "n" represents any integer, is transcribed from the DNA genetic constructs disclosed herein. In some aspects, "(N)n" comprises a nucleotide sequence of an exonic sequences. In some aspects, "(N)n" comprises a nucleotide sequence of an exonic sequences and a nucleotide sequence of an IRES.

[0213] Non-limiting examples of constructs of the present and of RNA transcripts transcribed from the DNA genetic constructs disclosed herein are shown in **Table 11**.

Table 11. Exemplary genetic constructs and exemplary RNA transcripts transcribed from the DNA genetic constructs disclosed herein.

[illegible]

[illegible]

III. Vectors

[0214] In some aspects, the genetic DNA or RNA genetic constructs are comprised in a vector. In some aspects, the vector is a DNA vector. In some aspects, the vector is a RNA vector. In some aspects, the vector is a DNA/RNA hybrid vector. In some aspects, the vector is a plasmid.

[0215] In some aspects, the vector is produced by any of the method known in the art for the production of vectors comprising genetic constructs. In some aspects, the vector is produced by chemical synthesis. In some aspects, the vector is produced by molecular cloning. In some aspects, the vector comprises naturally occurring nucleobases, naturally occurring sugar moieties, naturally occurring phosphates groups, naturally occurring nucleic acids backbones, naturally occurring nucleosides, naturally occurring internucleoside linkages, naturally occurring nucleotides, modified nucleobases, modified sugar moieties, modified phosphates groups, modified nucleic acids backbones, modified nucleosides, modified internucleoside linkages, modified nucleotides, or any combination thereof. In some aspects, the genetic construct or the vector is suitable for *in vivo* transcription. In some aspects, the vector is suitable for *in vitro* transcription. In some aspects, the vector is suitable for *in vivo* and *in vitro* and transcription.

[0216] In some aspects, the genetic construct (e.g., comprised in a vector) described herein comprises one or more additional components that aid in the function of the DNA genetic construct, of the pre-circRNA, or of the circRNA produced as disclosed herein.

[0217] In some aspects, the genetic constructs of the present disclosure comprise one or more additional components, wherein the one or more additional components, for example, promotes the transcription of the pre-circRNAs from the DNA genetic constructs, promotes circularization of the pre-circRNAs, enhances the expression of an encoded payload, increases the stability of the payload sequence, or any combination thereof.

[0218] Non-limiting examples of additional components that are useful for the present disclosure include: (1) an internal ribosome entry site (IRES), (2) an untranslated region (UTR), (3) a sequence encoding a signal peptide, (4) a translation initiation sequence, (5) a sequence encoding a RNA binding protein, (6) a sequence encoding a 2A peptide, (7) a translation enhancer element, or (8) any combination of (1) to (7).

[0219] In some aspects, the vectors disclosed herein further comprise additional genetic elements. In some aspects, the additional genetic element is: a restriction enzymes recognition site, a selectable marker, an antibiotic resistance conferring sequences (e.g., an ampicillin or a kanamycin resistance element), a self-cleaving sites coding sequences (e.g., self-cleaving 2A peptides: P2A, T2A, and F2A either with or without furin cleavage site), comprises a translational enhancer element (TEE), origin of replication sites, and a promoter sequence (e.g., a T7, T3, or Sp6 phage polymerase promoter).

[0220] In some aspects, the vectors disclosed herein as disclosed herein comprises a translational enhancer element (TEE). As used herein, the term "**translational enhancer element**" refers to cis-acting sequences that increase the expression of a protein encoded by a nucleotide sequence. Non-limiting examples of TEEs that can be used with the present disclosure are known in the art, *see, e.g.*, US20130177581A, which is incorporated herein by reference in its entirety. When such a pre-circRNA or circRNA produced as disclosed herein is introduced into a target cell, the expression of the payload is increased, *e.g.*, as compared to a corresponding pre-circRNA or circRNA where the payload sequence does not comprise the TEE.

[0221] In some aspects, the TEE is positioned between the transcription promoter and the start codon of a sequence (*e.g.*, payload sequence). In some aspects, a TEE useful for the present disclosure has at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% sequence identity with any of the TEEs provided in U.S. Publication Number US 20140147454, US20090226470, US20070048776, US20130177581, US20110124100, WO1999024595, WO2012009644, WO2009075886, WO2007025008, U.S.

Pat. No. 6,310,197, U.S. Pat. No. 6,849,405, U.S. Pat. No. 7,456,273, U.S. Pat. No. 7,183,395, each of which is herein incorporated by reference in its entirety.

[0222] In some aspects, the vectors disclosed herein comprises multiple TEEs. For example, in some aspects, a pre-circRNA or circRNA produced as disclosed herein comprises a payload sequence, wherein the payload sequence comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, or more than 60 TEE sequences. In some aspects, the TEE sequences in the 5'UTR of the RNA (*e.g.*, modified RNA) are the same or different TEE sequences. In some aspects, the TEE sequences are in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times. In these patterns, each letter, A, B, or C represent a different TEE sequence at the nucleotide level.

[0223] In some aspects, the vectors disclosed herein comprise an antibiotic resistance conferring sequence. In some aspects, the genetic constructs disclosed herein comprise an antibiotic resistance conferring sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an antibiotic resistance conferring sequence of SEQ ID NO: 92 or 93. In some aspects, the genetic constructs (*e.g.*, comprised in a vector), precursor RNAs, or circular RNAs disclosed herein comprises an antibiotic resistance conferring sequence of SEQ ID NO: 92 or 93.

[0224] In some aspects, the DNA genetic constructs (*e.g.*, comprised in a vector), pre-circRNAs, and circRNA disclosed herein are between 300 and 10000, 400 and 9000, 500 and 8000, 600 and 7000, 700 and 6000, 800 and 5000, 900 and 5000, 1000 and 5000, 1100 and 5000, 1200 and 5000, 1300 and 5000, 1400 and 5000, and/or 1500 and 5000 nucleotides in length. In some aspects, the DNA genetic constructs (*e.g.*, comprised in a vector), pre-circRNAs, and circRNA disclosed herein are at least 300 nt, 400 nt, 500 nt, 600 nt, 700 nt, 800 nt, 900 nt, 1000 nt, 1100 nt, 1200 nt, 1300 nt, 1400 nt, 1500 nt, 2000 nt, 2500 nt, 3000 nt, 3500 nt, 4000 nt, 4500 nt, or 5000 nt in length. In some aspects, the DNA genetic constructs (*e.g.*, comprised in a vector), pre-circRNAs, and circRNA disclosed herein are no more than 3000 nt, 3500 nt, 4000 nt, 4500 nt, 5000 nt, 6000 nt, 7000 nt, 8000 nt, 9000 nt, or 10000 nt in length. In some aspects, the length of a DNA, linear RNA, and/or circular RNA polynucleotide provided herein is about 300 nt, 400 nt, 500 nt, 600 nt,

700 nt, 800 nt, 900 nt, 1000 nt, 1100 nt, 1200 nt, 1300 nt, 1400 nt, 1500 nt, 2000 nt, 2500 nt, 3000 nt, 3500 nt, 4000 nt, 4500 nt, 5000 nt, 6000 nt, 7000 nt, 8000 nt, 9000 nt, or 10000 nt.

IV. Modified Nucleotides

[0225] In some aspects, the genetic constructs disclosed herein comprises one or more modified nucleosides. In some aspects, the one or more modified nucleosides comprises 6-aza-cytidine, 2-thio-cytidine, α -thio-cytidine, pseudo-iso-cytidine, 5-aminoallyl-uridine, 5-iodo-uridine, N1-methyl-pseudouridine, 5,6-dihydrouridine, α -thio-uridine, 4-thio-uridine, 6-aza-uridine, 5-hydroxy-uridine, deoxy-thymidine, pseudo-uridine, inosine, α -thio-guanosine, 8-oxo-guanosine, O6-methyl-guanosine, 7-deaza-guanosine, N1-methyl adenosine, 2-amino-6-chloro-purine, N6-methyl-2-amino-purine, 6-chloro-purine, N6-methyl-adenosine, α -thio-adenosine, 8-azido-adenosine, 7-deaza-adenosine, pyrrolo-cytidine, 5-methyl-cytidine, N4-acetyl-cytidine, 5-methyl-uridine, 5-iodo-cytidine, and combinations thereof.

[0226] In some aspects, the genetic constructs disclosed herein comprises one or more uridines which have been replaced by a modified nucleoside. In some aspects, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine (m1 ψ) or 5-methyl-uridine (m5U).

V. Circular RNAs

[0227] In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have greater stability than a linear RNA (e.g., an mRNA) comprising a same sequence. In some aspects, circRNAs prepared by the pre-circRNA constructs or methods described herein have a stability at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 folds greater than that of a linear RNA (e.g., an mRNA) comprising a same sequence. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have greater stability than a linear RNA (e.g., an mRNA) comprising a same sequence when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have greater stability than a linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have a stability at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45,

50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 folds greater than that of a linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein has greater stability than a linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same protein when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have a stability greater than or equal to that of a pre-determined threshold value. In some aspects, the pre-determined threshold value is a stability of a reference linear RNA (e.g., an mRNA) comprising a same sequence as the circular RNA. In some aspects, the pre-determined threshold value is the stability of a reference linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload. In some aspects, the circRNAs have a stability greater than or equal to that of a pre-determined threshold value when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human.

[0228] In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein has greater half-life or functional half-life than a linear RNA (e.g., an mRNA) comprising a same sequence. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have a half-life or functional half-life at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 folds greater than that of a linear RNA (e.g., an mRNA) comprising a same sequence. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have greater half-life or functional half-life than a linear RNA (e.g., an mRNA) comprising a same when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have greater half-life or functional half-life than a linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have a half-life or functional half-life at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000

folds greater than that of a linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have greater half-life or functional half-life than a linear RNA (e.g., an mRNA) comprising a same sequence, a same modifications, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human. In some aspects, the circRNAs have a half-life or functional half-life in a cell greater than or equal to that of a pre-determined threshold value. In some aspects, the pre-determined threshold value is the half-life or functional half-life of a reference linear RNA (e.g., an mRNA) comprising a same sequence as the circRNAs. In some aspects, the pre-determined threshold value is a half-life or functional half-life of a reference linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload. In some aspects, the circRNAs have a half-life or functional half-life greater than or equal to that of a pre-determined threshold value when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human.

[0229] In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have a half-life or functional half-life of at least 5 hours, 10 hours, 15 hours, 20 hours, 30 hours, 40 hours, 50 hours, 60 hours, 70 hours or 80 hours. In some aspects, the circular RNA provided herein has a half-life of 5-80, 10-70, 15-60, and/or 20-50 hours. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have a half-life or functional half-life of at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, or 10 days. In some aspects, the half-life or functional half-life is determined by a nucleic acid assay. In some aspects the functional half-life is determined by a functional protein assay. In some aspects, the circular RNA comprises a nucleotide sequence encoding a reporter gene. In some aspects, the functional half-life is determined by an *in vitro* luciferase assay measuring the expression of the reporter gene. In some aspects, the functional half-life is determined by an *in vitro* luciferase assay, wherein the activity of Gaussia luciferase (GLuc) is measured in cells (e.g. HepG2) expressing the circular RNA polynucleotide every 1, 2, 6, 12, or 24 hours over 1, 2, 3, 4, 5, 6, 7, or 14 days. In some aspects, the functional half-life is determined by an *in vitro* fluorescence assay, wherein fluorescence (e.g., from a GFP protein) is measured in cells (e.g. HepG2) expressing the circular RNA every 1, 2, 6, 12, or 24 hours over 1, 2, 3, 4, 5, 6, 7, or 14 days. In other aspects,

the functional half-life is determined by an *in vivo* assay, wherein levels of a protein encoded by the expression sequence of the circular RNA polynucleotide are measured in patient serum or tissue samples every 1, 2, 6, 12, or 24 hours over 1, 2, 3, 4, 5, 6, 7, or 14 days.

[0230] In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have greater magnitude of expression than a linear RNA (e.g., an mRNA) comprising a same sequence. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have a magnitude of expression at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 folds greater than that of a linear RNA (e.g., an mRNA) comprising a same sequence. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have greater magnitude of expression than a linear RNA (e.g., an mRNA) comprising a same sequence when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have greater magnitude of expression than a linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have a magnitude of expression at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 folds greater than that of a linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload. In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein have greater magnitude of expression than a linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human. In some aspects, the circRNAs have a magnitude of expression greater than or equal to that of a pre-determined threshold value. In some aspects, the pre-determined threshold value is a magnitude of expression of a reference linear RNA (e.g., an mRNA) comprising a same sequence as the circRNAs. In some aspects, the pre-determined threshold value is a magnitude of expression of a reference linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload. In some aspects, the circRNAs have a magnitude of expression greater than or equal

to that of a pre-determined threshold value when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human.

[0231] In some aspects, the circRNAs prepared by the pre-circRNA constructs or methods described herein has less immunogenicity than a linear RNA (e.g., an mRNA) comprising a same sequence. In some aspects, the circRNAs described herein have an immunogenicity at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 folds lower than that of a linear RNA (e.g., an mRNA) comprising a same sequence. In some aspects, the circular RNA described herein have less immunogenicity than a linear RNA (e.g., an mRNA) comprising a same sequence when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human. In some aspects, the circRNAs provided herein have less immunogenicity than a linear RNA (e.g., an mRNA) comprising a same sequence, a same modifications, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload. In some aspects, the circRNAs provided herein have an immunogenicity at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 folds lower than that of a linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload. In some aspects, the circRNAs provided herein have less immunogenicity than a linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human. In some aspects, the circRNAs have an immunogenicity lower than or equal to that of a pre-determined threshold value. In some aspects, the pre-determined threshold value is an immunogenicity of a reference linear RNA (e.g., an mRNA) comprising a same sequence as the circRNAs. In some aspects, the pre-determined threshold value is an immunogenicity of a reference linear RNA (e.g., an mRNA) comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload. In some aspects, the circRNAs have an immunogenicity lower than or equal to that of a pre-determined threshold value when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human.

[0232] In some aspects, the circular RNA described herein is less immunogenic than an equivalent linear RNA (i.e., a linear RNA comprising a same sequence as the circular RNA, or a

linear RNA comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding the same protein as the circular RNA (e.g., mRNA)) when exposed to an immune system of an organism or a certain type of immune cell.

[0233] In some aspects, the circRNAs described herein do not comprise an excision scar and have less immunogenicity than a same circRNA comprising an excision scar and comprising a same sequence. In some aspects, the circRNAs provided herein have an immunogenicity at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 folds lower than that of a same circRNA comprising an excision scar and comprising a same sequence. In some aspects, the circRNAs provided herein have less immunogenicity than a same circRNA comprising an excision scar and comprising a same sequence when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human. In some aspects, the circRNAs provided herein have less immunogenicity than a same circRNA comprising an excision scar and comprising a same sequence, a same modification, a same optimized UTR, and encoding a same payload. In some aspects, the circRNAs provided herein have an immunogenicity at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 folds lower than that of a same circRNA comprising an excision scar and comprising a same sequence, a same modification, a same optimized UTR, and encoding a same payload. In some aspects, the circRNAs provided herein have less immunogenicity than a same circRNA comprising an excision scar and comprising a same sequence, a same modification, a same optimized UTR, and encoding a same payload when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human. In some aspects, the circRNAs have an immunogenicity lower than or equal to that of a pre-determined threshold value. In some aspects, the pre-determined threshold value is an immunogenicity of a reference circRNA comprising an excision scar and comprising a same sequence as the circRNAs. In some aspects, the pre-determined threshold value is an immunogenicity of a reference circRNA comprising an excision scar and comprising a same sequence, a same modification, a same optimized UTR, and encoding a same payload. In some aspects, the circRNAs have an immunogenicity lower than or equal to that of a pre-determined threshold value when administered to a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is a human.

[0234] In some aspects, the circular RNA described herein is less immunogenic than an equivalent circRNA comprising an excision scar (i.e., a circRNA comprising an excision scar and comprising a same sequence, a same modifications, an same optimized UTR, and encoding the same payload) when exposed to an immune system of an organism or a certain type of immune cell.

[0235] In some aspects, the circRNAs provided herein are associated with modulated production of cytokines when exposed to an immune system of an organism or a certain type of immune cell. In some aspects, the circRNAs provided herein are associated with reduced production of IFN- β 1, RIG-I, IL-2, IL-6, IFN γ , and/or TNF α when exposed to an immune system of an organism or a certain type of immune cell as compared to an equivalent linear RNA (i.e., a linear RNA comprising a same sequence as the circRNAs, or a linear RNA comprising a same sequence, a same modification, a same optimized UTR, a cap, and/or a polyA tail, and encoding a same payload as the circular RNA (e.g., mRNA)).

[0236] In some aspects, the circRNAs provided herein does not comprise an excision scar. In some aspects, the circRNAs provided herein are associated with modulated production of cytokines when exposed to an immune system of an organism or a certain type of immune cell. In some aspects, the circRNAs provided herein are associated with reduced production of IFN- β 1, RIG-I, IL-2, IL-6, IFN γ , and/or TNF α when exposed to an immune system of an organism or a certain type of immune cell as compared to an equivalent circRNA comprising an excision scar (i.e., a circRNA comprising a same sequence as the circRNA, and further comprising an excision scar; or a circRNA comprising a same sequence, a same modification, a same optimized UTR, and encoding a same payload as the circular RNA (e.g., mRNA), and further comprising an excision scar).

[0237] In some aspects, the circular RNA provided herein is associated with less IFN- β 1, RIG-I, IL-2, IL-6, IFN γ , and/or TNF α transcript induction when exposed to an immune system of an organism or a certain type of immune cell as compared to an equivalent linear RNA (i.e., a linear RNA comprising the same expression sequence as the circular RNA, or a linear RNA comprising the same expression sequence, the same modifications, an optimized UTR, a cap, and/or a polyA tail, and encoding the same protein as the circular RNA (e.g., mRNA)).

[0238] In some aspects, the circular RNA provided herein is associated with less IFN- β 1, RIG-I, IL-2, IL-6, IFN γ , and/or TNF α transcript induction when exposed to an immune system of an organism or a certain type of immune cell as compared to an equivalent circRNA comprising

an excision scar (i.e., a circRNA comprising a same sequence as the circRNA, and further comprising an excision scar; or a circRNA comprising a same sequence, a same modification, a same optimized UTR, and encoding a same payload as the circular RNA (e.g., mRNA), and further comprising an excision scar).

[0239] In some aspects, transcription of the pre-circRNAs from the DNA genetic constructs (e.g., comprised in a vector), is *in vitro*. In some aspects, *in vitro* transcription of the pre-circRNAs from the DNA genetic constructs (e.g., comprised in a vector), is in a cell. In some aspects the cell is a prokaryotic cell. In some aspects the cell is a eukaryotic cell. *In vitro* transcription of the precursor RNA from the genetic construct (e.g., comprised in a vector) in a cell can be via added polymerases or polymerases encoded by nucleic acids transfected into the cell, or via endogenous (e.g., RNA polymerase II). In some aspects, the pre-circRNAs are transcribed in the cytoplasm of the cell. In some aspects, the pre-circRNAs are transcribed in the nucleus of the cell. In some aspects, *in vitro* transcription of the pre-circRNAs from the DNA genetic construct (e.g., comprised in a vector), is in a cell-free reaction. *In vitro* transcription of the precursor RNA from the genetic construct (e.g., comprised in a vector) in a cell free reaction can be by any RNA polymerase. In some aspects, the RNA polymerase is a bacteriophage RNA polymerase (e.g., T7, T3, or Sp6).

[0240] In some aspects, circularization of the pre-circRNAs to produce the circRNAs, is *in vitro*. In some aspects, *in vitro* circularization of the pre-circRNAs to produce the circRNAs, is in a cell. In some aspects the cell is a prokaryotic cell. In some aspects the cell is a eukaryotic cell. In some aspects, the circRNAs are produced in the cytoplasm of the cell. In some aspects, the circRNAs are produced in the nucleus of the cell. In some aspects, circularization of the pre-circRNAs to produce the circRNAs is in a cell-free reaction.

[0241] In some aspects, transcription of the pre-circRNAs from the DNA genetic constructs (e.g., comprised in a vector), is *in vivo*. In some aspects, DNA genetic constructs (e.g., comprised in a vector) is administered to a cell comprised in a multicellular organism and the pre-circRNAs are transcribed from the DNA genetic constructs (e.g., comprised in a vector) in the cell comprised in the multicellular organism.

[0242] In some aspects, circularization of the pre-circRNAs to produce the circRNAs is *in vivo*. In some aspects, DNA genetic constructs (e.g., comprised in a vector) are administered to a cell comprised in a multicellular organism, the pre-circRNAs are transcribed from the DNA genetic constructs (e.g., comprised in a vector) and the pre-circRNAs circularize in the cell comprised in

the multicellular organism. In some aspects, pre-circRNAs are administered to a cell comprised in a multicellular organism and the pre-circRNAs circularize in the cell comprised in the multicellular organism.

[0243] In certain aspects, the circRNA provided herein is administered to a cell. In some aspects the sequence encoding the payload comprised in the circular RNAs described herein is expressed in a cell. In some aspects, the cell is comprised in an organism. In some aspects, the organism is an animal. In some aspects, the animal is a human.

VI. *Pre-circRNA Circularization*

[0244] In some aspects, the pre-circRNA comprises a complete 5' splice site and a complete 3' splice site. In the aspects, circularization of the pre-circRNA takes place by a two-step transesterification reaction with an endogenous bulged adenosine located in domain VI with its 2'-OH acting as an initiating nucleophile. Binding of the bulged adenosine in the catalytic core in domain V positions the 2'-OH of adenosine to attack the 5' splice site. During the first transesterification step the adenosine is attached to the 5'-end of the intron RNA by a 2'-5' phosphodiester bond, creating a branched lariat between the 3' intron and the 5' intron. This step is facilitated by the binding of Intron Binding Site 1 (IBS1) to Exon Binding Site 1 (EBS1) - and the IBS2 with EBS2 in group IIA and IIB introns - which position the adenosine and 5' splice site in the catalytic core (triad). This step is followed by conformational changes aided by tertiary interactions – delta-delta' or IBS3-EBS3 for group IIA or B and C, respectively – that allow the 3' splice site to trade positions with the adenosine and occupy the catalytic core (triad) to initiate the second transesterification reaction. The 3'-OH of the exon attacks the 3' splice site promoting the ligation and therefore circularization of the exon and the release of the branched intron RNA.

[0245] In some aspects, the pre-circRNA comprises a complete 3' splice site and a free 3'-OH between the most 3' of the exon and 5' of the conserved GUGYG site. In the aspects, circularization of the pre-circRNA takes place by a one-step transesterification reaction where the exon's Y within the AY conserved region (3' splice site) occupies the catalytic core (triad) to initiate the transesterification reaction. The 3'-OH of the exon attacks the 3' splice site (an interaction facilitated by delta-delta' or IBS3-EBS3 for group IIA or B and C, respectively) promoting the ligation and circularization of the exon and the release of the intron RNA.

VIII. Compositions Comprising the Genetic Constructs and Methods of Making circRNAs

[0246] In some aspects, provided herein are compositions comprising the genetic constructs capable of forming circular RNAs disclosed herein (*i.e.*, pre-circRNAs or DNA genetic constructs from which pre-circRNAs can be transcribed) and one or more additional component.

[0247] In some aspects, the additional component is additional protein machinery (e.g., spliceosomal machinery including U1 snRNP, U2AF, U2 snRNP, and U4, U5, and U6 snRNPs). In some aspects, the protein machinery can be used in compositions comprising the pre-circRNAs disclosed herein to produce a circRNA.

[0248] In some aspects, the additional component is one or more metal ion. In some aspects, the one or more metal ion can be used in compositions comprising the pre-circRNAs disclosed herein to produce a circRNA. In some aspects the metal ion is a cation. In some aspects the cation is a monovalent cation. In some aspects, the monovalent cation is Na⁺, K⁺, NH₄⁺, which can be included in the form NaCl, KCl, NH₄Cl, Ammonium acetate, and Ammonium sulfate. In some aspects, the cation is a bivalent cation. In some aspects, the metal ion is Mg²⁺, Ca²⁺, Cu²⁺, Mn²⁺, Zn²⁺, Pb²⁺, Co²⁺, Cd²⁺, Sn²⁺, Ni²⁺, Fe²⁺, Ba²⁺, or Sr²⁺. In some aspects, the cation is a trivalent cation. In some aspects the metal ion is Ytterbium (Yb³⁺).

[0249] In some aspects, the additional component is a RNA polymerase. In some aspects, the RNA polymerase can be used in compositions comprising the DNA genetic constructs disclosed herein to produce the pre-circRNAs disclosed herein. In some aspects, the RNA polymerase is a T7 RNA polymerase, a T3 RNA polymerase, a SP6 RNA polymerase, or any combination thereof.

[0250] In some aspects, the additional component is a salt, a buffer, or any additional component that may facilitate the transcription of the DNA genetic constructs disclosed herein into the pre-circRNAs disclosed herein, and/or any additional component that may facilitate the circularization of the pre-circRNAs disclosed herein to produce the circRNAs disclosed herein.

[0251] Any suitable method known in the published literature can be used to transcribe the DNA genetic constructs disclosed herein into the pre-circRNAs disclosed herein, and/or to circularize the pre-circRNAs disclosed herein to produce the circRNAs disclosed herein.

[0252] The DNA genetic constructs disclosed herein can be transcribed into the pre-circRNAs disclosed herein in vitro, in vivo, or any combination thereof. Similarly, the pre-circRNAs disclosed herein in vitro or in vivo can be circularized to produce the circRNAs disclosed herein in vitro, in vivo, or any combination thereof.

[0253] The vectors comprising the DNA genetic constructs and the pre-circRNAs provided herein can be made using standard techniques of molecular biology. For example, the various elements of the vectors provided herein can be obtained by chemical synthesis or by recombinant methods. For example, the genetic elements comprised in the genetic constructs can be derived from cDNA derived from cells, or from a vector known to include the genetic element of interest. Alternatively, the genetic elements of the genetic constructs provided herein can also be produced synthetically, rather than cloned, based on the known sequences. The complete sequence can be assembled from overlapping oligonucleotides prepared by standard methods and assembled into the complete sequence. See, e.g., Edge, Nature (1981) 292:756; Nambair et al., Science (1984) 223 : 1299; and Jay et al., J. Biol. Chem. (1984) 259:631 1. Particular nucleotide sequences can be obtained from vectors harboring the desired sequences or synthesized completely or in part using various oligonucleotide synthesis techniques known in the art. Additionally, nucleotide sequences can be modified or amplified using any technique known in the art, such as site-directed mutagenesis and polymerase chain reaction (PCR) techniques where appropriate. One method of obtaining nucleotide sequences encoding the desired vector elements is by annealing complementary sets of overlapping synthetic oligonucleotides produced in a conventional, automated polynucleotide synthesizer, followed by ligation with an appropriate DNA ligase and amplification of the ligated nucleotide sequence via PCR. See, e.g., Jayaraman et al., Proc. Natl. Acad. Sci. USA (1991) 88:4084-4088. Additionally, oligonucleotide-directed synthesis (Jones et al., Nature (1986) 54:75-82), oligonucleotide directed mutagenesis of preexisting nucleotide regions (Riechmann et al., Nature (1988) 332:323-327 and Verhoeven et al., Science (1988) 239: 1534-1536), and enzymatic filling-in of gapped oligonucleotides using T4 DNA polymerase (Queen et al., Proc. Natl. Acad. Sci. USA (1989) 86: 10029-10033) can be used.

[0254] The pre-circRNAs provided herein can be generated by incubating a vector provided herein under conditions permissive of transcription of the precursor RNA encoded by the vector. In some aspects, a precursor RNA is synthesized by incubating a genetic construct (e.g., comprised in a vector) provided herein that comprises an RNA polymerase promoter with a compatible RNA polymerase enzyme (e.g., a T7, T3, or Sp6 RNA polymerase) under conditions permissive of *in vitro* transcription. In some aspects, a genetic construct (e.g., comprised in a vector) is incubated inside of a cell and transcribed by a bacteriophage RNA polymerase or by a RNA polymerase II.

[0255] In some aspects, provided herein is a method of generating pre-circRNA by performing *in vitro* transcription using a DNA genetic construct (e.g., comprised in a vector) provided herein as a template (e.g., a vector provided herein comprising a RNA polymerase promoter). In some aspects, the resulting pre-circRNA can be used to generate circRNAs provided herein by incubating it in the presence of magnesium and ammonium ions at a temperature at which RNA circularization occurs (e.g., between 20 °C and 60 °C).

[0256] In some aspects, provided herein is a method of making circular RNA. In some aspects, the method comprises synthesizing pre-circRNAs by transcription (e.g., run-off transcription) using a vector provided herein as a template, and incubating the resulting precursor RNA in the presence of divalent cations (e.g., magnesium ions) such that it circularizes to form circRNAs. Alternatively, the pre-circRNAs can be chemically synthesized and then incubated in the presence of divalent cations (e.g., magnesium ions) such that it circularizes to form circRNAs. In some aspects, pre-circRNAs disclosed herein is capable of circularizing in the absence of magnesium ions and GTP and/or without the step of incubation with magnesium ions and GTP. Without wishing to be bound to any theory, circRNAs has reduced immunogenicity relative to a corresponding mRNA, at least partially because the mRNA contains an immunogenic 5' cap. When transcribing a DNA vector from certain promoters (e.g., a T7 promoter) to produce a precursor RNA, the 5' end of the precursor RNA is generally GTP. To reduce the immunogenicity of a circular RNA composition that contains a low level of contaminant linear mRNA, an excess of GMP relative to GTP can be provided during transcription such that most transcripts contain a 5' GMP, which cannot be capped. Therefore, in some aspects, transcription is carried out in the presence of an excess of GMP. In some aspects, transcription is carried out where the ratio of GMP concentration to GTP concentration is within the range of about 3:1 to about 15:1, for example, about 3:1 to about 10:1, about 3:1 to about 5:1, about 3:1, about 4:1, or about 5:1.

[0257] In some aspects, a composition comprising circular RNA is purified. Circular RNA may be purified by any known method known in the art, such as column chromatography, gel filtration chromatography, and size exclusion chromatography. In some aspects, purification comprises one or more of the following steps: phosphatase treatment, HPLC size exclusion purification, and RNase R digestion. In some aspects, purification comprises the following steps in order: RNase R digestion, phosphatase treatment, and HPLC size exclusion purification. In some aspects, purification comprises reverse phase HPLC. In some aspects, a purified composition contains less double stranded RNA, DNA splints, triphosphorylated RNA, phosphatase proteins,

protein ligases, capping enzymes and/or nicked RNA than unpurified RNA. In some aspects, a purified composition is less immunogenic than an unpurified composition. In some aspects, immune cells exposed to a purified composition produce less IFN- β 1, RIG-I, IL-2, IL-6, IFN γ , and/or TNF α than immune cells exposed to an unpurified composition.

IX. Pharmaceutical Compositions

[0258] In some aspects, provided herein are pharmaceutical compositions comprising a DNA genetic construct (e.g., comprised in a vector), a pre-circRNA, or a circRNA disclosed herein. In some aspects, the DNA genetic constructs (e.g., comprised in a vector), the pre-circRNAs, or the circRNAs disclosed her disclosed herein can be used as therapeutic agents. In some aspects, the therapeutic agent is a DNA genetic construct (e.g., comprised in a vector) provided herein. In some aspects, the therapeutic agent is a pre-circRNA provided herein. In some aspects, the therapeutic agent is a circRNA provided herein. In some aspects the therapeutic agent is a vector comprising a genetic construct provided herein. In some aspects, the therapeutic agent is a cell comprising a genetic construct provided herein (e.g., a human cell, such as a human T cell). In certain aspects, the composition further comprises a pharmaceutically acceptable carrier. In some aspects, the pharmaceutical composition comprises pharmaceutically acceptable carriers, buffer agents, excipients, salts, or stabilizers in the form of lyophilized formulations or aqueous solutions, *See, e.g.*, Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover. "Acceptable", as used herein, means that the carrier must be compatible with the active ingredient of the composition and not deleterious to the subject to be treated. In some aspects, the carrier is capable of stabilizing the active ingredient.

[0259] Acceptable carriers and excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations used, and comprises buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl, or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium;

metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEENTM, PLURONICSTM, or polyethylene glycol (PEG).

[0260] In some aspects, the compositions provided herein comprise a therapeutic agent provided herein in combination with other pharmaceutically active agents or drugs, such as anti-inflammatory drugs or antibodies.

[0261] With respect to pharmaceutical compositions, the pharmaceutically acceptable carrier can be any of those conventionally used and is limited only by chemico-physical considerations, such as solubility and lack of reactivity with the active agent(s), and by the route of administration. The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. The pharmaceutically acceptable carrier be one which is chemically inert to the therapeutic agent(s) and one which has no detrimental side effects or toxicity under the conditions of use.

[0262] The choice of carrier will be determined in part by the particular therapeutic agent, as well as by the particular method used to administer the therapeutic agent. Accordingly, there are a variety of suitable formulations of the pharmaceutical compositions provided herein.

[0263] In some aspects, the pharmaceutical composition comprises a preservative. Suitable preservatives may include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. Optionally, a mixture of two or more preservatives may be used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about 2% by weight of the total composition. In some aspects, the pharmaceutical composition comprises a buffering agent. Suitable buffering agents may include, for example, citric acid, sodium citrate, phosphoric acid, potassium phosphate, and various other acids and salts. A mixture of two or more buffering agents optionally may be used. The buffering agent or mixtures thereof are typically present in an amount of about 0.001% to about 4% by weight of the total composition.

[0264] In some aspects, the concentration of therapeutic agent in the pharmaceutical composition can vary, *e.g.*, less than about 1%, or at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or about 50% or more by weight, and can be selected primarily by fluid volumes, and viscosities, in accordance with the particular mode of administration selected.

[0265] In some aspects, the therapeutic agents are formulated in time-released, delayed release, or sustained release delivery systems such that the delivery of the composition occurs prior

to, and with sufficient time to cause, sensitization of the site to be treated. Such systems can avoid repeated administrations of the therapeutic agent, thereby increasing convenience to the subject and the physician, and may be particularly suitable for certain composition aspects provided herein. In some aspects, the compositions of the invention are formulated such that they are suitable for extended-release of the circRNA contained therein. Such extended-release compositions may be conveniently administered to a subject at extended dosing intervals. In some aspects, the compositions of the present invention are administered to a subject twice day, daily or every other day. In some aspects, the compositions of the present invention are administered to a subject twice a week, once a week, every ten days, every two weeks, every three weeks, every four weeks, once a month, every six weeks, every eight weeks, every three months, every four months, every six months, every eight months, every nine months or annually.

[0266] In some aspects, a product (e.g., a protein) encoded by a circRNA disclosed herein, is produced by a target cell for sustained amounts of time. For example, the product (e.g., a protein) may be produced for more than one hour, more than four, more than six, more than 12, more than 24, more than 48 hours, or more than 72 hours after administration. In some aspects, the product (e.g., a protein) is expressed at a peak level about six hours after administration. In some aspects, the expression of the product (e.g., a protein) is sustained at least at a therapeutic level. In some aspects, the product (e.g., a protein) is expressed at least at a therapeutic level for more than one, more than four, more than six, more than 12, more than 24, more than 48, or more than 72 hours after administration. In some aspects, the product (e.g., a protein) is detectable at a therapeutic level in patient serum or tissue. In some aspects, the level of detectable product (e.g., a protein) is from continuous expression from the circRNA composition over periods of time of more than one, more than four, more than six, more than 12, more than 24, more than 48, or more than 72 hours after administration.

[0267] Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the nanoparticles which matrices are in the form of shaped articles, e.g., films or microcapsules. Examples of sustained-release matrices include, but are not limited to, polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPROM DEPOT™ (injectable microspheres

composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

[0268] In some aspects, suitable surface-active agents include, but are not limited to, non-ionic agents, such as polyoxyethylenesorbitans (*e.g.*, TWEEN™ 20, 40, 60, 80 or 85) and other sorbitans (*e.g.*, SPAN™ 20, 30, 60, 80, or 85). In some aspects, compositions with a surface-active agent comprise between 0.05 and 5% surface-active agent. In some aspects the composition comprises 0.1 and 2.5%. It will be appreciated that other ingredients can be added, for example mannitol or other pharmaceutically acceptable vehicles, if necessary.

[0269] In some aspects, a product (*e.g.*, a protein) encoded by a circRNA disclosed herein is produced at levels above normal physiological levels. The level of the product (*e.g.*, a protein) may be increased as compared to a control. In some aspects, the control is the baseline physiological level of the protein in a normal individual or in a population of normal individuals. In some aspects, the control is the baseline physiological level of the protein in an individual having a deficiency in the relevant protein or in a population of individuals having a deficiency in the relevant protein. In some aspects, the control can be the normal level of the relevant protein in the individual to whom the composition is administered. In some aspects, the control is the expression level of the protein upon other therapeutic intervention, *e.g.*, upon direct injection of the corresponding protein, at one or more comparable time points.

[0270] In some aspects, the levels of a product (*e.g.*, a protein) encoded by a circRNA disclosed herein are detectable at 3 days, 4 days, 5 days, or 1 week or more after administration. Increased levels of secreted protein may be observed in the serum and/or in a tissue (*e.g.*, liver or lung).

[0271] In some aspects, the method yields a sustained circulation half-life a product (*e.g.*, a protein) encoded by a circRNA disclosed herein. For example, the product (*e.g.*, a protein) may be detected for hours or days longer than the half-life observed via subcutaneous injection of the protein or mRNA encoding the protein. In some aspects, the half-life of the product (*e.g.*, a protein) is 1 day, 2 days, 3 days, 4 days, 5 days, or 1 week or more.

[0272] In some aspects, the pharmaceutical composition is in unit dosage forms such as tablets, pills, capsules, powders, granules, solutions or suspensions, or suppositories, for oral, parenteral, or rectal administration, or administration by inhalation or insufflation.

[0273] For preparing solid compositions such as tablets, the principal active ingredient can be mixed with a pharmaceutical carrier, *e.g.*, conventional tableting ingredients such as corn starch,

lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other pharmaceutical diluents, e.g., water, to form a solid preformulation composition containing a homogenous mixture of a compound of the present disclosure, or a non-toxic pharmaceutically acceptable salt thereof. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition can be readily subdivided into equally effective unit dosage forms such as tablets, pills, and capsules. This solid preformulation composition is then subdivided into unit dosage forms of the type described above containing from about 0.1 to about 500 mg of the active ingredient of the present disclosure. The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer that serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials include a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

[0274] Suitable emulsions can be prepared using commercially available fat emulsions, such as INTRALIPID™, LIPOSYN™, INFONUTROL™, LIPOFUNDIN™, and LIPIPHYSAN™. The active ingredient can be either dissolved in a pre-mixed emulsion composition or alternatively it can be dissolved in an oil (e.g., soybean oil, safflower oil, cottonseed oil, sesame oil, corn oil, or almond oil) and an emulsion formed upon mixing with a phospholipid (e.g., egg phospholipids, soybean phospholipids, or soybean lecithin) and water. It will be appreciated that other ingredients can be added, for example glycerol or glucose, to adjust tonicity of the emulsion. Suitable emulsions will typically contain up to about 20% oil, for example, between about 5 and about 20%. The fat emulsion can comprise fat droplets having a suitable size and can have a pH in the range of about 5.5 to about 8.0.

[0275] Pharmaceutical compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions can contain suitable pharmaceutically acceptable excipients as set out above. In some aspects, the composition is administered by the oral or nasal respiratory route for local or systemic effect.

[0276] Compositions in pharmaceutically acceptable solvents can be nebulized by use of gases. Nebulized solutions can be breathed directly from the nebulizing device or the nebulizing device can be attached to a face mask, tent or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions can be administered from devices which deliver the formulation in an appropriate manner.

[0277] The pharmaceutical compositions to be used for *in vivo* administration must be sterile. This is readily accomplished by, for example, filtration through sterile filtration membranes. The nanoparticles can be placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0278] In some aspects, the pharmaceutical composition can be formulated for intratumoral, intrathecal, intramuscular, intravenous, subcutaneous, inhalation, intradermal, intralymphatic, intraocular, intraperitoneal, intrapleural, intraspinal, intravascular, nasal, percutaneous, sublingual, submucosal, transdermal, or transmucosal administration. In some aspects of the disclosure, the pharmaceutical composition can be formulated for intratumoral injection. Intratumoral injection, as used herein, refers to direct injections into the tumor. A high concentration of composition can be achieved *in situ*, while using small amounts of drugs. Local delivery of immunotherapies allows multiple combination therapies, while preventing significant system exposure and off-target toxicities.

[0279] In some aspects, the pharmaceutical composition can be formulated for intramuscular injection, intravenous injection, or subcutaneous injection.

X. Therapeutic Applications

[0280] In some aspects of the disclosure, the DNA genetic construct from which a pre-circRNA can be transcribed, a pre-circRNA, or a circRNA, and/or pharmaceutical compositions described herein (also collectively referred to herein as "therapeutic compositions") are used to treat a disease or disorder. As is apparent from the present disclosure, any of the therapeutic compositions provided herein can be used to treat a wide range of diseases or disorders. Any suitable disease or disorder whether in a therapeutic agent can be encoded by the payload sequence of a genetic construct or circRNA provided herein. Accordingly, some aspects of the present disclosure relates to a method of treating a disease or disorder in a subject in need thereof, comprising administering to the subject any of the therapeutic compositions provided herein (*e.g.*, circRNA).

[0281] In some aspects, any of the therapeutic compositions described herein is administered to a subject in need thereof via a suitable route, such as intratumoral administration, intravenous administration (e.g., as a bolus or by continuous infusion over a period of time), by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, inhalation, or topical routes. Commercially available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers are useful for administration. Liquid formulations can be nebulized and lyophilized powder can be nebulized after reconstitution. In some aspects, the pharmaceutical composition described herein is aerolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder. In some aspects, the pharmaceutical composition described herein is formulated for intratumoral injection. In some aspects, the pharmaceutical composition described herein is administered to a subject via a local route, for example, injected to a local site such as a tumor site or an infectious site. In some aspects, the subject is a human.

[0282] As will be apparent from the present disclosure, in some aspects, the therapeutic compositions described herein are administered to a subject in an effective amount to confer a therapeutic effect, either alone or in combination with one or more other active agents. In some aspects, the therapeutic compositions are administered to a subject suffering from a cancer, and the therapeutic effect comprises reduced tumor burden, reduction of cancer cells, increased immune activity, or combinations thereof. Whether the administered therapeutic composition (e.g., a nanoparticle, such as a LNP or LLN) achieved the therapeutic effect can be determined using any suitable methods known in the art (e.g., measuring tumor volume and/or T cell activity). Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size, gender and weight, the duration of the treatment, the nature of the concurrent therapy (if any), the specific route of administration and like factors within the knowledge of expertise of the health practitioner.

[0283] Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. Frequency of administration can be determined and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of a target disease/disorder. Alternatively, sustained continuous release formulations of a therapeutic composition described herein (e.g., circRNA) can be appropriate. Various formulations and devices for achieving sustained release are known in the art.

[0284] In some aspects of the disclosure, the treatment is a single injection of the therapeutic composition disclosed herein. In some aspects, the single injection is administered intratumorally to the subject in need thereof.

[0285] In some aspects of the disclosure, dosages for a therapeutic composition described herein can be determined empirically in individuals who have been given one or more administration(s) of the therapeutic composition (*e.g.*, circRNA). In some aspects, the individuals are given incremental dosages of the therapeutic composition described herein. To assess efficacy of the therapeutic composition herein, an indicator of disease/disorder can be followed. For repeated administrations over several days or longer, depending on the condition, in some aspects, the treatment is sustained until a desired suppression of symptoms occurs or until sufficient therapeutic levels are achieved to alleviate a target disease or disorder, or symptom thereof.

[0286] In some aspects of the disclosure, the method comprises administering to a subject in need thereof one or multiple doses of a therapeutic composition described herein.

[0287] In some aspects, the therapeutic composition described herein is co-administered with at least one additional suitable therapeutic agent. In some aspects, the therapeutic composition described herein and the at least one additional therapeutic agent are administered to the subject in a sequential manner, *i.e.*, each therapeutic agent is administered at a different time. In some aspects, the therapeutic composition described herein and the at least one additional therapeutic agent are administered to the subject in a substantially simultaneous manner.

[0288] In some aspects, a therapeutic application described herein comprises producing the encoded payload in a target cell. Accordingly, in some aspects, the present disclosure relates to a method of selectively producing a payload in a target cell. In some aspects, the method comprises contacting a target cell with any of the genetic constructs, circRNAs, or compositions described herein (*e.g.*, circRNA) under conditions suitable for producing the encoded payload. In some aspects, the contacting occurs *in vivo* (*e.g.*, by administering the circRNA to a subject). In some aspects, the contacting occurs *ex vivo* (*e.g.*, by culturing cells with the circRNA *in vitro*). Cells (*e.g.*, host cells) comprising the genetic constructs, the circRNA, or any of the compositions described herein are encompassed herein. Non-limiting examples of cells that can be used include immortal hybridoma cell, NS/0 myeloma cell, 293 cell, Chinese hamster ovary (CHO) cell, HeLa cell, human amniotic fluid-derived cell (CapT cell), COS cell, or combinations thereof.

XI. Kits for Use in Therapy

[0289] The present disclosure also provides kits for use in therapy. In some aspects, the kit includes one or more containers comprising a composition described herein.

[0290] In some aspects, the kit comprises instructions for use in accordance with any of the methods described herein. For example, the included instructions can comprise a description of administration of the pharmaceutical composition described herein to treat, delay the onset, or alleviate a target disease. In some aspects, the instructions comprise a description of administering the composition described herein to a subject at risk of a target disease.

[0291] In some aspects, the instructions comprise dosage information, dosing schedule, and route of administration. In some aspects, the containers are unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. In some aspects, the instructions are written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit). In some aspects, the instructions are machine-readable instructions (*e.g.*, instructions carried on a magnetic or optical storage disk).

[0292] In some aspects, the kits described herein are in suitable packaging. In some aspects, suitable packing comprises vials, bottles, jars, flexible packaging (*e.g.*, seal Mylar or plastic bags), or combinations thereof. In some aspects, the packaging comprises packages for use in combination with a specific device such as an inhaler, nasal administration device (*e.g.*, an atomizer), or an infusion device such as a minipump. In some aspects, the kit comprises a sterile access port (for example the container can be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). In some aspects, the container can also have a sterile access port (for example the container can be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). In some aspects, at least one active agent is a composition as described herein.

[0293] In some aspects, the kits further comprise additional components such as buffers and interpretive information. In some aspects, the kit comprises a container and a label or package insert(s) on or associated with the container. In some aspects, the disclosure provides articles of manufacture comprising the contents of the kits described herein.

XII. General Techniques

[0294] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Molecular Cloning: A Laboratory Manual, second edition (Sambrook, et al., 1989) Cold Spring Harbor Press;

Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R. I. Freshney, ed. 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Giffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; Method of Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel, et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis, et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty, ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane, Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanette and J.D. Capra, eds., Harwood Academic Publishers, 1995). Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present disclosure to its fullest extent. All publications cited herein (including those listed above and elsewhere in the present disclosure) are incorporated by reference in their entirety.

Table 12: Sequence Table (N indicates any nucleotide (A, T, U, C, or G); "n: indicates any integer; "^" indicates the location of the phosphodiester bond that is cleaved during splicing).

SEQ ID NO	DESCRIPTION	SEQUENCE
11	<i>Pylaiella littoralis</i> 1 Plasmid (DNA)	CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTA ATCTGCTGCTTGCAAACAAAAAACACCGCTACCAGCGGTGGTTTGTGTTG CCGGATCAAGAGCTACCAACTCTTTTCCGAAGGTAAGTGGCTTCAGCAGA GCGCAGATACCAAATACTGTCTCTTAGTGTAGCCGTAGTTAGGCCACCAC TTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTA CCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGTTGGACTCA AGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGTTTCG TGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTA CAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGAC AGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTT CCAGGGGGAAACGCCCTGGTATCTTTATAGTCCTGTGCGGTTTCGCCACCTC TGACTTGAGCGTCGATTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGG AAAAAAGCCAGCAACGCGGCCTTTTACGGTTCTTGGCCTTTTGTGGCCT TTTGCTCACATGTTCTTCTCCTGCGTTATCCCTGATTCGTGTGGATAACCGT ATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGAGCCGAACGACCGAG CGCAGCGAGTCAGTGAGCGAGGAAGCGGAGGAGCGCCCAATACGCAACCG CCTCTCCCGCGCGTTGGCCGATTCAATATGCAGCTGGCAGCAGAGGTTT CCCGACTGGAAGCGGGCAGTGAGCGCAACGCAATTAAATACGCGTACCGCT AGCCAGGAAGAGTTTGTAGAAACGCAAAAAGGCCATCCGTACAGGATGGCCT TCTGCTTAGTTTGTATGCTGGCAGTTTATGGCGGGCGTCTGCCCGCCACC CTCCGGGCGCTTGCTTCAACAGTTCAAATCCGCTCCCGGCGGATTTGTTC

		TACTCAGGAGAGCGTTTACCGACAAAACAACAGATAAAACGAAAGGCCAGT CTTCCGACTGAGCCTTTTCGTTTTATTGATGCCTGGCAGTTCCTACTCTC GCGTTAACGCTAGCATGGATGTTTTCCAGTCACGACGTTGTAAAACGACG GCCAGTCTTAAGCTCGGCTCTTCGCACTAATACGACTCACTATAGggAGAC CCTCGGGTTGTTGGAGATCTCTGGCgtcccttccctgggaacttggtaagcc caatagactccctctgggaaaccagaggaggagtaagagcaatcttactta tcgtctagtgggtaaaagacataaataggaagcaaatgcttggttgtaacga tcgagatagaatctgttgatttaagctgaaaggctgcagacttattaaatg gggtgttctgctgtatggcgcttcgcgcctaagcagcagaatgcgtgagccgt gtgcatgaaagtgcgaagcacggttctgatggggggaaaacgagagatcg tctacctAtccaactCacaAGCTGAGACGGCGCCGATGGATAGCGGACTTT CGGTCAACCACAATTCCCCACGAGACAGGTCTCTGCCGTAAAAACAAAAAC AAAAAAACAAAAAAACAAAAAAACAAAAACACATTAAAAACAGCCTGT GGGTGATCCCAACACAGGCCCATTTGGGCGCTAGCACTCTGGTATCACGG TACCTTTGTGCGCCTGTTTATACCCCTCCCCCACTGTAACCTTAGAAGT AACACACACCGATCAACAGTCAGCGTGGCACACACAGCCACGTTTGGATCAA GCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCGGTGAA GGAGAAAGCGTTTCGTTATCCGGCCAACCTACTTCGAAAAAACCTAGTAAACAC GTGGAAGTTGCAGAGTGTTCGCTCAGCACTACCCAGTGTAGATCAGGTC GATGAGTCACCGCATTTCCCAACGGGCGACCGTGGCGGTGGCTGCGTTGGCG GCCTGCCCATGGGGAAACCCATGGGACGCTCTAATACAGACATGGTGCGAA GAGTCTATTGAGCTAGTTGGTAGTCTCCGGCCCCCTGAATGCGGCTAATCC TAACTGCGGAGCACACACCTCAAGCCAGAGGGCAGTGTGTCTGTAACGGGC AACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTCATTTC TATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGCTATT GGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTTGGGT TTATACCCTTAGCTTGAAAGAGGTTAAAAACATTACAATTTCATTGTTAAGT TGAATACAGCAAAatggtgagcaaggcgaggagctgttcaccgggggtggt gcccacctctggtcgagctggacggcgacgtaaacggccacaagtccagcgt gtccggcgagggcgaggcgatgccacctacggcaagctgacctgaagtt catctgcaccacggcaagctgcccgtgcccgtgcccacccctcgtgaccac cctgacctacggcgtgcagtgttccagccgctaccccgaccacatgaagca gcacgactcttcaagtcggccatgcccgaaggctacgtccaggagcgcac catcttcttcaaggacgacggcaactacaagaccgcgcgaggtgaagtt cgagggcgacacccctggtgaaccgcacgagctgaaggcgatcgacttcaa ggaggacggcaacatcctggggcacaagctggagtacaactacaacagcca caacgtctatatcatggccgacaagcagaagaacggcatcaaggtgaactt caagatccgccacaacatcgaggacggcagcgtgcagctcgccgaccacta ccagcagaacaccccccatcggcgacggccccgtgctgctgcccgaacaacca ctacctgagcaccagtcggccctgagcaaaagaccccaacgagaagcgcgga tcacatggtcctgctggagtccgtgaccgcgcgaggatcactctcgccat ggacgagctgtacaagtaaAAAAAACAAAAACAAACCTTCTCATGACTG AACTCGCGAGGGTCGTGATGTCGGTTCCTTCAAAGGTTAAAGAACAAGGC TTACTGactgtttattaaaaaGTGCGACAAGAAGTTCAGGAAGGGATTGAG GTGAAAGTCTCTCCCGAATCGTTTCATGGGAGAGTCTATCCAGACTTGCG TAGCGAGTAATCGCTAGGTGAGAAGCTCTGGAGACAATGTACCTGCCCTTC AATTGGAGGTGCCAGGGCTAGGCTTTGTTCTATGGCTAGCGAAAGCGAAT ACAGGTTATTAGGCGTCGTGAAAACAAAActcttccgacataaggggagagg ctcgtagatgagttaccctcctttttatggggtaactgaacgatcaatctc taccggcgtgactgtgagcctaaggaacgaatgaaagtGCCAGAGATC TCCAACAACCATAAGAAGAGCATCCCATGGTCATAGCTGTTTCCTGGCAGC TCTGGCCCGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAAATA TATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAGGG GTGTTATGAGCCATATTCAACGGGAACGTGAGGCCGCGATTAATTTCCA ACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTGGGC AATCAGGTGCGACAATCTATCGCTTGATGGGAAGCCCGATGCGCCAGAGT TGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGA TGGTCAGACTAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGC ATTTTATCCGTACTCCTGATGATGCATGGTTACTACCACTGCGATCCCCG
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		GAAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTGAGGTGAAAAATA TTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTGATTCTCTGTTT GTAATTGTCTCTTTTAACAGCGATCGCGTATTTCTGCTCGCTCAGGCGCAAT CACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTA ATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCAT TCTCACCAGATTTCAGTCGTCACCTCATGGTGATTTCTCACTTGATAACCTTA TTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAA TCGCAGACCGATACCAGGATCTTGCCATCCTATGGAAGTGCCTCGGTGAGT TTTCTCTTCATTACAGAAACGGCTTTTCAAAAAATATGGTATTGATAATC CTGATATGAATAAAATTGCAGTTTCATTTGATGCTCGATGAGTTTCTCTAAT CAGAATTGGTTAATTGGTTGTAACACTGGCAGAGCATTACGCTGACTTGAC GGGACGGCGCAAGCTCATGACCAAAATCCCTTAACGTGAGTTACGCGTCGT TCCACTGAGCGTCAGAC
12	<i>Pyraliella</i> <i>littoralis</i> 2 Plasmid (DNA)	CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTA ATCTGCTGCTTGCAAACAAAAAACCCGCTACCAGCGGTGGTTTGTGTTG CCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGA GCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCAC TTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTGCTTAATCTGTTA CCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTTCGCACTCA AGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTTCG TGCACACAGCCCAGCTTGAGCGAACGACCTACACGAACTGAGATACCTA CAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGAC AGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTT CCAGGGGGAAACGCTTGGTATCTTTTATAGTCCTGTGCGGGTTTCGCCACCTC TGACTTGAGCGTCGATTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGG AAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGTGCGCT TTTGCTCACATGTTCTTCTGCGTTATCCCTGATTTCTGTGGATAACCGT ATTACCGCTTTGAGTGAGCTGATACCGCTCGCCGAGCCGAACGACCGAG CGCAGCGAGTCAGTGAGCGAGGAAGCGGAGGAGCGCCCAATACGCAAAACCG CCTCTCCCGCGCGCTTGGCCGATTCAATTAATGCAGCTGGCAGCAGAGTTT CCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATACGCGTACCGCT AGCCAGGAAGAGTTGTAGAAACGCAAAAGGCCATCCGTGAGGATGGCCT TCTGCTTAGTTTGATGCCTGGCAGTTTATGGCGGGCGTCTGCCCGCCACC CTCCGGGCGCTTGCTTCACAACGTTCAAATCCGCTCCCGGCGGATTTGTCC TACTCAGGAGAGCGTTACCCGACAAACAACAGATAAAACGAAAGGCCAGT CTTCCGACTGAGCCTTTCTGTTTTATTTGATGCCTGGCAGTTCCCTACTCTC GCGTTAACGCTAGCATGGATGTTTTCCAGTCACGACGTTGTAAACGACG GCCAGTCTTAAGCTCGGCTCTTCGCACTAATACGACTCACTATAGggAGAC CCTCGGGTTGTTGGAGATCTCTGGCCgcgccctaacgcagcagaatgcgtagag ccgtgtgcatgaaagtgcgaagcacggttctgatgggggggaaacgagag atcgctctacctatccaactCacaAGCTGAGACGGCGCGGATGGATAGCGGA CTTTCGGTCAACCACAATTTCCCCACGAGACAGGTCTGCGGTAAAAACAAA AAACAAAAAAACAAAAAAACAAAAAAACAAAAACATTTAAACAGC CTGTGGGTTGATCCCACCCACAGGCCCATTTGGGCGCTAGCACTCTGGTATC ACGGTACCTTTGTGCGCTGTTTTATACCCCTCCCCCACTGTAACTTAG AAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACGTTTTGA TCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCAGCGGT TGAAGGAGAAAGCGTTCTGTTATCCGGCCAACACTTTCGAAAAACCTAGTAA CACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCACTACCCAGTGTAGATCA GGTCGATGAGTCACCGCATTTCCCCACGGGCGACCGTGGCGGTGGCTGCGTT GGCGGCCCTGCCCATGGGGAAACCATGGGACGCTCTAATACAGACATGGTG CGAAGAGTCTATTGAGCTAGTTGGTAGTCTCTCCGGCCCCCTGAATGCGGCTA ATCCTAAGTGCAGGACACACACCTCAAGCCAGAGGCGAGTGTGTCGTAAC GGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTTTA TTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATAGC TATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTGTT GGGTTTATACCACTTAGCTTGAAAGAGGTTAAACATTACAATTCAATTGTT AAGTTGAATACAGCAAAatggtgagcaagggcgaggagctgttcacggggg tggtgcccaccttggtcgagctggacggcgacgtaaacggccacaagttca

		gcgtgtccggcgagggcgagggcgatgccacctacggcaagctgacctga agttcatctgcaccaccggcaagctgcccggtgccttgcccacctcgta ccacctgacctacggcggtgcagtgttcagccgctaccccaccacatga agcagcacgacttcttcaagtcgcccatgccgaaggctacgtccaggagc gcaccatcttcttcaaggacgacggcaactacaagacccgcgcgaggtga agttcgagggcgacacctggtgaaccgcacgcagctgaagggcacgcact tcaaggaggacggcaacatcctggggcacaagctggagtacaactacaaca gccacaacgtctatatcatggcgcgacaagcagaagaacggcatcaagggtga acttcaagatccgccacaacatcgaggacggcagcgtgcagctcgccgacc actaccagcagaacaccccccatcggcgcacggccccgtgctgctgccgaca accactacctgagcaccagtcggccctgagcaaaagaccccaacgagaagc gcgatcacatggtcctgctggagttcgtgaccgcgcgggatcactctcg gcatggacgagctgtacaagttaaAAAAACAAAAACAAAACTTCTCATG ACTGAACTCGCGAGGGTCGTGATGTCGGTTCCTTCAAAGGTTAAAGAACAA AGGCTTACTGactgtttattaaaaaGTGCGACAAGAGTTTCAGGAAGGGAT TGAGGTGAAAGTCCTCCTCCCAATCGTTCATGGGAGAGTCTATCCAGACT TGCGTAGCGAGTAATCGCTAGGTGAGAAGCTCTGGAGACAATGTACCTGCC CTTCAATTGGAGGTGCCAGGGCTAGGCTTTGTTCTCTAGCTAGCAAGAC GAATACAGGTTATTAGGCGTCGTGAAAACAAAActcttctcgacataaggga gaggctcgtagatgagttacctcctttttatggggttaactgaacgatcaa tatctaccggcgtagactgtgagcctaaaggaaacgaatgaaagtgtccct tcctgggaacttggttaagcccaatagactccctctgggaaaccagaggagg agtaagagcaatcttacttatcgtctagtgggtaaaagacataataggaag caaatgcttggttgtaacgatcgagatagaatctgttgatttaagctgaaa ggctgcagacttattaatgggtgttctgctgtatggcggtGCCAGAGATC TCCAACAACCATAAGAAGAGCATCCCATGGTCATAGCTGTTTCTGGCAGC TCTGGCCCGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAAATA TATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAGGG GTGTTATGAGCCATATTCACGGGAAACGTCGAGGCCGCGATTAAATTTCCA ACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGC AATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAGT TGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGA TGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGC ATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCG GAAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTGAGGTGAAAAATA TTGTTGATGCGCTGGCAGTGTTCTTGCGCCGGTTGCATTTCGATTCTGTTT GTAATTGTCCTTTTAACAGCGATCGCGTATTTTCGTCTCGCTCAGGCGCAAT CACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTA ATGGCTGGCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCAT TCTCACCGGATTTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTA TTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAA TCGACAGACCGATACCAGGATCTTGCCATCCTATGGAACCTGCCCTCGGTGAGT TTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATC CTGATATGAATAAATTGCAGTTTCATTGATGCTCGATGAGTTTCTTAAT CAGAATTGGTTAATTGGTTGTAAACACTGGCAGAGCATTACGCTGACTTGAC GGGACGGCGCAAGCTCATGACCAAAATCCCTTAACGTGAGTTACGCGTCGT TCCACTGAGCGTCAGAC
13	<i>Pylaiella</i> <i>littoralis</i> 1 DNA of transcribed region	ggAGACCTCGGGTTGTTGGAGATCTCTGGCgtcccttcctgggaacttgg taagcccaatagactccctctgggaaaccagaggaggagtaagagcaatct tacttatcgtctagtgggtaaaagacataataggaagcaaatgcttggttg taacgatcgagatagaatctgttgatttaagctgaaaggctgcagacttat taaatgggtgttctgctgtatggcggttcgcgcctaagcagcagaatgcgtg agccgtgtcgatgaaagtgcgaagcacgggttctgatggggggaaaacgag agatcgtctacctAtccaactCacaAGCTGAGACGGCGCCGATGGATAGCG GACTTTCGGTCAACCACAATTCCCCACGAGACAGGTCTGCCGTAAAAACA AAAAACAAAAAAACAAAAAAACAAAAAAACAAAAACACATTTAAACA GCCTGTGGGTTGATCCCAACACAGGCCCATTTGGGCGCTAGCACTCTGGTA TCACGGTACCTTTGTGCGCCTGTTTTATACCCCTCCCCCACTGTAACCTT AGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCAGTTTT

		GATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCACGCG GTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACCTAGT AACACCGTGGAAGTTGCAGAGTGTTCGCTCAGCACTACCCAGTGTAGAT CAGGTCGATGAGTCACCGCATTCCCCACGGGCGACCGTGGCGGTGGCTGCG TTGGCGGCCTGCCATGGGGAAACCCATGGGACGCTCTAATACAGACATGG TGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCCTGAATGCGGC TAATCCTAACTGCGGAGCACACACCCCTCAAGCCAGAGGGCAGTGTGTGTA ACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTCATTT TATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCATATA GCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATCCCTTTG TTGGGTTTATACCACTTAGCTTGAAAGAGGTTAAACATTACAATTCAATTG TTAAGTTGAATACAGCAAAatggtgagcaagggcgaggagctgttcaccgg ggtggtgcccacctcctggtcgagctggacggcgacgtaaacgggccacaagtt cagcgtgtccggcgagggcgagggcgatgccacctacggcaagctgacct gaagttcatctgcaccacgggcaagctgcccggtgccctggcccaccctcgt gaccaccctgacctacggcgtgcagtgttcagccgctaccccagccacat gaagcagcacgacttcttcaagtcgcccatgccgaaggtacgtccagga gcgaccatcttcttcaaggacgacggcaactacaagaccgcgcgaggt gaagttcgagggcgacacccctggtgaaccgcatcgagctgaagggcgcga cttcaaggaggacggcaacatcctggggcacaagctggagtacaactaaa cagccacaacgtctatatcatggccgacaagcagaagaacggcatcaaggt gaacttcaagatccgccacaacatcgaggacggcagcgtgcagctgcgcga ccactaccagcagaacaccccccatcggcgacggccccctgctgctgccga caaccactacctgagcacccagtcgcacctgagcaaaagacccccaacgagaa gcgcgatcacatggtcctgctggagttcgtgaccgcgcgcgggatcactct cggcatggacgagctgtacaagtaaAAAAACAAAAACAAAACCTTCTCA TGACTGAACTCGCGAGGGTCGTGATGTCGGTTCCTTCAAAGGTTAAAGAAC AAAGGCTTACTGactgtttattaaaaaGTGCGACAAGAAGTTCAGGAAGGG ATTGAGGTGAAAGTCCTCCTCCGAATCGTTCATGGGAGAGTCTATCCAGA CTTGCGTAGCGAGTAATCGCTAGGTGAGAAGCTCTGGAGACAATGTACCTG CCCTTCAATTGGAGGTGCCAGGGCTAGGCTTTGTTCCTATGGCTAGCGAAA GCGAATACAGGTTATTAGGCGTCGTGAAAACAAAActctttcgacataagg gagaggctcgtagatgagttaccctccttttatgggtaactgaacgatc aatatctaccggcgtagactgtgagcctaaaggaaacgaatgaaagtGCCA GAGATCTCCAACAACC
14	<i>Pyraliella littoralis</i> 2 DNA of transcribed region	ggAGACCCTCGGGTTGTTGGAGATCTCTGGCcgcgccctaagcagcagaatg cgtgagccgtgtgcatgaaagtcgcaagcacggttctgatggggggaaaa cgagagatcgtctacctAtccaaactCacaAGCTGAGACGGCGCCGATGGAT AGCGGACTTTCGGTCAACCACAATTCCCCACGAGACAGGTCTCGCGTAAA AACAAAAACAAAAAAACAAAAAAACAAAAAAACAAAACACATTAA AACAGCCTGTGGGTTGATCCCACCACAGGCCCATTTGGGCGCTAGCACTCT GGTATCACGGTACCTTTGTGCGCCTGTTTATACCCCTCCCCCACTGTA ACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAGCCACG TTTTGATCAAGCACTTCTGTTACCCCGGACTGAGTATCAATAGACTGCTCA CGCGGTTGAAGGAGAAAGCGTTCGTTATCCGGCCAACTACTTCGAAAAACC TAGTAACACCGTGGAAGTTGCAGAGTGTTCGCTCAGCACTACCCAGTGT AGATCAGGTGATGAGTACCGCATTCCCCACGGGCGACCGTGGCGGTGGC TGCGTTGGCGGCCTGCCATGGGGAAACCCATGGGACGCTCTAATACAGAC ATGGTGCGAAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCCTGAATG CGGCTAATCCTAACTGCGGAGCACACACCCCTCAAGCCAGAGGGCAGTGTGT CGTAACGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCCGTGTTTC ATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTACCA TATAGCTATTGGATTGGCCATCCGGTGACTAATAGAGCTATTATATATACCC TTTGTGGGTTTATACCACTTAGCTTGAAAGAGGTTAAACATTACAATTTC ATTGTTAAGTTGAATACAGCAAAatggtgagcaagggcgaggagctgttca ccgggggtggtgcccacctcctggtcgagctggacggcgacgtaaacggccaca agttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctga ccctgaagttcatctgcaccacgggcaagctgcccggtgccctggcccacc tcgtgaccaccctgacctacggcgtgcagtgttcagccgctaccccagacc

		acatgaagcagcagcacttcttcaagtccgccatgccgaaggctacgtcc aggagcgcaccatcttcttcaaggacgacggcaactacaagaccgcgcg aggtgaagttcgagggcgacaccctggtgaaccgcatcgagctgaagggca tcgacttcaaggaggacggcaacatcctggggcacaagctggagtacaact acaacagccacaacgtctatatcatggccgacaagcagaagaacggcatca aggtgaacttcaagatccgccacaacatcgaggacggcagcgtgcagctcg ccgaccactaccagcagaacacccccatcggcgacggccccgtgctgctgc ccgacaaccactacctgagcaccagtcgccttgagcaaagacccccaacg agaagcgcgcatcacatggtcctgctggagttcgtgaccgcgcgcgggatca ctctcgcatggacgagctgtacaagtaaAAAAACAAAAACAAACCTT CTCATGACTGAACTCGCGAGGGTCGTGATGTGGTTCTTCAAGGTTAA GAACAAAGGCTTACTGactgtttattaaaaaGTGCGACAAGAAGTTTCAGGA AGGGATTGAGGTGAAAGTCTCTCTCCGAATCGTTTCATGGGAGAGTCTATC CAGACTTGCGTAGCGAGTAATCGCTAGGTGAGAAGCTCTGGAGACAATGTA CCTGCCCTTCAATTGGAGGTGCCAGGGCTAGGCTTTGTTCCTATGGCTAGC GAAAGCGAATACAGGTATATTAGGCGTCGTGAAAACAAAActctttcgacat aagggagaggtcgttagatgagttaccctctttttatggggtaactgaac gatcaatatctaccggcgttagactgtgagcctaaggaacgaatgaaagt gtcccttctgggaacttggttaagcccaatagactccctctgggaaccag aggaggagtaagagcaatcttacttatcgtctagtgggtaaaagacataat aggaagcaaatgcttggttgtaacgatcgagatagaatctgttgatttaag ctgaaagggtgcagacttattaaatgggtgttctgctgtatggcgttGCCA GAGATCTCCAACAACC
15	<i>Pyraliella littoralis</i> 1 RNA of transcribed region	ggAGACCCUCGGGUUGUUGGAGAUUCUCUGGCgucccuuccugggaaacuugg uaagcccaauagacuccucugggaaaccagaggagaguaagagcaauacu uacuuauucgucuguggguaaaagacauauaggaagcaauugcuugguug uaacgaucgagauagaauucugugauuuuagcugaagggcugcagacuau uaauugggugucugcuguguauggcguucgcgcuaagcagcagaauugcug agccgugugcgaugaaagucgcaagcacggcuucugauggggggaaaaacgag agaucgucuaaccuAuccaacuCacaAGCUGAGAGCGCGCCGAUGGAUAGCG GACUUUCGGUCAACCACAAUUCCCCACGAGACAGGUCCUGCCGUAAAAACA AAAAACAAAAAAACAAAAAAACCAAAAAACAAAAACACAUUAAAACA GCCUGUGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUA UCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCUCCCCCAACUGUAACUU AGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUU GAUCAAGCACUUCUGUUUACCCCGGACUGAGUAUCAUAGACUGUACACGCG GUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGU AACACCGUGGAAGUUGCAGAGUGUUUCGUCAGCACUACCCAGUGUAGAU CAGGUCGAGAGUACCCGAUUCCCCACGGGCGACCGUGGCGGUGGCUGCG UUGGCGGCCUGCCCAUGGGGAAACCAUGGGACGCUCUAUACAGACAUGG UGCGAAGAGUCUAUUGAGCUGUUGGUGAGUCCUCCGGCCCCUGAAUGCGGC UAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUA ACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUU UAUUCUUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUUGUACCAUAUA GCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUACCCUUUG UUGGGUUUAUACCACUUAAGCUUGAAAGAGGUUAAAAACAUUACAAUUAUUG UUAAGUUGAAUACAGCAAAauggugagcaagggcgaggagcuguuacccgg gguggugcccauccugugcagcuggacggcgacguaaacggccacaaguu cagcguguccggcgagggcgagggcgauccaccuacggcaagcugacccu gaagucaucugcaccaccggcaagcugcccugcccugcccaccuccu gaccaccugaccuacggcgugcagugcuucagccguaccccgaccacau gaagcagcacgacuucuaaguccgccaugcccgaaggcuacguccagga gcgaccacuucuaagacgacggaacuaacaagaccgcgcgcgaggu gaaguucgagggcgacaccuccggugaaccgcaucgagcugaaggcgauca cuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuaaa cagccacaacgucuaaucaugggcgacaagcagaagaacggcaucaaggu gaacuucagaucggccacaacaucgaggacggcagcgugcagcucgcgga ccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccga caaccacuaccugagcaccaguccgcccugagcaaagaccccaacgagaa

[illegible]

36	SP6 RNAP (DNA)	ATTTAGGTGACACTATAG
37	SP3 RNAP (DNA)	AATTAACCCCTCACTAAAG
38	T7 RNAP (RNA)	UAAUACGACUCACUAUAG
39	SP6 RNAP (RNA)	AUUUAGGUGACACUAUAG
40	SP3 RNAP (RNA)	AAUUAACCCUCACUAAAG
41	Enhanced green fluorescent protein (eGFP)	atggtgagcaagggcgaggagctgttcacccggggtggtgcccacctcctgggtc gagctggacggcgacgtaaacgggccacaagttcagcgtgtccggcgaggggc gagggcgatgccacctacggcaagctgacctgaagttcatctgcaccacc ggcaagctgcccgtgcctggcccaccctcgtgaccaccctgacctacggc gtgcagtgttcagccgctaccccgaccacatgaagcagcagcacttcttc aagtccgcatgcccgaaggctacgtccaggagcgcaccatcttcttcaag gacgacggcaactacaagaccgcgcggagggtgaagttcgagggcgacacc ctggtgaaccgcctcgagctgaagggcatcgacttcaaggaggacggcaac atcctggggcacaagctggagtacaactacaacagccacaacgtctatatc atggccgacaagcagaagaacggcatcaaggtgaacttcaagatccgccac aacatcgaggacggcagcgtgcagctcgccgaccactaccagcagaacacc cccatcgcgacggcccggtgctgctgcccgcacaaccactacctgagcacc cagtcgccttgagcaaagaccccaacgagaagcgcgatcacatggtcctg ctggagttcgtgaccgcgcgggatcactctcgcatggacgagctgtac aagtaa
92		ATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGATTAAATTTCCAACATG GATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCCGGCAATCA GGTGCGACAATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTTT CTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTC AGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTT ATCCGTACTCCTGATGATGCATGGTTACTCACCCTGCGATCCCCGGAAAA ACAGCATTTCCAGGTATTAGAAGAATATCCTGATTGAGGTGAAAAATTGTT GATGCGCTGGCAGTGTTCCTGCGCCGTTGCATTGATTCCTGTTTGTAAT TGTCTTTTAACAGCGATCGCGTATTTCTGCTCTCGCTCAGGCGCAATCACGA ATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGC TGGCCTGTTGAACAAGTCTGGAAGAAATGCATAAACTTTTGCCATTCTCA CCGGATTTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTT GACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCA GACCGATACCAGGATCTTGCCATCCTATGGAAC TGCTCGGTGAGTTTCT CCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGAT ATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTCTCTAA
93		AUGAGCCAUAUUAACGGGAAACGUCGAGGCCGCGAUUAAAUCCAACAU GAUGCUGAUUUUAUUGGUUAUAAAUGGGCUCGCGAUAAUGUCGGGCAAUCA GGUGCGACAAUUAUCGCUUGUAUGGGAAGCCCGAUGC GCCAGAGUUGUUU CUGAAACAUGGCAAAGGUAGCGUUGCCAAUGAUGUUA CAGAUGAGAUGGUC AGACUAAACUGGCUGACGGAUUUAUGCCUCUUCGACCAUCAAGCAUUUU AUCCGUACUCCUGAUGAUGCAUGGUUACUACCACUGCGAUCCCCGGAAAA ACAGCAUUC CAGGUAAUAGAAGAAUUAUCCUGAUUCAGGUGAAAAUUAUGUU GAUGCGCUGGCAGUGUUCUGCGCCGGUUGCAUUCGAUUCUGUUUGUAAU UGUCCUUUAACAGCGAUUCGCGUAUUUCGUCUCGUCAGGCGCAAUCACGA AUGAAUAACGGUUUGGUUGAUGCGAGUGAUUUUGAUGACGAGCGUAAUGGC UGGCCUGUUGAAACAAGUCUGGAAAGAAUUGCAUAAACUUUUGCCAUUCUCA CCGGAUUCAGUCGUCACUCAUGGUGAUUUUCACUUGAUAAACUUUUUUU GACGAGGGGAAAUAAUAGGUUGUAUUUGAUGUUGGACGAGUCGGAUUCGCA GACCGAUACCAGGAUCUUGCCAUCCUAUGGAACUGCCUCGGUGAGUUUUCU CCUUAUUAACAGAAACGGCUUUUUCAAAAUAUGGUUAUUGAUAAUCCUGAU AUGAAUAAAUUGCAGUUUCAUUUGAUGCUCGAUGAGUUUUUCUAA
94	Bacterial origin of replication for plasmid	CCCGTAGAAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTA ATCTGCTGCTTGCAAACAAAAAACACCGCTACCAGCGGTGGTTTGTGTTG CCGGATCAAGAGCTACCAACTCTTTTTTCCGAAGGTAAGTGGCTTCAGCAGA GCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCAC TTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCCTGTTA

	maintenance (pUC ORI)	CCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCA AGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTTCG TGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTA CAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAAGCGGAC AGGTATCCGGTAAGCGGCAGGGTCGGAAACAGGAGAGCGCACGAGGGAGCTT CCAGGGGGAAACGCTTGGTATCTTTATAGTCCTGTCTGGGTTTTCGCCACCTC TGACTTGAGCGTCGATTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGG AAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGTGGCCT TTTGCTCACAT
95	U1 snRNP	AUACUUACCUGGCAGGGGAGAUACCAUGAUCACGAAGGUGGUUUUCCAGG GCGAGGCUUAUCCAUUGCACUCCGGAUGUGCUGACCCUGCGAUUUCCCCA AAUGUGGGAAACUCGACUGCAUAAUUUGUGGUAGUGGGGGACUGCGUUCG GCUUCCCCUG
96	U2AF2	TCGGAGCAAGTCCGTGGAAGAAGCCAAAGACTGGGACGGATTGAATTGTTG GAAGAACCCGAACCTCGCAGAGGGGACTGGGCGCAGTGGCACACGAGGACAC ACGGAAACCTCCGAACGTTGAGGAGATAATCTCGGAAAAGTTACGGAAACC TGGGAAGTCGGCGAAACCTACGTTCCGGGTGTTTTCGGAAGCAGCCGAAGC GGCCGCTGCTTCGCCCTTCGTCCAGTATCCGGAGATAGCCGAGGCGCTTCG GAGCCAGGCTCGGGGGGAGGGGCAAGCTCGCGGGCGGGCGGGCTGAGG GGGCGGAGCCCGGGCGGGGAGGGCTGCCGAGGGCTTGATTAGGTAG GAGGTGGCGAAGCGCCGGCGGGCGCCGAAGTAGCCGAAGCCAGCGGCGGA AGTAGCCGAAGCGGCTGGAGCGGGCGGCAAGGCGAGGCGAAAGCTGCACAG GGCCCTACGCGGCCGCCCTCAGCATGTCGGACTTCGACGAGTTCGAGCGGCA GCTCAACGAGAATAAACAAAGGTGAGGGCACCGGGTCCGCGGGCGGAGGTG TGGGCGGCTCCTCGCGTCGCTCTTTGCCCCCGCCATTTTCTCCCTCGCT TCCCCCACTTCACGGCCGCGCGGCGCCCCCAACCCTGGTTCCGTGGCGC GCGCCTCGTTCACGTGACGTCCCCAGCGTTCCGCGCGCGCTAGGCGAGGG GAAGGGGGCGGCGGGGCGCGGGCCCGTGACGCATGCGCACGGCGGCTGTCC CTGCGCCCCACGTGGTCTCGCGGAGGTGGCGCGGGGACGCGGCACGAGC GCGCCAGATGCCTCGACGTGAACCTTTGTTTTTTAAATCTTTTTGGTTTTT TCTGAAAAGGAGATTATGTTAGTCCCCGTAATCTCTTTTCTCCCGCTGCCT TGGCACTTCCGCTTCCGGTCCGGGCCACGGCAGCGTCTCCACGCCCCCTTT GTTTCCAAAATGGCGGTTGTGAGAACTTGGTGGGGGGGCCCCCTTTCCTTCC TTCACGTGGATGAGGGCAGAGCCGTTTCGGCGGATGCTTGGAGGCCACGGG CCCCCTCGCGTACCCCTTTTCTCTTTTGTGATTGCTCTGATCTCTCCCGC CAGGTGGTTTTCTGCCCCGCTGTGTTGGTAGTGGGGCGCCTCATATTAACTG TTTGGCACATGTTGCCTAAAAGCATCTGCATTCTTTGAGTGTCCGCACGCA CACATCTGCATATGCGGGGCTTTTGGCGGTCCCGAGAATCGCGATATGGA GTGTTTGAGACAAACCTCAAATCATTTACAATCCCAATCTCAGGGCACCTG ATAATACTCAGTCTCAGATCTCAATATAGAGATAAG
97	U2 SnRNP	AUCGCUUCUCGGCCUUUUGGCUAAGAUCAAGUGUAGUAUCUGUUCUUAUCA GUUUAAUAUCUGAUACGUCCUCUAUCCGAGGACAAUUAUAAAUGGAUUU UUGGAGCAGGGAGAUGGAAUAGGAGCUUGCUCGUGCCACUCCACGCAUCGA CCUGGUUUUAGCAUACCUCAGGAACGGUGCACCC
98	U4	AGCUUUGCACAGUGGCAGUAUCAAACCCAGUGAGGUUUUAUCUGUGGCAUGA UUAUUGCUAAUUGAUACUCCCCUCUACAGGAAUUUAAACUUUCCUCUUCAG GAAUGAAAGAUUGGGGUGGUGGGGAACUUCUCAAUACCCUGCCGGGAUGAU UUGUAACACAGUUGGCAUUGGCAUUUUUUGACAGUCUCUAUAGAGA
99	U5	UUACUCUAGUUUCUCUUAUUAUCGCAUAAAUCUUUCGCCUUUUACUAAAGA UUUCCGUGGAGAGGAAUAAUUCUGAGUUUAAACCAUUUUUAGAGGUCU UGCAUUUUUUAGCAAGGC
100	U6	GUUCUGCACUUGUAUCCAGAAACUUGAAAUAUUUUUUAAAAAGAAUA UUAAAAAAGAAUACUCACUUGGGCAGGUCACAUACUGAAAUUGGAGUU AGCACGGCCCCUGUGCAAGGAUGCCAAGCAAUCUGUGAAGCGUUUCGUAU UUUU
101	U2AF2	MSDFDEFERQLNENKQERDKENRHRKRSHSRSRSRDRKRSRSRDRNRDQ RSASRDRRRRSKPLTRGAKEEHGGLIRSPRHEKKKKVRYWDVPPGFEHI TPMQYKAMQAAGQIPATALLPTMTPDGLAVTPTPVVVGSMTRQARRLYV GNIPFGITEAMMDFFNQMRLLGLTQAPGNPVLAVQINQDNKFAFLEFRS

		VDETTQAMAFDGIIFQGGSLKIRRPDHYQPLPGMSENPSVYVPGVVSTVVP DSAHKLFIGGLPNYLNDQVKELLTSFGPLKAFNLVKDSATGLSKGYAFCE YVDINVTDAQAIAGLNGMQLGDKLLVQRASVGAKNATLSTINQTPVTLQVP GLMSSQVQMGGHPTEVLC LMNMVLP EELLDD EYE EIVEDVRDECSKYGLV KSIEIPRPVDGVEVPGCGKIFVEFTSVFDCQKAMQGLTGRKFANRVVVTKY CDPDSYHRRDFW
	U1 snRNP	>URS000032B6B6 snRNA from 96 species
	U2AF2	>ref NC_000019.10 :55654624-55655985 Homo sapiens chromosome 19, GRCh38.p14 Primary Assembly
	U2 SnRNP	>URS00003EE995 snRNA from 3 species
	U4	>URS000067B062 snRNA from 2 species
	U5	>URS0000631BD4 snRNA from 2 species
	U6	>URS00006767A8 snRNA from 2 species

Examples

[0295] DNA genetic constructs comprising a 3' intronic sequence and a 5' intronic sequence of SEQ ID NO 1-5 were chemically synthesized in the form of double-stranded DNA.

[0296] The genetic constructs were cloned in a DNA vector (pUC plasmid vector). The genetic constructs comprise SEQ ID NOs: 11-12. The intron performs a catalytic splicing reaction that removes the 3' intronic sequence and the 5' intronic sequence from the transcribed RNA sequence and circularizes the exons sequence that is bookended by the introns.

[0297] With respect to the genetic construct depicted in Fig. 1, the mechanism the circularization reaction is as follows:

- 1) an unpaired adenosine located within the group II intron binds to the catalytic core (triad) in the permuted 3' intron,
- 2) the adenosine performs a nucleophilic attack using it's 2'-OH on the 5' splice site of the permuted 5' intron, cleaving the intron from the exonic sequence, forming a branched lariat between the permuted 3' and 5' introns, and
- 3) the resulting 3'-OH of the exonic sequence then performs a nucleophilic attack of the phosphate between the permuted 3' intron and the exonic sequence at the 3' splice site, cleaving the 3' intron from the sequence and ligating the exon ends together into a circular RNA (Fig. 2).

[0298] The intron comprised in the generated genetic constructs are a group II introns. The 5' splice site, the 3' splice site, and the catalytic core (triad) are all involved in coordinating the sequential cleavage-ligation reactions of group II introns. The endogenous A first binds the catalytic core (triad) in a base triple with the intron. Magnesium ions help coordinate the nucleophilic attack of the 2-OH of adenosine on the phosphate backbone in the 5' splice site and later with the 3' splice site. The 5' splice site is located in a region known as Domain I. Intronic and

exonic sequences base pair with each other in this region, which creates the specificity needed for proper cleavage at the 5' splice site. The intron component is known as the exon binding site (EBS) – ~6 bp of complementarity to the intron binding site (IBS). The IBS sequence is the exonic sequence complementary to the EBS.

[0299] It is to be appreciated that the Detailed Description section, and not the Summary and Abstract sections, is intended to be used to interpret the claims. The Summary and Abstract sections can set forth one or more but not all exemplary aspects of the present disclosure as contemplated by the inventor(s), and thus, are not intended to limit the present disclosure and the appended claims in any way.

[0300] The present disclosure has been described above with the aid of functional building blocks illustrating the implementation of specified functions and relationships thereof. The boundaries of these functional building blocks have been arbitrarily defined herein for the convenience of the description. Alternate boundaries can be defined so long as the specified functions and relationships thereof are appropriately performed.

[0301] The foregoing description of the specific aspects will so fully reveal the general nature of the disclosure that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific aspects, without undue experimentation, without departing from the general concept of the present disclosure. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed aspects, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[0302] The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary aspects, but should be defined only in accordance with the following claims and their equivalents.

What is claimed is:

1. A genetic construct comprising a formula of:

5'-H-A-E-B-H'-3',

wherein:

H comprises a 5' homology arm;

A comprises a 3' intronic sequence that is optionally derived from a Group II intron;

E comprises an exonic sequence (i.e., one or more exons or fragments thereof);

B comprises a 5' intronic sequence that is optionally derived from a Group II intron; and

H' comprises a 3' homology arm;

wherein at least one of the 3' intronic sequence and the 5' intronic sequence is derived from a Group II intron and wherein the genetic construct is capable of forming a circular RNA.

2. A genetic construct comprising a formula of:

5'-P-H-A-3S-E-5S-B-H'-3',

wherein:

P comprises a promoter (e.g., T7, T3, SP6, etc.);

H comprises a 5' homology arm;

A comprises a 3' intronic sequence that is optionally derived from a Group II intron;

3S comprises a 3' splice site;

E comprises an exonic sequence (i.e., one or more exons or fragments thereof);

5S comprises a 5' splice site;

B comprises a 5' intronic sequence that is optionally derived from a Group II intron;

H' comprises a 3' homology arm;

wherein the promoter (P) is to be understood to be comprised in a DNA genetic construct, but not in a RNA genetic construct, and

wherein at least one of the 3' intronic sequence and the 5' intronic sequence is derived from a Group II intron.

3. The genetic construct of claim 1 or 2, wherein the 3' intronic sequence and the 5' intronic sequence are derived from a Group II intron.

4. The genetic construct of any one of claims 1-3, wherein the 3' intronic sequence and the 5' intronic sequence are derived from a same Group II intron.

5. The genetic construct of any one of claims 1-3 wherein the 3' intronic sequence and the 5' intronic sequence are derived from different Group II introns.
6. The genetic construct of any one of claims 1 to 5, wherein the 3' intronic sequence and the 5' intronic sequence together are less than the full intron.
7. The genetic construct of any one of claims 1 to 6, wherein the circular RNA has a reduced immunogenicity compared to a circular RNA that contains an excision scar.
8. The genetic construct of any one of claims 1 to 7, wherein the 5' intronic sequence and/or the 3' intronic sequence are derived from an intron of *Pylaiella littoralis* 1-2, *Amoebidium parasiticum*, *Mesostigma viridae*, *Lactococcus lactis*, or *Oceanobacillus iheyensis*, or any combination thereof.
9. The genetic construct of any one of claims 1 to 8, wherein the 5' splice site comprises a site between two nucleotides comprising A and N, where N is any nucleotide.
10. The genetic construct of claim 9, wherein the N is A, T, U, G, or C, e.g., G.
11. The genetic construct of any one of claims 1 to 10, wherein the 3' splice site comprises a site between two nucleotides comprising T or U and N, wherein N is any nucleotide.
12. The genetic construct of claim 11, wherein the N is A or C.
13. The genetic construct of any one of claims 1 to 12, wherein the catalytic core comprises AGC, CGC, or both.
14. The genetic construct of any one of claims 1 to 13, wherein the circRNA formed by the genetic construct comprises an excision scar.
15. The genetic construct of any one of claims 1 to 13, wherein the circRNA formed by the genetic construct does not comprise an excision scar.
16. The genetic construct of any one of claims 1 to 15, wherein the 3' intronic sequence comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to a 3' intronic sequence set forth in SEQ ID NOs: 17, 18, 19, or 20.

17. The genetic construct of any one of claims 1 to 16, wherein the 5' intronic sequence comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 95%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to a 5' intronic sequence set forth in SEQ ID NO: 21, 22, 23, or 24.
18. The genetic construct of any one of claims 1 to 17, which is RNA.
19. The genetic construct of any one of claims 1 to 17, which is DNA.
20. The genetic construct of any one of claims 1 to 19, which further comprises a promoter.
21. The genetic construct of any one of claims 1 to 20, wherein the promoter comprises a T7 promoter, a T3 promoter, an SP6 promoter, or any combination thereof.
22. The genetic construct of any one of claims 1 to 21, which further comprises an internal ribosomal entry site ("IRES").
23. The genetic construct of claim 22, wherein the IRES comprises a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to any sequence set forth in SEQ ID NO: 44 to 67 if the genetic construct is DNA and to any sequence set forth in SEQ ID NO: 68 to 91 if the genetic construct is RNA.
24. The genetic construct of any one of claims 1 to 23, wherein the exonic sequence further comprises a binding site for a polyA binding protein at the 5' terminus.
25. The genetic construct of any one of claims 1 to 24, wherein the 5' homology arm comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the sequence as set forth in SEQ ID NO: 27 or 29.
26. The genetic construct of any one of claims 1 to 25, wherein the 3' homology arm comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least

about 98%, at least about 99%, or about 100% sequence identity to the sequence as set forth in SEQ ID NO: 28 or 30.

27. The genetic construct of any one of claims 1 to 26, wherein the exonic sequence is linked to one or more untranslated regions at the 3' and/or 5' of the exonic sequence.

28. The genetic construct of any one of claims 1 to 27, which further comprises a polyA.

29. The genetic construct of claim 28, wherein the polyA comprises a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the sequence as set forth in SEQ ID NO: 42, 43, or any combination thereof.

30. The genetic construct of any one of claims 1 to 29, which further comprise a spacer sequence.

31. The genetic construct of claim 30, wherein the spacer comprises a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 95%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to any sequence as set forth in SEQ ID NO: 31, 32, 33, 34, or any combination thereof.

32. The genetic construct of any one of claims 1 to 31, wherein the exonic sequence encodes a protein.

33. The genetic construct of any one of claims 1 to 32, wherein the circular RNA exhibits an increased expression of the protein, a prolonged expression of the protein in vitro and/or in vivo, a reduced activation of an immune signaling pathway, an increased immune activation, or any combination thereof.

34. The genetic construct of claim 32 or 33, wherein the protein comprises a therapeutic protein.

35. The genetic construct of any one of claims 32 to 34, wherein the protein comprises a cytokine, a receptor, a ligand, an immunomodulatory, a growth factor, an antigen, an RNA binding domain, or any combination thereof.
36. The genetic construct of any one of claims 32 to 35, wherein the protein comprises an antibody, a fusion protein, or any combination thereof.
37. The genetic construct of any one of claims 32 to 36, wherein the protein comprises CD3, CD4, CD5, CD19, CD20, CD22, CD30, CD34, HER2, HER3, HER4, LFA-I, MoI, p150, 95, VLA-4, ICAM-I, VCAM, alpha v/beta 3 integrin, human macrophage inflammatory protein (MIP-1 - alpha), erythropoietin (EPO), NGF-beta, platelet-derived growth factor (PDGF), fibroblast growth factors, epidermal growth factor (EGF), transforming growth factors (TGF), insulin-like growth factors-I and -II (IGF-I and IGF-II), des(1-3)-IGF-I (brain IGF-1), factor VIII, tissue factor, von Willebrands factor, protein C, alpha-1-antitrypsin, plasminogen activators, such as urokinase and tissue plasminogen activator ("t-PA"), bombazine, thrombin, thrombopoietin, M-CSF, GM-CSF, G-CSF, flk2/flt3 receptor, obesity (OB) receptor, LDL receptor, growth hormone receptors, thrombopoietin receptors ("TPO-R," "c-mpl"), glucagon receptors, interleukin receptors, interferon receptors, T-cell receptors, stem cell factor receptors, OX40L, bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), relaxin A-chain, relaxin B-chain, prorelaxin, interleukins and interleukin receptors, including IL-1 to IL-33 and IL-1 to IL-33 receptors, such as the IL-8 receptor, viral antigens, lipoproteins, calcitonin, glucagon, atrial natriuretic factor, lung surfactant, tumor necrosis factor-alpha and -beta, enkephalinase, RANTES (regulated on activation normally T-cell expressed and secreted), mouse gonadotropin-associated peptide, DNase, inhibin, activin, PUF domain, Cas protein, or any combination thereof.
38. A composition comprising the genetic construct of any one of claims 1 to 37.
39. A method of making a circular RNA, comprising initiating the genetic construct of any one of claims 1 to 37 to form a circular RNA.
40. A circular RNA prepared by the method of claim 39.
41. A method of treating a disease or condition in a subject in need thereof, comprising administering the circular RNA of claim 40 to the subject.