(12) STANDARD PATENT (11) Application No. **AU 2017372731 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Compositions and methods for enhancing gene expression

(51) International Patent Classification(s)
C12N 15/86 (2006.01)

(21) Application No: **2017372731** (22) Date of Filing: **2017.12.04**

(87) WIPO No: **WO18/106615**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/486,361	**2017.04.17**	**US**
62/587,954	**2017.11.17**	**US**
62/430,250	**2016.12.05**	**US**

(43) Publication Date: **2018.06.14**
(44) Accepted Journal Date: **2024.05.23**

(71) Applicant(s)
Janssen Pharmaceuticals, Inc.

(72) Inventor(s)
Kamrud, Kurt Iver;Win, Maung Nyan;Wang, Nathaniel Stephen;Dehart, Jason

(74) Agent / Attorney
Spruson & Ferguson, GPO Box 3898, Sydney, NSW, 2001, AU

(56) Related Art
SJÖBERG, E. M. et al., "A significantly improved Semliki Forest virus expression system based on translation enhancer segments from the viral capsid gene.", 1994. BIOTECHNOLOGY, VOL.12, pages 1127-31.
WO 2005/026316 A2
WO 95/31565 A1
FROLOV, I. et al., "TRANSLATION OF SINDBIS VIRUS MRNA: ANALYSIS OF SEQUENCES DOWNSTREAM OF THE INITIATING AUG CODON THAT ENHANCE TRANSLATION", 1996. JOURNAL OF VIROLOGY, Vol.70, No.2, pages 1182-1190.
VENTOSO, I. et al., "Translational resistance of late alphavirus mRNA to eIF2 phosphorylation: a strategy to overcome the antiviral effect of protein kinase PKR", 2006. GENES AND DEVELOPMENT, Vol.20, No.1, pages 87-100.
ATKINS, G.J. et al., "Therapeutic and prophylactic applications of alphavirus vectors", 2008. EXPERT REVIEWS IN MOLECULAR MEDI, Vol.10, No.1, pages e33/1-18.
US 2014/0079734 A1
WO 2004/055161 A2
KELLEY, B.J. et al., "Potential of alphavirus vectors in the treatment of advanced solid tumors", 2007. RECENT PATENTS ON ANTI-CANCER DRUG DISCOVERY, Vol.2, No.2, pages 159-166.
KIM, D.Y. et al., "Enhancement of protein expression by alphavirus replicons by designing self-replicating subgenomic RNAs", 2014. PNAS, Vol.111, No.29, pages 10708-10713.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 June 2018 (14.06.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/106615 A3

(51) International Patent Classification:
C12N 15/86 (2006.01)

(21) International Application Number:
PCT/US2017/064561

(22) International Filing Date:
04 December 2017 (04.12.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/430,250	05 December 2016 (05.12.2016)	US
62/486,361	17 April 2017 (17.04.2017)	US
62/587,954	17 November 2017 (17.11.2017)	US

(71) Applicant: **SYNTHETIC GENOMICS, INC.** [US/US]; 11149 North Torrey Pines Road, La Jolla, CA 92037 (US).

(72) Inventors: **KAMRUD, Kurt, Iver**; 11149 North Torrey Pines Road, La Jolla, CA 92037 (US). **WIN, Maung, Nyan**; 11149 North Torrey Pines Road, La Jolla, CA 92037 (US). **WANG, Nathaniel, Stephen**; 11149 North Torrey Pines Road, La Jolla CA 92037 (US). **DEHART, Jason**; 11149 North Torrey Pines Road, La Jolla, CA 92037 (US).

(74) Agent: **MALLON, Joseph, J.**; Knobbe, Martens, Olson & Bear, LLP, 2040 Main Street, 14th Floor, Irvine, CA 92614 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

(54) Title: COMPOSITIONS AND METHODS FOR ENHANCING GENE EXPRESSION

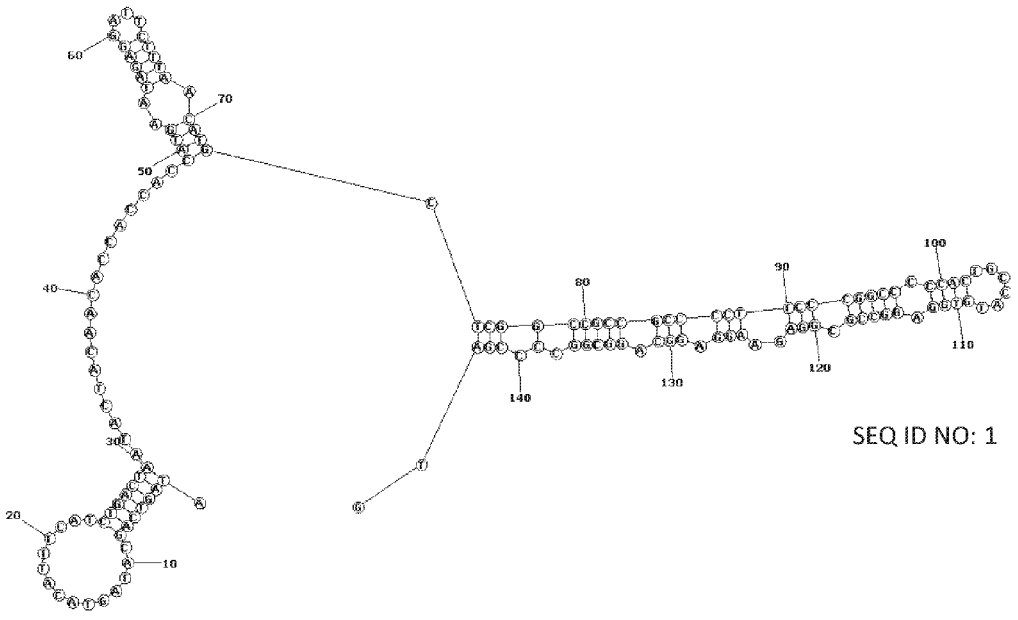


FIG 1

(57) Abstract: The present disclosure generally relates to nucleic acid molecules for use in regulating gene expression. Disclosed herein include nucleic acid molecules containing one or more structural elements of the viral capsid enhancer operably linked to a coding sequence of a gene of interest. In some embodiments, the viral capsid enhancer comprises a Downstream Loop (DLP) from a viral capsid protein, or a variant of the DLP.

WO 2018/106615 A3

[Continued on next page]

EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
09 August 2018 (09.08.2018)

COMPOSITIONS AND METHODS FOR ENHANCING GENE EXPRESSION

RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Application Serial No. 62/430,250, filed on December 5, 2016; U.S. Provisional Application Serial No. 62/486,361, filed on April 17, 2017; and U.S. Provisional Application Serial No. 62/587,954, filed on November 17, 2017. The contents of the above-referenced applications are hereby expressly incorporated by reference in their entireties.

INCORPORATION OF THE SEQUENCE LISTING

[0002] The material in the accompanying sequence listing is hereby incorporated by reference into this application. The accompanying sequence listing text file, named SGI012WO_SeqListing.txt, was created on December 4, 2017 and is 169 KB.

FIELD

[0003] The present disclosure relates to the field of molecular biology and genetic engineering, including nucleic acid molecules useful for regulating gene expression, and the use of the nucleic acid molecules for, for example, production of desired products in suitable host cells in cell culture or in a subject, and for conferring beneficial characteristics to the host cells or subjects.

BACKGROUND

[0004] Advances in biotechnology and molecular biology have offered many opportunities to develop recombinant cells and organisms with commercially desirable characteristics or traits. In particular, modern genetic engineering techniques have greatly accelerated the introduction of genes and hence new traits into recombinant cells and organisms. Proper expression level of a desirable gene in, for example, a host cell or a transgenic organism is helpful to achieve this goal.

[0005] However, despite the availability of many molecular tools, genetic modifications of host cells and organisms are often constrained by insufficient expression

level or uncontrolled expression of the gene of interest. Thus, there is still a need for regulatory elements capable of enhancing transgene expression in host cells and organisms. The identification of novel molecular tools including regulatory elements, expression vector, and expression systems that function in various types of organisms can be useful in developing genetically enhanced cells and organisms.

[0005a] Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

[0005b] Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

SUMMARY

[0006] This section provides a general summary of the present application, and is not comprehensive of its full scope or all of its features.

[0007] The present disclosure relates generally to methods and compositions useful for regulating, for example increasing, gene expression *in vitro*, *ex vivo*, or *in vivo*. The gene expression can be, for example, in animal cells and other eukaryotic cells. The gene can be, for example, a heterologous gene encoding a protein of interest.

[0007a] In one aspect, the present disclosure provides a nucleic acid molecule, comprising a modified viral RNA replicon, wherein the modified viral RNA replicon comprises: a first nucleic acid sequence encoding a viral capsid enhancer; and a second nucleic acid sequence encoding at least one nonstructural viral protein encoding a replicase, wherein the first nucleic acid sequence is operably linked upstream to the second nucleic acid sequence, wherein the modified viral RNA replicon is derived from a virus species belonging to the Togaviridae family or from a virus species belonging to the Arterivirus genus of the Arteriviridae family; and the viral capsid enhancer comprises a nucleotide sequence having a sequence identity of at least 80% to RNA corresponding to any one of SEQ ID Nos: 1 and 46-52.

[0007b] In another aspect, the present disclosure provides a nucleic acid molecule comprising a modified viral RNA replicon, wherein the modified viral RNA replicon comprises, ordered from the 5'- to 3'-end, (1) a 5' untranslated region (5'-UTR), (2) a nucleotide sequence encoding an amino-terminal fragment of the nsp1 of the VEEV, (3) a downstream loop (DLP) motif derived from Sindbis virus (SINV), (4) a nucleotide sequence encoding a 2A protease sequence (P2A), and (5) a nucleotide sequence encoding a polyprotein comprising the sequences of at least one of the non-structural proteins nsp1, nsp2, nsp3 and nsp4 of the VEEV.

[0007c] In another aspect, the present disclosure provides nucleic acid molecule comprising a nucleic acid sequence encoding the modified viral RNA replicon as described herein.

[0007d] In another aspect, the present disclosure provides a recombinant cell comprising a nucleic acid molecule of the invention.

[0007e] In another aspect, the present disclosure provides a method for producing a polypeptide of interest in a cell, comprising introducing a nucleic acid molecule of the invention into the cell, thereby producing the polypeptide encoded by at least the first GOI in the cell.

[0007f] In another aspect, the present disclosure provides a composition, comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier.

[0007g] In another aspect, the present disclosure provides a method for producing a polypeptide of interest in a subject, comprising administering to the subject a nucleic acid molecule of the invention.

[0008] In another aspect, some embodiments disclosed herein relate to a nucleic acid molecule, including (i) a first nucleic acid sequence encoding one or more RNA stem-loops of a viral capsid enhancer or a variant thereof; and (ii) a second nucleic acid sequence operably linked to the first nucleic acid sequence, wherein the second nucleic acid sequence comprises a coding sequence for a gene of interest (GOI).

[0009] Implementations of embodiments of the nucleic acid molecule according to the present disclosure can include one or more of the following features. In some embodiments, the first nucleic acid sequence is operably linked upstream to the coding

sequence for the GOI. In some embodiments, the nucleic acid molecule further includes a promoter operably linked upstream to the first nucleic acid sequence. In some embodiments, the nucleic acid molecule further includes a 5' UTR sequence operably linked upstream to the first nucleic acid sequence. In some embodiments, the 5' UTR sequence is operably linked downstream to the promoter and upstream to the first nucleic acid sequence. In some embodiments, the nucleic acid molecule further includes a coding sequence for an autoprotease peptide operably linked upstream to the second nucleic acid sequence. In some embodiments, the coding sequence for the autoprotease peptide is operably linked downstream to the first nucleic acid sequence and upstream to the second nucleic acid

sequence . In some embodiments, the autoprotease peptide comprises a peptide sequence selected from the group consisting of porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), and a combination thereof. In some embodiments, the nucleic acid molecule further includes a 3' UTR sequence operably linked downstream to the second sequence nucleic acid sequence.

[0010] In some embodiments, the viral capsid enhancer is derived from a capsid gene of a virus species belonging to the *Togaviridae* family. In some embodiments, the virus species belongs to the *Alphavirus* genus of the *Togaviridae* family. In some embodiments, the alphavirus species is Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), Everglades virus (EVEV), Mucambo virus (MUCV), Semliki forest virus (SFV), Pixuna virus (PIXV), Middleburg virus (MIDV), Chikungunya virus (CHIKV), O'Nyong-Nyong virus (ONNV), Ross River virus (RRV), Barmah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAGV), Bebaru virus (BEBV), Mayaro virus (MAYV), Una virus (UNAV), Sindbis virus (SINV), Aura virus (AURAV), Whataroa virus (WHAV), Babanki virus (BABV), Kyzylagach virus (KYZV), Western equine encephalitis virus (WEEV), Highland J virus (HJV), Fort Morgan virus (FMV), Ndumu (NDUV), Salmonid alphavirus (SAV), or Buggy Creek virus. In some embodiments, the viral capsid enhancer comprises a downstream loop (DLP) motif of the virus species, and wherein the DLP motif comprises at least one of the one or more RNA stem-loops. In some embodiments, the viral capsid enhancer comprises a nucleic acid sequence exhibiting at least 80% sequence identity to at least one of SEQ ID NOs: 1 and 46-52. In some embodiments, the nucleic acid sequence exhibits at least 95% sequence identity to at least one of SEQ ID NOs: 1 and 46-52.

[0011] In some embodiments, the coding sequence for the GOI encodes a polypeptide. In some embodiments, the polypeptide is a therapeutic polypeptide, a prophylactic polypeptide, a diagnostic polypeptide, a nutraceutical polypeptide, an industrial enzyme, a reporter polypeptide, or a combination thereof. In some embodiments, the

polypeptide is an antibody, an antigen, an immune modulator, a cytokine, an enzyme, or a combination thereof.

[0012] In some embodiments, the nucleic acid molecule of the disclosure further includes a third nucleic acid sequence encoding one or more RNA stem-loops of a second viral capsid enhancer or a variant thereof; and a fourth nucleic acid sequence operably linked to the third nucleic acid sequence, wherein the fourth nucleic acid sequence comprises a coding sequence for a second gene of interest (GOI). In some embodiments, the nucleic acid molecule further includes a coding sequence for a second autoprotease peptide operably linked downstream to the third nucleic acid sequence and upstream to the fourth nucleic acid sequence.

[0013] In some embodiments, the nucleic acid molecule of the disclosure is an mRNA molecule or an RNA replicon. In some embodiments, the nucleic acid molecule is an expression vector or a transcription vector. In some embodiments, the expression vector or a transcription vector further includes one or more additional transcription regulatory sequences. In some embodiments, the expression vector or a transcription vector further includes one or more additional transcription regulatory sequences. In some embodiments, the expression vector or a transcription vector further includes one or more additional translation regulatory sequences. In some embodiments, the nucleic acid molecule is a plasmid, a bacteriophage vector, a cosmid, a fosmid, a viral replicon, a shuttle vector, or a combination thereof. In some embodiments, the nucleic acid molecule is a prokaryotic vector or a eukaryotic vector. In some embodiments, the nucleic acid molecule is produced via de novo synthesis.

[0014] Also disclosed in some embodiments include a method for producing a polypeptide of interest in a cell, which includes introducing a nucleic acid molecule of according to the present disclosure into a cell, thereby producing a polypeptide encoded by the GOI in the cell. In yet another related aspect, some embodiments disclosed herein related to a method for producing a polypeptide of interest in a cell, which includes introducing a RNA molecule into the cell, wherein the RNA molecule comprises one or more RNA stem-loops of a viral capsid enhancer or a variant thereof, and a coding sequence for the polypeptide of interest, thereby producing the polypeptide of interest in the cell.

[0015] In some embodiments, the RNA molecule is a messenger RNA (mRNA) molecule or a replicion RNA molecule. In some embodiments, the RNA molecule is produced via *de novo* synthesis and/or *in vitro* transcription before being introduced into the cell. In some embodiments, the RNA molecule comprises a downstream loop (DLP) motif of a virus species, and wherein the DLP motif comprises at least one of the one or more RNA stem-loops of the viral capsid enhancer. In some embodiments, the RNA molecule further comprises a coding sequence for an autoprotease peptide downstream to at least one of the one or more RNA stem-loops and upstream to the coding sequence for the polypeptide of interest. In some embodiments, the autoprotease peptide comprises a peptide sequence selected from the group consisting of porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), and a combination thereof. In some embodiments, the polypeptide is a therapeutic polypeptide, a prophylactic polypeptide, a diagnostic polypeptide, a nutraceutical polypeptide, an industrial enzyme, a reporter polypeptide, or a combination thereof. In some embodiments, the polypeptide is an antibody, an antigen, an immune modulator, a cytokine, an enzyme, or a combination thereof. In some embodiments, the cell is present in a tissue, an organ, or a subject. In some embodiments, the subject is human, horse, pig, primate, mouse, ferret, rat, cotton rat, cattle, swine, sheep, rabbit, cat, dog, bird, fish, goat, donkey, hamster, or buffalo.

[0016] Some embodiments disclose a method for producing a messenger RNA (mRNA) in a cell. The method, in some embodiments, includes administering to the cell a nucleic acid molecule comprising a first nucleic acid sequence encoding one or more RNA stem-loops of a viral capsid enhancer or a variant thereof, and a second nucleic acid sequence operably linked to the first nucleic acid sequence, wherein the second nucleic acid sequence comprises a coding sequence for a gene of interest (GOI), thereby producing a mRNA of the GOI.

[0017] In some embodiments, the first nucleic acid sequence is operably linked upstream to the coding sequence for the GOI. In some embodiments, the nucleic acid molecule further includes a promoter operably linked upstream to the first nucleic acid

sequence. In some embodiments, the nucleic acid molecule further includes a 5' UTR sequence operably linked upstream to the first nucleic acid sequence. In some embodiments, the 5' UTR sequence is operably linked downstream to the promoter and upstream to the first nucleic acid sequence. In some embodiments, the nucleic acid molecule further includes a coding sequence for an autoprotease peptide operably linked upstream to the second nucleic acid sequence. In some embodiments, the coding sequence for the autoprotease peptide is operably linked downstream to the first nucleic acid sequence and upstream to the second nucleic acid sequence. In some embodiments, the autoprotease peptide comprises a peptide sequence selected from the group consisting of porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), and a combination thereof. In some embodiments, the nucleic acid molecule further includes a 3' UTR sequence operably linked downstream to the second sequence nucleic acid sequence.

[0018] In some embodiments disclosed herein, the viral capsid enhancer is derived from a capsid gene of a virus species belonging to the *Togaviridae* family. In some embodiments, the virus species belongs to the *Alphavirus* genus of the *Togaviridae* family. In some embodiments, the alphavirus species is Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), Everglades virus (EVEV), Mucambo virus (MUCV), Semliki forest virus (SFV), Pixuna virus (PIXV), Middleburg virus (MIDV), Chikungunya virus (CHIKV), O'Nyong-Nyong virus (ONNV), Ross River virus (RRV), Barmah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAGV), Bebaru virus (BEBV), Mayaro virus (MAYV), Una virus (UNAV), Sindbis virus (SINV), Aura virus (AURAV), Whataroa virus (WHAV), Babanki virus (BABV), Kyzylagach virus (KYZV), Western equine encephalitis virus (WEEV), Highland J virus (HJV), Fort Morgan virus (FMV), Ndumu (NDUV), Salmonid alphavirus (SAV), or Buggy Creek virus. In some embodiments, the viral capsid enhancer comprises a downstream loop (DLP) motif of the virus species, and wherein the DLP motif comprises at least one of the one or more RNA stem-loops. In some embodiments, the viral capsid enhancer comprises a nucleic acid sequence exhibiting at least 80% sequence identity to at least one of SEQ ID NOs: 1 and 46-

52. In some embodiments, the nucleic acid sequence exhibits at least 95% sequence identity to at least one of SEQ ID NOs: 1 and 46-52.

[0019] In some embodiments disclosed herein, the coding sequence for the GOI encodes a polypeptide. In some embodiments, the polypeptide is selected from the group consisting of a therapeutic polypeptide, a prophylactic polypeptide, a diagnostic polypeptide, a nutraceutical polypeptide, an industrial enzyme, a reporter polypeptide, and a combination thereof. In some embodiments, the polypeptide is an antibody, an antigen, an immune modulator, a cytokine, an enzyme, or a combination thereof. In some embodiments of the method for producing a messenger RNA (mRNA) according to the present disclosure, the nucleic acid molecule further includes a third nucleic acid sequence encoding one or more RNA stem-loops of a second viral capsid enhancer or a variant thereof; and a fourth nucleic acid sequence operably linked to the third nucleic acid sequence, wherein the fourth nucleic acid sequence comprises a coding sequence for a second gene of interest (GOI). In some embodiments, the nucleic acid molecule further includes a coding sequence for a second autoprotease peptide operably linked downstream to the third nucleic acid sequence and upstream to the fourth nucleic acid sequence.

[0020] The nucleic acid molecule of the present disclosure can be, in some embodiments, an RNA replicon. In some embodiments, the nucleic acid molecule is an expression vector or a transcription vector. In some embodiments, the nucleic acid molecule further comprises one or more additional transcription regulatory sequences. In some embodiments, the nucleic acid molecule further comprises . In some embodiments, one or more additional translation regulatory sequences. In some embodiments, the nucleic acid molecule is an expression vector selected from the group consisting of a plasmid, a bacteriophage vector, a cosmid, a fosmid, a viral replicon, a shuttle vector, and a combination thereof. In some embodiments, the nucleic acid molecule is a prokaryotic expression vector or a eukaryotic expression vector. In some embodiments, the cell is present in a tissue, an organ, or a subject. In some embodiments, the subject is human, horse, pig, primate, mouse, ferret, rat, cotton rat, cattle, swine, sheep, rabbit, cat, dog, bird, fish, goat, donkey, hamster, or buffalo. In some embodiments of the method for producing a messenger RNA (mRNA) according to the present disclosure further includes producing a polypeptide encoded by the

mRNA of the GOI in the cell. In some embodiments, the method further includes obtaining the produced mRNA of the GOI and ntroducing the obtained mRNA into a second cell for expression of a polypeptide encoded by the mRNA of the GOI in the second cell.

[0021] In one aspect, some embodiments of the disclosure relate to nucleic acid molecule comprising a nucleic acid sequence encoding a modified viral RNA replicon, wherein the modified viral RNA replicon comprises (i) a first nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer or a variant thereof, wherein the viral capsid enhancer is heterologous to the viral RNA replicon, and (ii) a second nucleic acid sequence encoding at least one nonstructural viral protein or a portion thereof, wherein the first nucleic acid sequence is operably linked upstream to the second nucleic acid sequence.

[0022] In some embodiments, at least one of the one or more structural elements of the viral capsid enhancer comprises one or more RNA stem-loops. In some embodiments, the viral capsid enhancer is derived from a capsid gene of a virus species belonging to the *Togaviridae* family. In some embodiments, the virus species belongs to the *Alphavirus* genus of the *Togaviridae* family. In some embodiments, the alphavirus species Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), Everglades virus (EVEV), Mucambo virus (MUCV), Semliki forest virus (SFV), Pixuna virus (PIXV), Middleburg virus (MIDV), Chikungunya virus (CHIKV), O'Nyong-Nyong virus (ONNV), Ross River virus (RRV), Barmah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAGV), Bebaru virus (BEBV), Mayaro virus (MAYV), Una virus (UNAV), Sindbis virus (SINV), Aura virus (AURAV), Whataroa virus (WHAV), Babanki virus (BABV), Kyzylagach virus (KYZV), Western equine encephalitis virus (WEEV), Highland J virus (HJV), Fort Morgan virus (FMV), Ndumu (NDUV), or Buggy Creek virus. In some embodiments, the viral capsid enhancer comprises a downstream loop (DLP) motif of the virus species, and wherein the DLP motif comprises at least one of the one or more RNA stem-loops. In some embodiments, the viral capsid enhancer comprises a nucleic acid sequence exhibiting at least 80% sequence identity to at least one of SEQ ID NOs: 1 and 46-52. In some embodiments, the nucleic acid sequence exhibits at least 95% sequence identity to at least one of SEQ ID NOs: 1 and 46-52.

[0023] In some embodiments, the nucleic acid sequence encoding the modified viral RNA replicon further comprising a coding sequence for an autoprotease peptide operably linked downstream to the first nucleic acid sequence and upstream to the second nucleic acid sequence. In some embodiments, the autoprotease peptide comprises a peptide sequence selected from the group consisting of porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), or a combination thereof. In some embodiments, the first nucleic acid sequence is operably positioned within a region of about 1 to 1000 nucleotides downstream of the 5'-terminus of the modified viral RNA replicon. the second nucleic acid sequence comprises substantially all the coding sequence for the native viral nonstructural proteins of the corresponding unmodified viral RNA replicon.

[0024] In some embodiments disclosed herein, the modified viral RNA replicon comprises a modified RNA replicon derived from a virus species belonging to the *Alphavirus* genus of the *Togaviridae* family or to the *Arterivirus* genus of the *Arteriviridae* family.

[0025] In some embodiments, the arterivirus virus species is Equine arteritis virus (EAV), Porcine respiratory and reproductive syndrome virus (PRRSV), Lactate dehydrogenase elevating virus (LDV), or Simian hemorrhagic fever virus (SHFV). In some embodiments, the first nucleic acid sequence is operably positioned upstream to a second nucleic acid sequence encoding a portion or the entire pp1ab nonstructural protein of the modified arterivirus RNA replicon. In some embodiments, the nucleic acid sequence encoding the modified arterivirus RNA replicon further comprising one or more expression cassettes, wherein at least one of the one or more expression cassettes comprises a promoter operably linked to a coding sequence for a gene of interest (GOI). In some embodiments, the modified arterivirus RNA replicon comprises at least two, three, four, five, or six expression cassettes. In some embodiments, at least one of the one or more expression cassettes is operably linked downstream of the second nucleic acid sequence encoding a portion or the entire pp1ab nonstructural protein of the modified arterivirus RNA replicon. In some embodiments, at least one of the one or more expression cassettes is operably positioned downstream to a transcriptional regulatory sequence (TRS) of the modified arterivirus RNA

replicon, wherein the TRS is TRS1, TRS2, TRS3, TRS4, TRS5, TRS6, or TRS7. In some embodiments, at least one of the one or more expression cassettes further comprises a third nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer, wherein the third nucleic acid sequence is operably linked upstream to the coding sequence for the GOI.

[0026] In some embodiments, the nucleic acid sequence encoding the modified arterivirus RNA replicon further comprises a coding sequence for an autoprotease peptide operably linked downstream to the third nucleic acid sequence and upstream to the coding sequence for the GOI. In some embodiments, the coding sequence for the GOI encodes a therapeutic polypeptide, a prophylactic polypeptide, a diagnostic polypeptide, a nutraceutical polypeptide, an industrial enzyme, a reporter polypeptide, or any combination thereof. In some embodiments, the coding sequence for the GOI encodes an antibody, an antigen, an immune modulator, a cytokine, an enzyme, or any combination thereof.

[0027] In some embodiments, the modified viral RNA replicon comprises a modified RNA replicon derived from an alphavirus virus species selected from the group consisting of Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), Everglades virus (EVEV), Mucambo virus (MUCV), Semliki forest virus (SFV), Pixuna virus (PIXV), Middleburg virus (MIDV), Chikungunya virus (CHIKV), O'Nyong-Nyong virus (ONNV), Ross River virus (RRV), Barmah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAGV), Bebaru virus (BEBV), Mayaro virus (MAYV), Una virus (UNAV), Sindbis virus (SINV), Aura virus (AURAV), Whataroa virus (WHAV), Babanki virus (BABV), Kyzylagach virus (KYZV), Western equine encephalitis virus (WEEV), Highland J virus (HJV), Fort Morgan virus (FMV), Ndumu (NDUV), Salmonid alphavirus (SAV), and Buggy Creek virus. In some embodiments, the first nucleic acid sequence is operably positioned upstream to a second nucleic acid sequence encoding one or more nonstructural proteins nsp1-4 or a portion thereof of the modified alphavirus RNA replicon. In some embodiments, the nucleic acid sequence encoding the modified alphavirus RNA replicon further comprises one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a coding sequence for a gene of interest (GOI). In some embodiments, the modified alphavirus RNA replicon comprises at least two,

three, four, five, or six expression cassettes. In some embodiments, t least one of the one or more expression cassettes is operably linked downstream of a nucleic acid sequence encoding one or more nonstructural proteins nsp1-4 or a portion thereof of the modified alphavirus RNA replicon. In some embodiments, at least one of the one or more expression cassettes further comprises a third nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer, wherein the third nucleic acid sequence is operably linked upstream of the coding sequence for the GOI. In some embodiments, the nucleic acid sequence encoding the modified alphavirus RNA replicon further comprises a coding sequence for an autoprotease peptide operably linked downstream to the third nucleic acid sequence and upstream to the coding sequence for the GOI. In some embodiments, the coding sequence for the GOI encodes a therapeutic polypeptide, a prophylactic polypeptide, a diagnostic polypeptide, a nutraceutical polypeptide, an industrial enzyme, a reporter polypeptide, or a combination thereof. In some embodiments, the coding sequence for the GOI encodes an antibody, an antigen, an immune modulator, an enzyme, a cytokine, or a combination thereof.

[0028] In one aspect, some embodiments of the disclosure relate to nucleic acid molecule comprising a nucleic acid sequence encoding a modified non-alphavirus RNA replicon, wherein the modified non-alphavirus RNA replicon comprising a first nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer or a variant thereof. In some embodiments, the nucleic acid sequence encoding the modified non-alphavirus RNA replicon further comprises a second nucleic acid sequence encoding at least one nonstructural viral protein or a portion thereof, wherein the first nucleic acid sequence is operably linked upstream to the second nucleic acid sequence. In some embodiments, nucleic acid sequence encoding the modified non-alphavirus RNA replicon further comprises a coding sequence for an autoprotease peptide operably linked downstream to the first nucleic acid sequence and upstream to the second nucleic acid sequence. In some embodiments, the autoprotease peptide comprises a peptide sequence selected from the group consisting of porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), or a combination thereof. In some embodiments, the nucleic acid sequence encoding the modified non-

alphavirus RNA replicon comprises a modified RNA replicon derived from a positive-strand RNA virus. In some embodiments, the positive-strand RNA virus is a virus species belonging to a family selected from the group consisting of *Togaviridae* family, *Flaviviridae* family, *Orthomyxoviridae* family, Rhabdoviridae family, and *Paramyxoviridae* family. In some embodiments, the positive-strand RNA virus is a virus species belonging to the *Arterivirus* genus of the *Arteriviridae* family.

[0029] In some embodiments disclosed herein, the nucleic acid sequence encoding the modified non-alphavirus RNA replicon further comprising one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a coding sequence for a gene of interest (GOI). In some embodiments, the modified non-alphavirus RNA replicon comprises at least two, three, four, five, or six expression cassettes. In some embodiments, at least one of the one or more expression cassettes is operably linked downstream of the second nucleic acid sequence encoding the at least one nonstructural viral protein or a portion thereof. In some embodiments, at least one of the one or more expression cassettes further comprises a third nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer, wherein the third nucleic acid sequence is operably linked upstream to the coding sequence for the GOI. In some embodiments, the nucleic acid sequence encoding the modified non-alphavirus RNA replicon further comprising a coding sequence for an autoprotease peptide operably linked downstream to the third nucleic acid sequence and upstream to the coding sequence for the GOI. In some embodiments, the nucleic acid molecule is produced via *de novo* synthesis.

[0030] In one aspect, some embodiments disclosed herein relate to a recombinant cell including a nucleic acid molecule as disclosed herein. In some embodiments, the recombinant cell is a prokaryotic cell or a eukaryotic cell. In some embodiments, the recombinant cell is an animal cell. In some embodiments, the nucleic acid molecule comprises a nucleic acid sequence encoding a modified RNA replicon, and wherein expression of the modified replicon RNA confers a resistance to innate immune response in the recombinant cell. In a related aspect, some embodiments disclosed herein relate to a cell culture which includes at least one recombinant cell as disclosed herein.

[0031] In some aspects, some embodiments disclosed herein relate to a method for conferring a resistance to the innate immune system in a subject which includes administering to the subject a nucleic acid molecule comprising a nucleic acid sequence which encodes a modified viral RNA replicon, wherein the modified viral RNA replicon comprises (i) a first nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer or a variant thereof, wherein the viral capsid enhancer is heterologous to the viral RNA replicon, and (ii) a second nucleic acid sequence encoding at least one nonstructural protein or a portion thereof, wherein the first nucleic acid sequence is operably linked upstream to the second nucleic acid sequence, and wherein expression of the modified replicon RNA encoded by the nucleic acid molecule confers a resistance to innate immune response in the subject. In some embodiments, the subject is selected from the group consisting of human, horse, pig, primate, mouse, ferret, rat, cotton rat, cattle, swine, sheep, rabbit, cat, dog, bird, fish, goat, donkey, hamster, and buffalo

[0032] In some aspect, some embodiments disclosed herein relate to a method for producing a polypeptide of interest in a subject which includes administering to the subject a nucleic acid molecule comprising a nucleic acid sequence which encodes a modified viral RNA replicon, wherein the modified viral RNA replicon comprises (i) a first nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer or a variant thereof, wherein the viral capsid enhancer is heterologous to the viral RNA replicon, and (ii) a second nucleic acid sequence encoding at least one nonstructural protein or a portion thereof, wherein the first nucleic acid sequence is operably linked upstream to the second nucleic acid sequence. In some embodiments, the subject is human, horse, pig, primate, mouse, ferret, rat, cotton rat, cattle, swine, sheep, rabbit, cat, dog, bird, fish, goat, donkey, hamster, or buffalo.

[0033] In some aspect, some embodiments disclosed herein relate to a method for producing a polypeptide of interest, which includes ulturing a host cell comprising a nucleic acid molecule which comprises a nucleic acid sequence encoding a modified viral RNA replicon, wherein the modified viral RNA replicon comprises (i) a first nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer or a variant thereof, wherein the viral capsid enhancer is heterologous to the viral RNA replicon, and (ii) a second

nucleic acid sequence encoding at least one nonstructural protein or a portion thereof, wherein the first nucleic acid sequence is operably linked upstream to the second nucleic acid sequence.

[0034] In some embodiments of the method for producing a polypeptide of interest according to the present disclosure, the subject is selected from the group consisting of human, horse, pig, primate, mouse, ferret, rat, cotton rat, cattle, swine, sheep, rabbit, cat, dog, bird, fish, goat, donkey, hamster, and buffalo. In some embodiments, at least one of the one or more structural elements of the viral capsid enhancer comprises one or more RNA stem-loops. In some embodiments, the viral capsid enhancer is derived from a capsid gene of a virus species belonging to the *Togaviridae* family. In some embodiments, the virus species belongs to the *Alphavirus* genus of the *Togaviridae* family. In some embodiments, the alphavirus species is Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), Everglades virus (EVEV), Mucambo virus (MUCV), Semliki forest virus (SFV), Pixuna virus (PIXV), Middleburg virus (MIDV), Chikungunya virus (CHIKV), O'Nyong-Nyong virus (ONNV), Ross River virus (RRV), Barmah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAGV), Bebaru virus (BEBV), Mayaro virus (MAYV), Una virus (UNAV), Sindbis virus (SINV), Aura virus (AURAV), Whataroa virus (WHAV), Babanki virus (BABV), Kyzylagach virus (KYZV), Western equine encephalitis virus (WEEV), Highland J virus (HJV), Fort Morgan virus (FMV), Ndumu (NDUV), or Buggy Creek virus. In some embodiments, the viral capsid enhancer comprises a downstream loop (DLP) motif of the virus species, and wherein the DLP motif comprises at least one of the one or more RNA stem-loops. In some embodiments, the viral capsid enhancer comprises a nucleic acid sequence exhibiting at least 80% sequence identity to at least one of SEQ ID NOs: 1 and 46-52. In some embodiments, the nucleic acid sequence exhibits at least 95% sequence identity to at least one of SEQ ID NOs: 1 and 46-52.

[0035] In some embodiments disclosed herein, the nucleic acid sequence encoding the modified viral RNA replicon further comprising a coding sequence for an autoprotease peptide operably linked downstream to the first nucleic acid sequence and upstream to the second nucleic acid sequence. In some embodiments, the autoprotease peptide comprises a peptide sequence selected from the group consisting of porcine

teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), or a combination thereof. In some embodiments, the first nucleic acid sequence is operably positioned within a region of about 1 to 1000 nucleotides downstream of the 5'-terminus of the modified viral RNA replicon. the second nucleic acid sequence comprises substantially all the coding sequence for the native viral nonstructural proteins of the corresponding unmodified viral RNA replicon.

[0036] In some embodiments, the modified viral RNA replicon comprises a modified RNA replicon derived from a virus species belonging to the *Alphavirus* genus of the *Togaviridae* family or to the *Arterivirus* genus of the *Arteriviridae* family. In some embodiments, the arterivirus virus species is Equine arteritis virus (EAV), Porcine respiratory and reproductive syndrome virus (PRRSV), Lactate dehydrogenase elevating virus (LDV), or Simian hemorrhagic fever virus (SHFV).

[0037] In some embodiments disclosed herein, the nucleic acid sequence encoding the modified arterivirus RNA replicon further comprises one or more expression cassettes, and wherein at least one of the expression cassettes comprises a promoter operably linked to a coding sequence for a gene of interest (GOI). In some embodiments, the virus species is an arterivirus, and wherein the first nucleic acid sequence is operably positioned upstream to a nucleic acid sequence encoding a portion or the entire pp1ab nonstructural protein of the modified arterivirus RNA replicon. In some embodiments, the modified arterivirus RNA replicon further comprises at least two, three, four, five, or six expression cassettes. In some embodiments, at least one of the one or more expression cassettes is operably linked downstream of the second nucleic acid sequence encoding a portion or the entire pp1ab nonstructural protein of the modified arterivirus RNA replicon. In some embodiments, at least one of the one or more expression cassettes is operably positioned downstream to a transcriptional regulatory sequence (TRS) of the modified arterivirus RNA replicon, wherein the TRS is TRS1, TRS2, TRS3, TRS4, TRS5, TRS6, or TRS7. In some embodiments, at least one of the one or more expression cassettes further comprises a third nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer,

wherein the third nucleic acid sequence is operably linked upstream to the coding sequence for the GOI. In some embodiments, the nucleic acid sequence encoding the modified arterivirus RNA replicon further comprising a coding sequence for an autoprotease peptide operably linked downstream to the third nucleic acid sequence and upstream to the coding sequence for the GOI. In some embodiments, the coding sequence for the GOI encodes a therapeutic polypeptide, a prophylactic polypeptide, a diagnostic polypeptide, a nutraceutical polypeptide, an industrial enzyme, a reporter polypeptide, or any combination thereof. In some embodiments, the coding sequence for the GOI encodes an antibody, an antigen, an immune modulator, a cytokine, an enzyme, or any combination thereof.

[0038] In some embodiments, the modified viral RNA replicon comprises a modified RNA replicon derived from an alphavirus virus species selected from the group consisting of Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), Everglades virus (EVEV), Mucambo virus (MUCV), Semliki forest virus (SFV), Pixuna virus (PIXV), Middleburg virus (MIDV), Chikungunya virus (CHIKV), O'Nyong-Nyong virus (ONNV), Ross River virus (RRV), Barmah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAGV), Bebaru virus (BEBV), Mayaro virus (MAYV), Una virus (UNAV), Sindbis virus (SINV), Aura virus (AURAV), Whataroa virus (WHAV), Babanki virus (BABV), Kyzylagach virus (KYZV), Western equine encephalitis virus (WEEV), Highland J virus (HJV), Fort Morgan virus (FMV), Ndumu (NDUV), Salmonid alphavirus (SAV), and Buggy Creek virus. In some embodiments, the first nucleic acid sequence is operably positioned upstream to a nucleic acid sequence encoding one or more nonstructural proteins nsp1-4 or a portion thereof of the modified alphavirus RNA replicon.

[0039] In some embodiments, the nucleic acid sequence encoding the modified alphavirus RNA replicon further comprises one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a coding sequence for a gene of interest (GOI). In some embodiments, the modified alphavirus RNA replicon comprises at least two, three, four, five, or six expression cassettes. In some embodiments, at least one of the one or more expression cassettes is operably linked downstream of a nucleic acid sequence encoding one or more nonstructural proteins nsp1-4 or a portion thereof of the modified alphavirus RNA replicon. In some embodiments, at least one of the one or more

expression cassettes further comprises a third nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer, wherein the third nucleic acid sequence is operably linked upstream of the coding sequence for the GOI. In some embodiments, the modified alphavirus RNA replicon further comprising a coding sequence for an autoprotease peptide operably linked downstream to the third nucleic acid sequence and upstream to the coding sequence for the GOI. In some embodiments, the coding sequence for the GOI encodes a therapeutic polypeptide, a prophylactic polypeptide, a diagnostic polypeptide, a nutraceutical polypeptide, an industrial enzyme, a reporter polypeptide, or any combination thereof. In some embodiments, the coding sequence for the GOI encodes an antibody, an antigen, an immune modulator, a cytokine, an enzyme, or any combination thereof.

[0040] In another aspect, some embodiments disclosed herein relate to a method for conferring a resistance to the innate immune system in a subject, comprising administering to the subject a nucleic acid molecule comprising a nucleic acid sequence encoding a modified non-alphavirus RNA replicon, wherein the modified non-alphavirus RNA replicon comprises a first nucleic acid sequence encoding one or more structural elements of an alphavirus capsid enhancer and wherein expression of the modified non-alphavirus RNA replicon encoded by the nucleic acid molecule confers a resistance to innate immune response in the subject. In some embodiments, the subject is selected from the group consisting of human, horse, pig, primate, mouse, ferret, rat, cotton rat, cattle, swine, sheep, rabbit, cat, dog, bird, fish, goat, donkey, hamster, and buffalo.

[0041] Also disclosed herein include a method for producing a polypeptide of interest in a subject, where the method comprises administering to the subject a nucleic acid molecule comprising a nucleic acid sequence encoding a modified non-alphavirus RNA replicon, wherein the modified non-alphavirus RNA replicon comprises a first nucleic acid sequence encoding one or more structural elements of an alphavirus capsid enhancer. In some embodiments, the subject is human, horse, pig, primate, mouse, ferret, rat, cotton rat, cattle, swine, sheep, rabbit, cat, dog, bird, fish, goat, donkey, hamster, or buffalo.

[0042] Some embodiments disclosed herein relate to a method for producing a polypeptide of interest, where the method comprises culturing a host cell comprising a nucleic acid molecule which comprises a nucleic acid sequence encoding a modified non-

alphavirus RNA replicon, wherein the modified non-alphavirus RNA replicon comprises a first nucleic acid sequence encoding one or more structural elements of an alphavirus capsid enhancer.

[0043] In some embodiments according to the above aspects of the disclosure, the modified non-alphavirus RNA replicon further comprising a second nucleic acid sequence encoding at least one nonstructural viral protein or a portion thereof, wherein the first nucleic acid sequence is operably linked upstream to the second nucleic acid sequence. In some embodiments, the modified non-alphavirus RNA replicon further comprises a coding sequence for an autoprotease peptide operably linked downstream to the first nucleic acid sequence and upstream to the second nucleic acid sequence. In some embodimentsthe autoprotease peptide comprises a peptide sequence selected from the group consisting of porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), and a combination thereof. In some embodiments, the modified non-alphavirus RNA replicon comprises a modified RNA replicon derived from a positive-strand RNA virus. In some embodiments, the modified non-alphavirus RNA replicon comprises a modified RNA replicon derived from a virus species belonging to *Togaviridae* family, *Flaviviridae* family, *Orthomyxoviridae* family, *Rhabdoviridae* family, or *Paramyxoviridae* family. In some embodiments, the modified non-alphavirus RNA replicon comprises a modified RNA replicon derived from a virus species belonging to the *Arterivirus* genus of the *Arteriviridae* family. In some embodiments, the sequence encoding the non-alphavirus modified RNA replicon further comprising one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a coding sequence for a gene of interest (GOI). In some embodiments, the modified non-alphavirus RNA replicon comprises at least two, three, four, five, or six expression cassettes. In some embodiments, at least one of the one or more expression cassettes is operably linked downstream of the second nucleic acid sequence encoding the at least one nonstructural viral protein or a portion thereof of the modified non-alphavirus RNA replicon. In some embodiments, at least one of the one or more expression cassettes further comprises a third nucleic acid sequence encoding one or more structural

elements of an alphavirus capsid enhancer, wherein the third nucleic acid sequence is operably linked upstream to the coding sequence for the GOI. In some embodiments, the modified non-alphavirus RNA replicon further comprises a coding sequence for an autoprotease peptide operably linked downstream to the third nucleic acid sequence and upstream to the coding sequence for the GOI.

[0044] In some aspects, some embodiments disclosed herein relate to recombinant polypeptides produced by a method in accordance with one or more embodiments described herein.

[0045] Some embodiments disclosed herein relate to a composition including a recombinant polypeptide as described herein and a pharmaceutically acceptable carrier.

[0046] Some embodiments disclosed herein relate to a composition including a nucleic acid molecule as disclosed herein and a pharmaceutically acceptable carrier.

[0047] In some embodiments, one or more of the compositions and/or molecules of the present application, *e.g.* nucleic acid molecules, RNA replicons, and polypeptides, is further formulated into a pharmaceutical formulation. In some embodiments, one or more of the compositions and/or molecules of the present application is formulated into a pharmaceutical formulation with covalent compounds, non-covalent compounds, physical compositions, or pharmaceutically acceptable buffers.

[0048] In some embodiments disclosed herein, one or more of the compositions and/or molecules of the present application, *e.g.* nucleic acid molecules, RNA replicons, and polypeptides, is further formulated for use as a protective composition (*e.g.*, vaccine) or therapeutic composition. In particular, protective compositions made in accordance with the present disclosure have a variety of uses including, but not limited to, use as vaccines and other therapeutic agents, use as diagnostic agents and use as antigens in the production of polyclonal or monoclonal antibodies.

[0049] The foregoing summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative embodiments and features described herein, further aspects, embodiments, objects and features of the application will become fully apparent from the drawings and the detailed description and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0050] **FIGURE 1** is a graphical illustration of a non-limiting exemplary stem-loop RNA structure of an alphavirus capsid enhancer.

[0051] **FIGURES 2A-2D** are graphical representations of four non-limiting exemplary nucleic acid molecules of the present disclosure, where each of the nucleic acid molecules comprises a coding sequence for an alphavirus capsid enhancer(*e.g.*, DLP motif) and a coding sequence for a gene of interest (GOI), *e.g.*, a red Firefly (rFF) reporter gene. **FIG. 2A**: rEx-DLP-rFF; **FIG. 2B**: rEx-DLP-pp1ab-rFF; **FIG. 2C**: rEx-DLP-2A-pp1ab-rFF; and **FIG. 2D**: rEx-DLP-2A-pp1ab-DLP-rFF. DLP: Downstream Loop sequence; 2A: autoprotease peptide; pp1ab: nonstructural polypeptide sequence; and rFF: coding sequence for red Firefly reporter gene.

[0052] **FIGURES 3A-3D** are graphical illustrations of four non-limiting exemplary nucleic acid molecules of the present disclosure, where each of the nucleic acid molecules comprises a coding sequence for an alphavirus capsid enhancer (*e.g.*, a DLP motif) and a coding sequence for a gene of interest (GOI), *e.g.*, a red Firefly (rFF) reporter gene. **FIG. 3A**: Alpha-R-rFF; **FIG. 3B**: Alpha-R-DLP-rFF; **FIG. 3C**: Alpha-R-DLP-2A-nsp-rFF; and **FIG. 3D**: Alpha-R-DLP-2A-nsp-DLP-rFF. DLP: Downstream Loop sequence; 2A: autoprotease peptide; nsp1-4: nonstructural polypeptide sequence; and rFF: coding sequence for red Firefly reporter gene.

[0053] **FIGURES 4A-4B** are graphical illustrations of two other non-limiting exemplary nucleic acid molecules of the present disclosure, where each of the nucleic acid molecules comprises encoding coding sequence for an alphavirus capsid enhancer (*e.g.*, a DLP motif) and a coding sequence for a gene of interest (GOI), *e.g.*, a red Firefly (rFF) reporter gene. **FIG. 4A**: Alpha-R-DLP-2A-rFF; and **FIG. 4B**: Alpha-R-DLP-2A-nsp-DLP-2A-rFF. DLP: Downstream Loop sequence; 2A: autoprotease peptide; nsp1-4: nonstructural polypeptide sequence; and rFF: coding sequence for red Firefly reporter gene.

[0054] **FIGURES 5A-5B** graphically summarizes the results of flow cytometry analysis and bulk luciferase analyses performed to demonstrate that incorporating a DLP motif upstream of nucleic acid sequence encoding either EAV nonstructural protein genes or a gene of interest positioned in the subgenomic RNA, *i.e.* rFF reporter gene, did not

negatively impact genomic RNA replication. In these experiments, FACS analysis (**FIG. 5A**) and bulk-cell luciferase assays (**FIG. 5B**) were carried out on electroporated cells.

[0055] **FIGURES 6A-B** graphically summarize the results of another exemplary flow cytometry analysis and bulk luciferase analysis performed to demonstrate that modified arterivirus replicon RNAs with a DLP motif incorporated upstream of the sequence encoding nonstructural protein genes can replicate and express efficiently in host cells that had been treated with IFN to induce the cellular innate immune system. In these experiments, FACS analysis (**FIG. 6A**) and bulk-cell luciferase assays (**FIG. 6B**) were carried out on electroporated cells. IFN was added to cell culture media five hours post electroporation. Samples were collected in triplicate eighteen hours post electroporation for analysis.

[0056] **FIGURES 7A-C** graphically summarizes the results of another exemplary bulk luciferase analysis performed to demonstrate that modified alphavirus replicon RNAs with a DLP motif incorporated upstream of the sequence encoding nonstructural protein genes can replicate and express efficiently in host cells that had been treated with IFN to induce the cellular innate immune system. In these experiments, bulk-cell luciferase assays were carried out on electroporated cells. IFN was added to cell culture media immediately after electroporation or three hours post electroporation. Samples were collected in triplicate eighteen hours post electroporation for analysis. **FIG. 7A**: α-rFF versus alpha-R-rFF construct; **FIG. 7B**: α-rFF versus α-DLP-2A-nsp-rFF; and **FIG. 7C**: α-rFF versus alpha-R-DLP-2A-nsp-rFF construct.

[0057] **FIGURE 8** graphically summarizes the results of exemplary *in vivo* experiments performed to demonstrate that modified alphavirus replicon RNAs with a DLP motif incorporated upstream of the sequence encoding nonstructural protein genes can replicate and express efficiently in Balb/c mice. In these experiments, whole body imaging of animals that had been injected with a modified alphavirus replicon RNA was conducted. Each animal received 7.5 μg of replicon RNA injected intramuscularly. Individual animals were imaged on day 1, day 3, and day 7. Original: mice injected with the alpha-R-rFF construct; DLP: mice injected with the alpha-R-DLP-2A-nsp-rFF construct.

[0058] **FIGURE 9** schematically depicts a non-limiting exemplary alphavirus genomic structure and genome expression (adapted from Strauss *et al.*, Microbiological

Reviews, pp. 491-562, September 1994). Genome organization of a Sindbis virus (SINV) is shown. The names of the nonstructural genes and structural protein genes are given. Referenced to the nomenclature of the genes and proteins can be found in Strauss *et al.*, supra, 1994. The 49S genomic RNA is illustrated schematically in the center, with its translated ORF shown as an open box. Small black boxes are conserved sequence elements; the open diamond denotes the leaky opal termination codon. The nonstructural polyproteins and their processed products are shown above. Termination at the opal codon produces P123, whose major function in replication is believed to be as a proteinase that acts in trans to process the polyproteins in active RNA replicases; this proteinase domain is found in the nsP2 region. Read-through of the opal stop codon produces P1234, which can form an active replicase. The 26S subgenomic mRNA is expanded below to show the structural ORF and its translation products. Polypeptides present in the virion are shaded. vcRNA is the minus-strand complement of the genomic RNA.

[0059] **FIGURE 10** schematically depicts EAV genomic structure and genome expression strategy. The names of the replicase gene and structural protein genes are given (references to the nomenclature of genes and proteins can be found in Snijder *et al.*, 2005). Below the genome organization, the structural relationships of the genome and sg mRNAs are depicted. The leader sequence and TRSs found at the 5' end of the EAV mRNAs are indicated as blue and orange boxes, respectively. The ribosomal frameshifting element (RFS) found in the genome-length mRNA1 is indicated and the translated region of each mRNA is highlighted by a green line, whereas translationally silent regions are indicated by a red line. Only the translated open reading frames are indicated for each mRNA. The right-hand panels show a typical pattern of EAV mRNAs isolated from infected cells, visualized by hybridization to a probe complementary to the 3' end of the genome and therefore recognizing all viral mRNA species.

[0060] **FIGURES 11A-B** schematically show the predicted stem-loop RNA structure of the 5' CDS region of alphavirus mRNA 26S with a valley-peak topology. Two dimensional (2D) models of RNA structure based for the first 70–140 nucleotides of the CDS from seven representative Alphavirus mRNAs (SINV, SFV, RRV, SAGV, GETV, MIDV, UNAV, BEBV, MAYV and AURAV). The sequences were numbered from the initiation

codon (AUGi), with A being the +1 position. The predicted structures are constructed based on SHAPE (selective 2'-hydroxyl acylation and primer extension) data (Toribio *et al.*, 2016).

[0061] **FIGURES 12A-C** graphically summarize the results of exemplary *in vivo* experiments performed to demonstrate that modified alphavirus replicon RNAs with a DLP motif effect on immunogenicity in Balb/c mice. In this experiment, 6-8 week old BALB/c animals were primed at Days 0 and 42 using varying doses of the replicon RNA. Spleens and serum were collected on Day 56, and (a) flow cytometry for HA-specific T cell memory ($CD8^+CD44^+CD62L^{Lo}KLRG\text{-}1^{Lo}IL\text{-}7Ra^{Hi}CXCR3^{Hi}$) using Dextramers for detection (H-2 Kd [IYSTVASSL; SEQ ID NO: 44]) and (b,c) IFN-γ ELISpot to quantify $CD8^+$ and $CD4^+$ T cell effector responses. Statistics were one using multiple comparisons between matched doses using an ordinary one-way analysis of variance (ANOVA). **FIG. 12A**: A significant increase in memory precursor effector cells (MPECs) was observed in constructs containing the DLP motif compared with each comparable dose of unmodified replicon. **FIG. 12B**: Effector T cell responses were measured by the number of antigen-specific HA cells that were secreting IFN-γ following stimulation with a CD8+ T cell peptide. **FIG.12C**: Effector T cell responses were measured by the number of antigen-specific HA cells that were secreting IFN-γ following stimulation with a CD4+ T cell peptide.

[0062] **FIGURE 13** graphically summarizes the results of exemplary *in vivo* experiments performed to demonstrate that modified alphavirus replicon RNAs with a DLP motif incorporated upstream of the sequence encoding nonstructural protein genes effectively prevent suppression of immune response upon pre-treatment with agents that simulate viral infection in Balb/c mic. 6-8 week old BALB/c animals were pre-treated with 20 μg of Poly(I:C) or saline administered via hydrodynamic tail vein injection 24 hours before vaccination to simulate an ongoing viral infection. Mice were then primed at Day 0 and boosted at Day 28 using a 1.5 μg dose of RNA replicon encoding HA. Serum was collected on Day 42, and HA-specific antibodies were measured in the serum. Serum antibody concentrations were calculated by interpolation of dilution versus optical density on a four-parametic logistic regression and using the 8D2 HA-specific monoclonal antibody as a standard. Statistics between individual groups were conducted using a Mann-Whitney (non-parametric) test.

[0063] **FIGURES 14A-14C** graphically summarize the results of *in vivo* experiments performed to demonstrate that the DLP-containing replicons according to the present disclosure are compatible with LNP (cationic lipid nanoparticle) formulations. In this experiment, 6-8 week old BALB/c animals were primed at Days 0 and boosted at Day 28 using varying doses of an RNA replicon encoding HA. Spleens and serum were collected on Day 42. **FIG. 14A**: HA-specific antibodies were measured in the serum. Serum antibody titer is the inverse of the EC20% and was calculated by interpolation of dilution versus optical density on a four-parametric logistic regression. **FIG. 14B:** IFN-γ ELISpot used to quantify CD8+ cell effector responses. For detection of antigen-specific CD8+ T cells, splenocytes were incubated with the H-2 Kd (IYSTVASSL; SEQ ID NO: 44) peptide. **FIG. 14C:** IFN-γ ELISpot used to quantify CD4+ T cell effector responses. For detection of antigen-specific CD4+ T cells, splenocytes were incubated with H2-D restricted CD4 T cell epitope KSSFFRNVVWLIKKN (SEQ ID NO: 45). Statistics between individual groups were conducted using a Mann-Whitney (non-parametric) test.

[0064] **FIGURE 15** graphically illustrates of a non-limiting exemplary configuration of DLP-containing mRNA, in which a Sindbis virus DLP element is placed upstream of a coding sequence for a gene of interest (GOI; dsGFP), and a 5' UTR sequence is placed immediately downstream of a T7 promoter and upstream of the Sindbis virus DLP sequence. The coding sequence for dsGFP is linked to the DLP element via a P2A signal, which is an autocatalytic self-cleaving peptide (*e.g.*, autoprotease peptide) derived from the porcine teschovirus-1. Also shown at the bottom portion of the figure is another non-limiting exemplary configuration of DLP-containing mRNA, in which a coding sequence for a destabilized form of EGFP reporter gene (dsGFP) used as a GOI is operably linked to the proteolytic PEST degradation signal derived from a mouse ornithine decarboxylase gene (MODC).

[0065] **FIGURES 16A-D** graphically summarize the results of experiments performed to demonstrate that DLP-containing modified mRNAs can confer interferon resistance. **FIG. 16A:** inclusion of DLP in mRNA results in a statistically significant increase in the frequency of GFP positive cells in the presence of IFN. Mean with 95% confidence intervals in Kruskai-Wallist test (non-parametric). **FIG. 16B:** unmodified mRNA is sensitive

to IFN treatment (mean with 95% confidence intervals in 2-way ANOVA. Interaction: p=0.0083. Row: p=<0.0001. Column: p=0.0273. Sidak's multiple comparison test with * p=0.0217 and # p=<0.0241). **FIG. 16C:** DLP modified mRNA yields a statistically significant 30% increase in protein production per cell compared to unmodified mRNA in the presence of IFN (mean with 95% confidence intervals in 2-way ANOVA: p=<0.0001. Sidak's multiple comparison test with *** p=<0.0002 and **** p=<0.0001). **FIG. 16D:** DLP modified mRNA in the presence of IFN produces an equivalent amount of protein compared to unmodified mRNA in the absence of IFN treatment (mean with 95% confidence intervals in 2-way ANOVA. Interaction: p=<0.0001. Row: p=<0.0001. Column: p=0.0023. Sidak's multiple comparison test with **** p=<0.0001 and ** p=<0.0023).

[0066] The foregoing and other features of the present disclosure will become more fully apparent from the following description and appended claims, taken in conjunction with the accompanying drawings. Understanding that these drawings depict only several embodiments in accordance with the disclosure and are not to be considered limiting of its scope; the disclosure will be described with additional specificity and detail through use of the accompanying drawings.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0067] The present disclosure generally relates to compositions and methods for use in regulating gene expression in cells. Some embodiments of the disclosure relate to expression systems, such as viral-based expression systems, with superior expression potential which are suitable for expressing heterologous molecules such as, for example, vaccines and therapeutic polypeptides, in recombinant cells. For example, some embodiments of the disclosure relate to nucleic acid molecules containing one or more structural elements of a viral capsid enhancer or a variant thereof. In some embodiments, at least one of the one or more structural elements comprises a RNA stem-loop. In some embodiments, at least one of the one or more structural elements is operably linked to a coding sequence of a gene of interest. Some embodiments of the disclosure relate to nucleic acid molecules such as transcription and/or expression constructs and vectors, containing a nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer.

Also disclosed herein in some embodiments are transcription vectors and expression vectors, such as viral-based vectors, comprising a coding sequence of a gene of interest. In some embodiments, the nucleic acid molecules of the present disclosure, *e.g.*, messenger (mRNA) and RNA replicon, are generated via *de novo* synthesis and/or in vitro transcription. Recombinant cells that are genetically modified to include one or more of the nucleic acid molecules disclosed herein, as well as biomaterials and recombinant products derived from such cells are also within the scope of the application. Further provided herein are compositions and kits that include one or more of the nucleic acid molecules and/or recombinant cells disclosed herein, as well as methods for conferring a resistance to the innate immune system in a host cell.

[0068] In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative alternatives described in the detailed description, drawings, and claims are not meant to be limiting. Other alternatives may be used, and other changes may be made, without departing from the spirit or scope of the subject matter presented here. It will be readily understood that the aspects, as generally described herein, and illustrated in the Figures, can be arranged, substituted, combined, and designed in a wide variety of different configurations, all of which are explicitly contemplated and make part of the present application.

[0069] Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this application pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. Many of the techniques and procedures described or referenced herein are well understood and commonly employed using conventional methodology by those skilled in the art.

Some Definitions

[0070] The singular form “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes one or more cells, comprising mixtures thereof.

[0071] The term “about”, as used herein, has its ordinary meaning of approximately. If the degree of approximation is not otherwise clear from the context, “about” means either within plus or minus 10% of the provided value, or rounded to the nearest significant figure, in all cases inclusive of the provided value. Where ranges are provided, they are inclusive of the boundary values.

[0072] The terms, "cells", "cell cultures", "cell line", "recombinant host cells", "recipient cells" and "host cells" as used herein, include the primary subject cells and any progeny thereof, without regard to the number of transfers. In some situations, a progeny is not exactly identical to the parental cell (due to deliberate or inadvertent mutations or differences in environment); however, such altered progeny is included in these terms, so long as the progeny retain the same or substantially similar functionality as that of the originally transformed cell.

[0073] As used herein, the term "construct" is intended to mean any recombinant nucleic acid molecule such as an expression cassette, plasmid, cosmid, fosmid, viral replicon, shuttle vector, autonomously replicating polynucleotide molecule, bacteriophage, or linear or circular, single-stranded or double-stranded, DNA or RNA polynucleotide molecule, derived from any source, capable of genomic integration or autonomous replication, comprising a nucleic acid molecule where nucleic acid sequences are linked in a functionally operative manner, *e.g.* operably linked.

[0074] The term "derived from" used herein refers to an origin or source, and may include naturally-occurring, recombinant, unpurified or purified molecules. The molecules of the present disclosure may be derived from viral or non-viral molecules. A protein or polypeptide derived from an original protein or polypeptide may include the original protein or polypeptide, in part or in whole, and may be a fragment or variant of the original protein or polypeptide.

[0075] The term "gene" is used broadly to refer to any segment of nucleic acid molecule that encodes a protein or that can be transcribed into a functional RNA. Genes may

include sequences that are transcribed but are not part of a final, mature, and/or functional RNA transcript, and genes that encode proteins may further comprise sequences that are transcribed but not translated, for example, 5' untranslated regions, 3' untranslated regions, introns, *etc.* Further, genes may optionally further comprise regulatory sequences required for their expression, and such sequences may be, for example, sequences that are not transcribed or translated. Genes can be obtained from a variety of sources, including cloning from a source of interest or synthesizing from known or predicted sequence information, and may include sequences designed to have desired parameters.

[0076] The term "native" is used herein to refer to nucleic acid sequences or amino acid sequences as they naturally occur in the host. The term "non-native" is used herein to refer to nucleic acid sequences or amino acid sequences that do not occur naturally in the host, or are not configured as they are naturally configured in the host. A nucleic acid sequence or amino acid sequence that has been removed from a host cell, subjected to laboratory manipulation, and introduced or reintroduced into a host cell is considered "non-native." Synthetic genes or partially synthetic genes introduced into a host cell or organism are "non-native." Non-native genes further include genes endogenous to the host cell operably linked to one or more heterologous regulatory sequences that have been recombined into the host genome, or genes endogenous to the host cell or organism that are in a locus of the genome other than that where they naturally occur.

[0077] The terms “naturally-occurring” and “wild-type”, as used herein, refer to a form found in nature. For example, a naturally-occurring or wild-type nucleic acid molecule, nucleic acid sequence or protein may be present in and isolated from a natural source, and is not intentionally modified by human manipulation. As described in detail below, the nucleic acid molecules according to some embodiments of the present disclosure are non-naturally occurring nucleic acid molecules.

[0078] The term "heterologous" when used in reference to a polynucleotide, a gene, or a nucleic acid molecule refers to a polynucleotide, gene, or a nucleic acid molecule that is not derived from the host species. For example, "heterologous gene" or "heterologous nucleic acid sequence" as used herein, refers to a gene or nucleic acid sequence from a different species than the species of the host organism it is introduced into. When referring

to a gene regulatory sequence such as, for example, an enhancer sequence, or to an auxiliary nucleic acid sequence used for manipulating expression of a gene sequence (*e.g.* a 5' untranslated region, 3' untranslated region, poly A addition sequence, *etc.*) or to a nucleic acid sequence encoding a protein domain or protein localization sequence, "heterologous" means that the regulatory or auxiliary sequence or sequence encoding a protein domain or localization sequence is from a different source than the gene with which the regulatory or auxiliary nucleic acid sequence or nucleic acid sequence encoding a protein domain or localization sequence is juxtaposed in a genome. Thus, a promoter operably linked to a gene to which it is not operably linked to in its natural state (for example, in the genome of a non-genetically engineered organism) is referred to herein as a "heterologous promoter," even though the promoter may be derived from the same species (or, in some cases, the same organism) as the gene to which it is linked. For example, in some embodiments disclosed herein, a coding sequence of a heterologous gene of interest (GOI) is not linked to the recombinant RNA replicon sequence in its natural state. In some embodiments, the coding GOI sequence is derived from another organism, such as another virus, bacteria, fungi, human cell (tumor Ag), parasite (malaria), *etc.*)

[0079] The terms "nucleic acid molecule" and "polynucleotide" are used interchangeably herein, and refer to both RNA and DNA molecules, including nucleic acid molecules comprising cDNA, genomic DNA, synthetic DNA, and DNA or RNA molecules containing nucleic acid analogs. Nucleic acid molecules can have any three-dimensional structure. A nucleic acid molecule can be double-stranded or single-stranded (*e.g.*, a sense strand or an antisense strand). Non-limiting examples of nucleic acid molecules include genes, gene fragments, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, siRNA, micro-RNA, tracrRNAs, crRNAs, guide RNAs, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, nucleic acid probes and nucleic acid primers. A nucleic acid molecule may contain unconventional or modified nucleotides. The terms “polynucleotide sequence” and “nucleic acid sequence” as used herein interchangeably refer to the sequence of a polynucleotide molecule. The nomenclature for nucleotide bases as set forth in 37 CFR §1.822 is used herein. The nucleic acid molecules of the present disclosure can be synthesized *ex vitro* by any means known in the art, for example, using one

or more chemical or enzymatic techniques (for example, by use of chemical nucleic acid synthesis, or by use of enzymes for the replication, polymerization, exonucleolytic digestion, endonucleolytic digestion, ligation, reverse transcription, transcription, base modification (including, *e.g.*, methylation), or recombination (including homologous and site-specific recombination) of nucleic acid molecules. In some embodiments, the nucleic acid molecules of the present disclosure are generated from *de novo* synthesis. In some embodiments, nucleic acid molecules can be synthesized *de novo* in whole or in part, using known chemical methods, known enzymatic techniques, or any combination thereof. For example, the component nucleic acid sequences can be synthesized by solid phase techniques, removed from the resin, and purified by preparative high performance liquid chromatography followed by chemical linkage and/or enzymatic ligation to form a chimeric nucleic acid molecule. The composition of the synthetic nucleic acid molecules may be confirmed by nucleic acid analysis or sequencing. In some embodiments, the nucleic acid molecules of the present disclosure can be enzymatically assembled from chemically synthesized oligonucleotides using techniques known in the art.

[0080] Nucleic acid molecules of the present disclosure can be nucleic acid molecules of any length, for example between about 0.5 Kb and about 1000 Kb, between about 0.5 Kb and about 500 Kb, between about 1 Kb and about 100 Kb, between about 2 Kb and about 50 Kb, or between about 5 Kb and about 20 Kb. In some embodiments, the nucleic acid molecule is, or is about, 0.5 Kb, 1 Kb, 2 Kb, 3 Kb, 4 Kb, 5 Kb, 6 Kb, 7 Kb, 8 Kb, 9 Kb, 10 Kb, 15 Kb, 20 Kb, 25 Kb, 30 Kb, 40 Kb, 50 Kb, 100 Kb, 200 Kb, 500 Kb, 1 Mb, or more, or a range between any two of these values.

[0081] The polynucleotides of the present disclosure can be "biologically active" with respect to either a structural attribute, such as the capacity of a nucleic acid to hybridize to another nucleic acid, or the ability of a polynucleotide sequence to be recognized and bound by one or more of a transcription factor, a ribosome, and a nucleic acid polymerase.

[0082] The term "recombinant" or "engineered" nucleic acid molecule as used herein, refers to a nucleic acid molecule that has been altered through human intervention. As non-limiting examples, a cDNA is a recombinant DNA molecule, as is any nucleic acid molecule that has been generated by *ex vitro* polymerase reaction(s), or to which linkers have

been attached, or that has been integrated into a vector, such as a cloning vector or expression vector. As non-limiting examples, a recombinant nucleic acid molecule: 1) has been synthesized or modified *ex vitro*, for example, using chemical or enzymatic techniques (for example, by use of chemical nucleic acid synthesis, or by use of enzymes for the replication, polymerization, exonucleolytic digestion, endonucleolytic digestion, ligation, reverse transcription, transcription, base modification (including, *e.g.*, methylation), or recombination (including homologous and site-specific recombination) of nucleic acid molecules; 2) includes conjoined nucleotide sequences that are not conjoined in nature, 3) has been engineered using molecular cloning techniques such that it lacks one or more nucleotides with respect to the naturally-occurring nucleic acid molecule sequence, and/or 4) has been manipulated using molecular cloning techniques such that it has one or more sequence changes or rearrangements with respect to the naturally-occurring nucleic acid sequence. As non-limiting examples, a cDNA is a recombinant DNA molecule, as is any nucleic acid molecule that has been generated by *ex vitro* polymerase reaction(s), or to which linkers have been attached, or that has been integrated into a vector, such as a cloning vector or expression vector. In some embodiments disclosed herein, the recombinant nucleic acid molecules of the present application are generated from *de novo* synthesis.

[0083] The term "variant" of a protein used herein refers to a polypeptide having an amino acid sequence that is the same or essentially the same as that of the reference protein except having at least one amino acid modified, for example, deleted, inserted, or replaced, respectively. The amino acid replacement may be a conservative amino acid substitution, preferably at a non-essential amino acid residue in the protein. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains are known in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), non-polar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.* , threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). A variant of a

protein may have an amino acid sequence at least about 80%, 90%, 95%, or 99%, preferably at least about 90%, more preferably at least about 95%, identical to the amino acid sequence of the protein. Preferably, a variant is a functional variant of a protein that retains the same function as the protein. The terms "variant", when used in reference to a nucleic acid sequence, refer to a nucleic acid sequence that differs by one or more nucleotides from another, usually related nucleotide acid sequence. As such, the term "variant" can refer to a change of one or more nucleotides of a reference nucleic acid which includes the insertion of one or more new nucleotides, deletion of one or more nucleotides, and substitution of one or more existing nucleotides. A "variation" is a difference between two different nucleotide sequences; typically, one sequence is a reference sequence. Broadly, the term "nucleotide variation" as used herein includes point mutation, multiple mutation, single nucleotide polymorphism (SNP), deletion, insertion, and translocation. The term "reference nucleic acid" is used herein to describe a nucleotide sequence having a known reference sequence of interest.

[0084] As used herein, the terms, "identical" or percent "identity", in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence over a comparison window. Unless otherwise specified, the comparison window for a selected sequence, *e.g.*, "SEQ ID NO: X" is the entire length of SEQ ID NO: X, and, *e.g.*, the comparison window for "100 bp of SEQ ID NO: X" is the stated 100 bp. The degree of amino acid or nucleic acid sequence identity can be determined by various computer programs for aligning the sequences to be compared based on designated program parameters. For example, sequences can be aligned and compared using the local homology algorithm of Smith & Waterman *Adv. Appl. Math.* 2:482-89, 1981, the homology alignment algorithm of Needleman & Wunsch *J. Mol. Biol.* 48:443-53, 1970, or the search for similarity method of Pearson & Lipman *Proc. Nat'l. Acad. Sci. USA* 85:2444-48, 1988, and can be aligned and compared based on visual inspection or can use computer programs for the analysis (for example, GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI).

[0085] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, *e.g.*, Karlin & Altschul, *Proc. Nat'l. Acad. Sci.* USA 90:5873-87, 1993). The smallest sum probability (P(N)), provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, preferably less than about 0.01, and more preferably less than about 0.001.

[0086] As used herein, the term "vector" refers to a recombinant polynucleotide construct designed for transfer to a host cell, or between host cells, and that may be used for the purpose of transformation, *e.g.* the introduction of heterologous DNA into a host cell. A vector can be, for example a replicon, such as a plasmid, bacteriophage, or cosmid, into which another DNA segment may be inserted so as to bring about the replication of the inserted segment. Generally, a vector is capable of replication when associated with the proper control elements. The term "vector" includes cloning vectors and expression vectors, as well as viral vectors and integrating vectors. An "expression vector" is a vector that includes a regulatory region, thereby capable of expressing DNA sequences and fragments, for example *ex vitro*, *ex vivo*, and *in vivo*. In some embodiments, the vector is a plasmid, a bacteriophage vector, a cosmid, a fosmid, a viral replicon, or a combination thereof. In some embodiments, the vector is a eukaryotic vector, a prokaryotic vector (*e.g.*, a bacterial plasmid), or a shuttle vector. An expression system can be, for example, an expression vector or an expression cassette. In some embodiments, the vector is a transcription vector. The term "transcription vector" refers to a vector capable of being transcribed but not translated. For example, transcription vectors can be used to amplify their insert.

[0087] Virus-based "replicon" expression vectors can be used as, for example, vaccines and therapeutic compositions. Replicon vectors may be utilized in several formats, including DNA, RNA, and recombinant viral particles. A wide body of literature has now demonstrated efficacy of viral replicon vectors for applications such as vaccines. Moreover, these terms may be referred to collectively as vectors, vector constructs or gene delivery vectors.

[0088] As will be understood by one having ordinary skill in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, *etc.* As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

Viral Capsid Enhancers

[0089] Some viruses have sequences capable of forming one or more stem-loop structures which regulate, for example increase, capsid gene expression. The term "viral capsid enhancer" is used herein to refer to a regulatory element comprising sequences capable of forming such stem-loop structures. In some examples, the stem-loop structures are formed by sequences within the coding sequence of a capsid protein and named Downstream Loop (DLP) sequence. As disclosed herein, these stem-loop structures or variants thereof can be used to regulate, for example increase, expression level of genes of interest. For example, these stem-loop structures or variants thereof can be used in a recombinant vector (*e.g.*, in a heterologous viral genome) for enhancing transcription and/or translation of coding sequence operably linked downstream thereto. As an example, members of the *Alphavirus* genus can resist the activation of antiviral RNA-activated protein kinase (PKR) by means of a prominent RNA structure present within in viral 26S transcripts, which allows an eIF2-independent translation initiation of these mRNAs. This structure, called the downstream loop (DLP), is located downstream from the AUG in SINV 26S mRNA and in other members of the *Alphavirus* genus. In the case of Sindbis virus, the DLP motif is found in the first ~150 nt of the Sindbis subgenomic RNA. The hairpin is located downstream of the

Sindbis capsid AUG initiation codon (AUG is collated at nt 50 of the Sindbis subgenomic RNA). Previous studies of sequence comparisons and structural RNA analysis revealed the evolutionary conservation of DLP in SINV and predicted the existence of equivalent DLP structures in many members of the *Alphavirus* genus (*see e.g.*, Ventoso, *J. Virol.* 9484-9494, Vol. 86, Sept. 2012).

[0090] PKR phosphorylates the eukaryotic translation initiation factor 2α (eIF2 α). Phosphorylation of eIF2 α blocks translation initiation of mRNA and in doing so keeps viruses from a completing a productive replication cycle. PKR is activated by interferon and double stranded RNA. Alphavirus replication in host cells is known to induce the double-stranded RNA-dependent protein kinase (PKR). For example, Sindbis virus infection of cells induces PKR that results in phosphorylation of eIF2 α yet the viral subgenomic mRNA is efficiently translated while translation of all other cellular mRNAs is restricted. The subgenomic mRNA of Sindbis virus has a stable RNA hairpin loop located downstream of the wild type AUG initiator codon for the virus capsid protein (*e.g.*, capsid enhancer). This hairpin loop, also called stem-loop, RNA structure is often referred to as the Downstream LooP structure (or DLP motif). It has been reported that the DLP structure can stall a ribosome on the wild type AUG and this supports translation of the subgenomic mRNA without the requirement for functional eIF2 α. Thus, subgenomic mRNAs of Sindbis virus (SINV) as well as of other alphaviruses are efficiently translated even in cells that have highly active PKR resulting in complete phosphorylation of eIF2α.

Structure of Alphavirus DLPs

[0091] The DLP structure was first characterized in Sindbis virus (SINV) 26S mRNA and also detected in Semliki Forest virus (SFV). Similar DLP structures have been reported to be present in at least 14 other members of the Alphavirus genus including New World (for example, MAYV, UNAV, EEEV (NA), EEEV (SA), AURAV) and Old World (SV, SFV, BEBV, RRV, SAG, GETV, MIDV, CHIKV, and ONNV) members. The predicted structures of these Alphavirus 26S mRNAs were constructed based on SHAPE (selective 2'-hydroxyl acylation and primer extension) data (Toribio *et al.*, *Nucleic Acids Res.* May 19; 44(9):4368-80, 2016), the content of which is hereby incorporated by reference). Stable stem-loop structures were detected in all cases except for CHIKV and ONNV, whereas

MAYV and EEEV showed DLPs of lower stability (*see* **FIGS. 11A-B** and Toribio *et al.*, 2016 supra). The highest DLP activities were reported for those Alphaviruses that contained the most stable DLP structures. In some instances, DLP activity depends on the distance between the DLP motif and the initiation codon AUG (AUGi). The AUG-DLP spacing in Alphavirus 26S mRNAs is tuned to the topology of the ES6S region of the ribosomal 18S rRNA in a way that allows the placement of the AUGi in the P site of the 40S subunit stalled by the DLP, allowing the incorporation of Met-tRNA without the participation of eIF2. Two main topologies were detected: a compact and stable structure in the SFV clade, and a more extended structure in the SINV group. In both cases, it was observed that DLP structures were preceded by a region of intense SHAPE reactivity, suggesting a single stranded conformation for the AUG-DLP stretch. Accordingly, this region showed a high content of A and a low content of G that resulted in a low propensity to form secondary structures when compared with equivalent positions in whole mouse mRNA transcriptome or in those Alphavirus mRNAs lacking DLPs. These results reported by Toribio *et al.* (2016, supra) suggest that the occurrence of DLPs in Alphavirus is probably linked to a flattening of the preceding region, resulting in a valley-peak topology for this region of mRNA.

[0092] In the case of Sindbis virus, the DLP motif is found in the first ~150 nt of the Sindbis subgenomic RNA. The hairpin is located downstream of the Sindbis capsid AUG initiation codon (AUG at nt 50 of the Sindbis subgenomic RNA) and results in stalling a ribosome such that the correct capsid gene AUG is used to initiate translation. This is because the hairpin causes ribosomes to pause eIF2α is not required to support translation initiation. Without being bound by any particular theory, it is believed that placing the DLP motif upstream of a coding sequence for any GOI typically results in a fusion-protein of N-terminal capsid amino acids that are encoded in the hairpin region to the GOI encoded protein because initiation occurs on the capsid AUG not the GOI AUG. In some embodiments disclosed herein, a porcine teschovirus-1 2A (P2A) peptide sequence was engineered in-frame immediately after the DLP sequence and in-frame immediately upstream of all GOI. The incorporation of the P2A peptide in the modified viral RNA replicons of the present disclosure allows release of a nearly pristine GOI protein from the capsid-GOI fusion; a single proline residue is added to all GOI proteins.

[0093] Without being bound by any particular theory, it is believed that the DLP allows translation to occur in an eIF2α independent manner, nucleic acid molecules and expression vectors (*e.g.*, RNA replicon vectors) engineered to use it to initiate translation of non-structural proteins have increased functionality in cells that are innate immune system activated. Therefore, it is contemplated that DLP-engineered nucleic acid molecules and expression vectors (*e.g.*, RNA replicon vectors) also function with more uniformity in different cells, individuals or populations of individuals because differences in the level of innate immune activation in each will naturally cause variability. In some embodiments, the DLP can assist in removing that variability because translation and replication of RNA replicon vectors (as well as GOI expression) can be less impacted by pre-existing innate immune responses. One of the significant values of the compositions and methods disclosed herein is that vaccine efficacy can be increased in individuals that are in a chronic or acute state of immune activation. Causes of chronic or acute immune activation could be found in individuals suffering from a subclinical or clinical infection or individuals undergoing medical treatments for cancer or other maladies (*e.g.*, diabetes, malnutrition, high blood pressure, heart disease, Crohn's disease, muscular scleroses, *etc.*).

[0094] As described herein, DLP-containing nucleic acid molecules (for example, transcription and expression vectors (*e.g.*, RNA viral replicons)) disclosed herein can be useful in conferring a resistance to the innate immune system in a subject. Unmodified RNA replicons are sensitive to the initial innate immune system state of cells they are introduced into. If the cells/individuals are in a highly active innate immune system state, the RNA replicon performance (*e.g.*, replication and expression of a GOI) can be negatively impacted. By engineering a DLP to control initiation of protein translation, particularly of non-structural proteins, the impact of the pre-existing activation state of the innate immune system to influence efficient RNA replicon replication is removed or lessened. The result is more uniform and/or enhanced expression of a GOI that can impact vaccine efficacy or therapeutic impact of a treatment.

Arteriviruses

[0095] The arteriviruses (Family *Arteriviridae*, Genus *Arterivirus*) encompass an important group of enveloped, single-stranded, positive-sense RNA viruses which infect

domestic and wild animals. *Arterivirus*es share a similar genome organization and replication strategy to that of members of the family *Coronaviridae* (genera *Coronavirus* and *Torovirus*), but differ considerably in their genetic complexity, genome length, biophysical properties, size, architecture, and structural protein composition of the viral particles (*e.g.*, virion). Currently, the *Arterivirus* genus is considered to include equine arteritis virus (EAV), porcine reproductive and respiratory syndrome virus (PRRSV), lactate dehydrogenase-elevating virus (LDV) of mice, simian hemorrhagic fever virus (SHFV), and wobbly possum disease virus (WPDV).

[0096] A typical arterivirus genome varies between 12.7 and 15.7 kb in length but their genome organization is relatively consistent with some minor variations. Exemplary genome organization and virion architecture of an arterivirus is shown in **FIG. 10**. The arterivirus genome is a polycistronic +RNA, with 5' and 3' non-translated regions (NTRs) that flank an array of 10–15 known ORFs. The large replicase ORFs 1a and 1b occupy the 5'-proximal three-quarters of the genome, with the size of ORF1a being much more variable than that of ORF1b. Translation of ORF1a produces replicase polyprotein (pp) 1a, whereas ORF1b is expressed by –1 programmed ribosomal frameshifting (PRF), which C-terminally extends pp1a into pp1ab. In addition, a short transframe ORF has been reported to overlap the nsp2-coding region of ORF1a in the +1 frame and to be expressed by –2 PRF. The 3'-proximal genome part has a compact organization and contains 8 to 12 relatively small genes, most of which overlap with neighboring genes. These ORFs encode structural proteins and are expressed from a 3'-co-terminal nested set of sg mRNAs. The organization of these ORFs is conserved, but downstream of ORF1b, SHFV and all recently identified SHFV-like viruses contain three or four additional ORFs (~1.6 kb) that may be derived from an ancient duplication of ORFs 2–4. Together with the size variation in ORF1a, this presumed duplication explains the genome size differences among arteriviruses.

[0097] With regard to equine arteritis virus (EAV), the wild-type EAV genome is approximately 12.7 Kb in size. The 5' three fourths of the genome codes for two large replicase proteins 1a and 1ab; the amino acid sequences of the two proteins are N-terminally identical but due to a ribosomal frameshift the amino acid sequence of the C-terminal region of 1ab is unique. The 3' one quarter of the EAV genome codes for the virus's structural

protein genes, all of which are expressed from subgenomic RNAs. The subgenomic RNAs form a nested set of 3' co-terminal RNAs that are generated via a discontinuous transcriptional mechanism. The subgenomic RNAs are made up of sequences that are not contiguous with the genomic RNA. All of the EAV subgenomic RNAs share a common 5' leader sequence (156 to 221 nt in length) that is identical to the genomic 5' sequence. The leader and body parts of the subgenomic RNAs are connected by a conserved sequence termed a transcriptional-regulatory sequence (TRS). The TRS is found on the 3' end of the leader (leader TRS) as well as in the subgenomic promoter regions located upstream of each structural protein gene (body TRS). Subgenomic RNAs are generated as the negative strand replication intermediate RNA is transcribed. As transcription occurs the replication complex pauses as it comes to each body TRS and then the nascent negative strand RNA become associated with the complementary positive strand leader TRS where negative strand RNA transcription continues. This discontinuous transcription mechanism results in subgenomic RNA with both 5' and 3' EAV conserved sequences. The negative strand subgenomic RNAs then become the template for production of the subgenomic positive sense mRNA.

[0098] Infectious cDNA clones, representing the entire genome of EAV, have been reported and they have been used to study EAV RNA replication and transcription for nearly two decades. In addition, infectious clones have been generated that contain the chloramphenicol acetyltransferase (CAT) gene inserted in place of ORF2 and ORF7, and CAT protein was shown to be expressed in cells electroporated with those RNAs. Modifications of the infectious clone via site directed mutagenesis and deletion of the structural protein gene regions has been used to determine the requirement for each structural gene in support of RNA replication (Molenkamp 2000). The study reported by Molenkamp 2000 concluded that the structural genes are not required to support RNA replication. Analysis of sequence homology requirements for TRS activity in subgenomic RNA production was conducted and used to better define how discontinuous transcription mechanistically occurs (van Marle 1999, Pasternak 2000, Pasternak 2001, Pasternak 2003, van den Born 2005) and defective interfering RNAs have been used to understand the minimal genomic sequences required for replication and packaging of RNA into virus particles (Molenkamp 2000a).

Alphaviruses

[0099] *Alphavirus* is a genus of genetically, structurally, and serologically related viruses of the group IV *Togaviridae* family which includes at least 30 members, each having single stranded RNA genomes of positive polarity enclosed in a nucleocapsid surrounded by an envelope containing viral spike proteins. Currently, the alphavirus genus comprises among others the Sindbis virus (SIN), the Semliki Forest virus (SFV), the Ross River virus (RRV), Venezuelan equine encephalitis virus (VEEV), and Eastern equine encephalitis virus (EEEV), which are all closely related and are able to infect various vertebrates such as mammal, rodents, fish, avian species, and larger mammals such as humans and horses as well as invertebrates such as insects. Transmission between species and individuals occurs mainly via mosquitoes making the alphaviruses a contributor to the collection of Arboviruses – or Arthropod-Borne Viruses. In particular, the Sindbis and the Semliki Forest viruses have been widely studied and, therefore, the life cycle, mode of replication, *etc.*, of these viruses are well characterized. In particular, alphaviruses have been shown to replicate very efficiently in animal cells which makes them valuable as vectors for production of protein and nucleic acids in such cells.

[0100] Alphavirus particles are enveloped, have a 70 nm diameter, tend to be spherical (although slightly pleomorphic), and have an approximately 40 nm isometric nucleocapsid. **FIG. 9** depicts a typical alphavirus genomic structure and genome expression. *Alphavirus* genome is single-stranded RNA of positive polarity of approximately 11- 12 kb in length, comprising a 5' cap, a 3' poly-A tail, and two open reading frames with a first frame encoding the nonstructural proteins with enzymatic function and a second frame encoding the viral structural proteins (*e.g.*, the capsid protein C, E1 glycoprotein, E2 glycoprotein, E3 protein and 6K protein).

[0101] The 5′ two-thirds of the alphavirus genome encodes a number of nonstructural proteins necessary for transcription and replication of viral RNA. These proteins are translated directly from the RNA and together with cellular proteins form the RNA-dependent RNA polymerase essential for viral genome replication and transcription of subgenomic RNA. Four nonstructural proteins (nsP1-4) are produced as a single polyprotein which constitutes the virus' replication machinery. The processing of the polyprotein occurs

in a highly regulated manner, with cleavage at the P2/3 junction influencing RNA template use during genome replication. This site is located at the base of a narrow cleft and is not readily accessible. Once cleaved, nsP3 creates a ring structure that encircles nsP2. These two proteins have an extensive interface. Mutations in nsP2 that produce noncytopathic viruses or a temperature sensitive phenotypes cluster at the P2/P3 interface region. P3 mutations opposite the location of the nsP2 noncytopathic mutations prevent efficient cleavage of P2/3. This in turn can affect RNA infectivity altering viral RNA production levels.

[0102] The 3' one-third of the genome comprises subgenomic RNA which serves as a template for translation of all the structural proteins required for forming viral particles: the core nucleocapsid protein C, and the envelope proteins P62 and E1 that associate as a heterodimer. The viral membrane-anchored surface glycoproteins are responsible for receptor recognition and entry into target cells through membrane fusion. The subgenomic RNA is transcribed from the p26S subgenomic promoter present at the 3′ end of the RNA sequence encoding the nsp4 protein. The proteolytic maturation of P62 into E2 and E3 causes a change in the viral surface. Together the E1, E2, and sometimes E3, glycoprotein "spikes" form an E1/E2 dimer or an E1/E2/E3 trimer, where E2 extends from the center to the vertices, E1 fills the space between the vertices, and E3, if present, is at the distal end of the spike. Upon exposure of the virus to the acidity of the endosome, E1 dissociates from E2 to form an E1 homotrimer, which is necessary for the fusion step to drive the cellular and viral membranes together. The alphaviral glycoprotein E1 is a class II viral fusion protein, which is structurally different from the class I fusion proteins found in influenza virus and HIV. The E2 glycoprotein functions to interact with the nucleocapsid through its cytoplasmic domain, while its ectodomain is responsible for binding a cellular receptor. Most alphaviruses lose the peripheral protein E3, while in Semliki viruses it remains associated with the viral surface.

[0103] Alphavirus replication has been reported to take place on membranous surface within the host cell. In the first step of the infectious cycle, the 5′ end of the genomic RNA is translated into a polyprotein (nsP1-4) with RNA polymerase activity that produces a negative strand complementary to the genomic RNA. In a second step, the negative strand is

used as a template for the production of two RNAs, respectively: (1) a positive genomic RNA corresponding to the genome of the secondary viruses producing, by translation, other nsp proteins and acting as a genome for the virus; and (2) subgenomic RNA encoding the structural proteins of the virus forming the infectious particles. The positive genomic RNA/subgenomic RNA ratio is regulated by proteolytic autocleavage of the polyprotein to nsp 1, nsp 2, nsp 3 and nsp 4. In practice, the viral gene expression takes place in two phases. In a first phase, there is main synthesis of positive genomic strands and of negative strands. During the second phase, the synthesis of subgenomic RNA is virtually exclusive, thus resulting in the production of large amount of structural protein.

Innate Immunity

[0104] Since innate immune activation can occur due to many different stimuli, vaccine approaches that rely on self-amplifying RNA replicons to express antigen or therapeutic GOI can be negatively impacted by the global host protein shutdown associated with PKR phosphorylation of eIF2α. Engineering RNA replicons to function in a cellular environment where host protein translation is repressed would provide those systems with a significant advantage over standard RNA replicon systems.

[0105] Accordingly, RNA replicon systems that are negatively impacted by innate immune responses, such as systems derived from alphaviruses and arteriviruses, can be more effective at expressing their encoded GOI when engineered to contain a DLP motif. The DLP motif confers efficient mRNA translation in cellular environments where cellular mRNA translation is inhibited. When a DLP is linked with translation of a replicon vectors non-structural protein genes the replicase and transcriptase proteins are capable of initiating functional replication in PKR activated cellular environments. When a DLP is linked with translation of subgenomic mRNAs robust GOI expression is possible even when cellular mRNA is restricted due to innate immune activation. Accordingly, engineering replicons that contain DLP structures to help drive translation of both non-structural protein genes and subgenomic mRNAs provides yet another powerful way to overcome innate immune activation.

[0106] Some embodiments of the disclosure relate to DLP structures that have been engineered to support translation of viral non-structural genes of replicon vectors

derived from two different viruses, Venezuelan equine encephalitis virus (VEEV) and equine arteritis virus (EAV), thus conveying innate immune response evasion to the systems. As described in greater detail below, incorporation of the DLP structures into the replicon vectors made them both resistant to interferon (IFN) treatment and unexpectedly also resulted in an overall increase in GOI expression potential. The combination of IFN resistance and superior protein expression potential imparted by engineering a DLP into the RNA replicon systems make them suitable for use in individuals or populations where innate immune activation is acutely or chronically present.

Nucleic Acid Molecules of the Disclosure

[0107] Some aspects of the present disclosure relate to nucleic acid molecules, such as synthetic or recombinant nucleic acid molecules, that include one or more DLP motifs, a coding sequence for one or more DLP motifs, or a combination thereof. In some embodiments, the nucleic acid molecules of the disclosure can include a coding sequence for a gene of interest (GOI) operably linked to DLP motif(s) and/or the coding sequence for the DLP motifs.

[0108] In one aspect, disclosed herein is a nucleic acid molecule, comprising (i) a first nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer or a variant thereof; and (ii) a second nucleic acid sequence operably linked to the first nucleic acid sequence, wherein the second nucleic acid sequence comprises a coding sequence for a gene of interest (GOI). In some embodiments, at least one of the one or more structural elements of the viral capsid enhancer comprises one or more RNA stem-loops. In some embodiments, at least one of the one or more RNA stem-loops is comprised by a DLP motif present in the first nucleic acid sequence. In some embodiments, at least one of the one or more structural elements of the viral capsid enhancer does not comprise any RNA stem-loop.

[0109] As described above, a viral capsid enhancer comprises sequences within the 5' non-coding and/or 5' coding sequences (preferably, the 5' coding sequences) of that enhance expression (*e.g.*, transcription and/or translation) of sequences operably linked therewith. In some embodiments of the present disclosure, the one or more structural elements of the viral capsid enhancer include one or two RNA stem-loops of the viral capsid

enhancer. In some embodiments, the viral capsid enhancer of the present disclosure includes the sequences containing the 26S subgenomic promoter. In some embodiments, the viral capsid enhancer of the disclosure contains the 5‘ coding sequences at about nucleotides 20 to 250, about nucleotides 20 to 200, about nucleotides 20 to 150, about nucleotides 20 to 100, or about nucleotides 50 to 250, about nucleotides 100 to 250, about nucleotides 50 to 200, about nucleotides 75 to 250, about nucleotides 75 to 200, about nucleotides 75 to 150, about nucleotides 77 to 139, or about nucleotides 100 to 250, about nucleotides 150 to 250, about nucleotides 100 to 150, about nucleotides 100 to 200 of the viral 26S RNA, which is capable of forming a hairpin structure. In some embodiments, the first nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer that are important for enhancing expression of a heterologous sequence operably linked thereto. In some embodiments, the first nucleic acid sequence includes encoding sequence for one or more RNA stem-loops of a viral capsid enhancer. In some embodiments, the first nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer that are important for enhancing translation of a heterologous sequence operably linked thereto. In some embodiments, the first nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer that are important for enhancing transcription of a heterologous sequence operably linked thereto.

[0110] In some embodiments, the first nucleic acid sequence of the nucleic acid molecule includes at least about 50, about 75, about 100, about 150, about 200, about 300, or more nucleotides from the 5′ coding sequence for a viral capsid protein. In some embodiments, the first nucleic acid sequence of the nucleic acid molecule includes about 50, about 75, about 100, about 150, about 200, about 300, or more, or a range between any two of these values, nucleotides from the 5′ coding sequence for a viral capsid protein. In some embodiments, the viral capsid enhancer is derived from a capsid gene of an alphavirus species selected from the group consisting of Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), Everglades virus (EVEV), Mucambo virus (MUCV), Semliki forest virus (SFV), Pixuna virus (PIXV), Middleburg virus (MIDV), Chikungunya virus (CHIKV), O’Nyong-Nyong virus (ONNV), Ross River virus (RRV), Barmah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAGV), Bebaru virus

(BEBV), Mayaro virus (MAYV), Una virus (UNAV), Sindbis virus (SINV), Aura virus (AURAV), Whataroa virus (WHAV), Babanki virus (BABV), Kyzylagach virus (KYZV), Western equine encephalitis virus (WEEV), Highland J virus (HJV), Fort Morgan virus (FMV), Ndumu (NDUV), and Buggy Creek virus. In some embodiments, the viral capsid enhancer is derived from a capsid gene of a Sindbis virus species or a Semliki Forest virus species. In some particular embodiments, the viral capsid enhancer is derived from a capsid gene of a Sindbis virus species. Additionally, one of ordinary skill in the art will appreciate that modifications may be made in the 5′ coding sequences from the viral capsid protein without substantially reducing its enhancing activities. More information in this regard can be found in, *e.g.*, Frolov *et al.*, *J. Virology* 70:1182, 1994; Frolov *et al.*, *J. Virology* 68:8111, 1994. In some embodiments, it can be advantage for such mutations to substantially preserve the RNA hairpin structure formed by the 5′ capsid coding sequences.

[0111] In some embodiments, the viral capsid enhancer disclosed herein does not contain one or more, or all, of the 5' coding sequences of the capsid protein that are upstream of the hairpin structure. In some embodiments, the viral capsid enhancer disclosed herein does not contain all of the 5′ coding sequences of the viral capsid protein that are upstream of the hairpin structure. In some embodiments, the viral capsid enhancer sequence may encode all or part of the capsid protein. Accordingly, in some embodiments disclosed herein, the capsid enhancer region will not encode the entire viral capsid protein. In some embodiments, the viral capsid enhancer sequence encodes an amino terminal fragment from the viral capsid protein. In those embodiments in which an otherwise functional capsid protein is encoded by the capsid enhancer sequence, it may be desirable to ablate the capsid autoprotease activity. Capsid mutations that reduce or ablate the autoprotease activity of the capsid protein are known in the art (see *e.g.*, WO1996/37616). In addition or alternatively, one or more of amino acid residues in the capsid protein may be altered to reduce capsid protease activity.

[0112] As indicated above, previous studies of sequence comparisons and structural RNA analysis revealed the evolutionary conservation of DLP motifs in many members of the *Alphavirus* genus (*see e.g.*, Ventoso, 2012 supra). Accordingly, in some further embodiments, the viral capsid enhancer sequence of the present disclosure can be of any other variant sequence such as, for example, a synthetic sequence or a heterologous

sequence, that can form an RNA hairpin functionally or structurally equivalent to one or more of the RNA stem-loops predicted for a viral capsid enhancer and which can act to enhance translation of RNA sequences operably linked downstream thereto (*e.g.*, coding sequence for a gene of interest). Non-limiting examples of RNA stem-loops which can act as a transcriptional and/or translational enhancer include those shown in **FIGS. 11A-B**. In some embodiments, the nucleic acid molecule of the disclosure includes an alphavirus capsid enhancer as derived from Sindbis virus (SINV; NC 001547.1), Aura virus (AURAV; AF126284), Chikungunya virus (CHIKV; NC 004162), O'Nyong-Nyong virus (ONNV; NC 001512), Eastern Equine Encephalitis virus (EEEV(SA); AF159559 and EEEV (NA); U01558), Mayaro virus (MAYV; DQ001069), Semliki Forest virus (SFV; NC 003215), Ross River virus (RRV; DQ226993 and Sagiyama virus (SAGV; AB032553), Getah virus (GETV; NC 006558), Middelburg virus (MIDV; EF536323), Una virus (UNAV; AF33948), or Bebaru virus (BEBV; AF339480) as described in Toribio *et al.*, 2016 supra, the content of which is hereby incorporated by reference in its entirety, or a variant thereof.

[0113] Nucleic acid molecules having a high degree of sequence identity (*e.g.*, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to the coding sequence for a viral capsid enhancer disclosed herein can be identified and/or isolated by using the sequence described herein (*e.g.*, SEQ ID NO: 1) or any others alphavirus capsid protein as they are known in the art, for example, by using the sequences of Sindbis virus (SINV; NC 001547.1), Aura virus (AURAV; AF126284), Chikungunya virus (CHIKV; NC 004162), O'Nyong-Nyong virus (ONNV; NC 001512), Eastern Equine Encephalitis virus (EEEV(SA); AF159559 and EEEV (NA); U01558), Mayaro virus (MAYV; DQ001069), Semliki Forest virus (SFV; NC 003215), Ross River virus (RRV; DQ226993 and Sagiyama virus (SAGV; AB032553), Getah virus (GETV; NC 006558), Middelburg virus (MIDV; EF536323), Una virus (UNAV; AF33948), and Bebaru virus (BEBV; AF339480), by genome sequence analysis, hybridization, and/or PCR with degenerate primers or gene-specific primers from sequences identified in the respective alphavirus genome. For example, the viral capsid enhancer can comprise, or consist of, a DLP motif from a virus species belonging to the *Togaviridae* family, for example an alphavirus species or a rubivirus species. In some embodiments, the nucleic acid molecule of

the disclosure includes a viral capsid enhancer having a nucleic acid sequence that exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the 5' CDS portion of an alphavirus capsid protein. In some embodiments, the 5' CDS portion of an alphavirus capsid protein comprises at least the first 25, 50, 75, 80, 100, 150, or 200 nucleotides of the coding sequence for the alphavirus capsid protein. In some embodiments, the nucleic acid molecule of the disclosure includes a viral capsid enhancer having a nucleic acid sequence that exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 1 and 46-52. In some embodiments, the nucleic acid molecule comprises a viral capsid enhancer having a nucleic acid sequence that exhibits 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a range between any two of these values, sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 1 and 46-52. In some embodiments, the nucleic acid molecule of the disclosure includes a viral capsid enhancer having a nucleic acid sequence that exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the sequence of SEQ ID NO: 1 disclosed herein. In some embodiments, the nucleic acid molecule of the disclosure includes a viral capsid enhancer having a nucleic acid sequence that exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any one of the sequences described in FIGS. 11A-B and/or Figure 1A in the publication by Toribio *et al.* (2016 supra), the content of which is hereby incorporated by reference in its entirety.

[0114] Accordingly, in some embodiments, the nucleic acid molecule of the disclosure includes a viral capsid enhancer having a nucleic acid sequence that exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the sequence of any one of SEQ ID NOS: 46-52 disclosed herein. In some embodiments, the nucleic acid molecule of the disclosure includes a viral capsid enhancer having a nucleic acid sequence that exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the sequence set forth at SEQ ID NO: 46 disclosed herein. In

some embodiments, the nucleic acid molecule of the disclosure includes a viral capsid enhancer having a nucleic acid sequence that exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the sequence set forth at SEQ ID NO: 47 disclosed herein. In some embodiments, the nucleic acid molecule of the disclosure includes a viral capsid enhancer having a nucleic acid sequence that exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the sequence set forth at SEQ ID NO: 48 disclosed herein. In some embodiments, the nucleic acid molecule of the disclosure includes a viral capsid enhancer having a nucleic acid sequence that exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the sequence set forth at SEQ ID NO: 49 disclosed herein. In some embodiments, the nucleic acid molecule of the disclosure includes a viral capsid enhancer having a nucleic acid sequence that exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the sequence set forth at SEQ ID NO: 50 disclosed herein. In some embodiments, the nucleic acid molecule of the disclosure includes a viral capsid enhancer having a nucleic acid sequence that exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the sequence set forth at SEQ ID NO: 51 disclosed herein. In some embodiments, the nucleic acid molecule of the disclosure includes a viral capsid enhancer having a nucleic acid sequence that exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the sequence set forth at SEQ ID NO: 52 disclosed herein.

[0115] In the nucleic acid molecule according to some embodiments of the present disclosure, the one or more RNA stem-loops are operably positioned upstream of the coding sequence for the GOI of the second nucleic acid sequence. In some embodiments, the one or more RNA stem-loops are operably positioned from about 1 to about 50 nucleotides, from about 10 to about 75 nucleotides, from about 30 to about 100 nucleotides, from about 40 to about 150 nucleotides, from about 50 to about 200 nucleotides, from about 60 to about 250 nucleotides, from about 100 to about 300 nucleotides, or from about 150 to about 500

nucleotides upstream of the coding sequence for the GOI. In some embodiments, the one or more RNA stem-loops are operably positioned from about 1, about 2, about 5, about 10, about 15, about 20, about 25, about 30, about 40, about 50, about 60, about 70, about 80, about 90, about 100, about 200, about 300, about 400, about 500, or a range between any two of these values, nucleotides upstream of the coding sequence for the GOI. In some embodiments, the one or more RNA stem-loops are operably positioned immediately upstream of the coding sequence for the GOI.

[0116] In some embodiments, the nucleic acid molecule further includes a 5'-unstranslated region (5'-UTR) sequence operably positioned upstream to the first nucleic acid sequence. In some embodiments, the 5'-UTR sequence is operably positioned from about 1 to about 50, from about 10 to about 75, from about 30 to about 100, from about 40 to about 150, from about 50 to about 200, from about 60 to about 250, from about 100 to about 300, or from about 150 to about 500 nucleotides upstream of the first nucleic acid sequence. In some embodiments, the 5'-UTR sequence is operably positioned from about 1, about 2, about 5, about 10, about 15, about 20, about 25, about 30, about 40, about 50, about 60, about 70, about 80, about 90, or 100 nucleotides upstream of the first nucleic acid sequence. In some embodiments, the 5'-UTR sequence is operably positioned immediately upstream of the first nucleic acid sequence.

[0117] In some embodiments, the 5' UTR sequence is operably positioned downstream to the promoter. In some embodiments, the 5'-UTR sequence is operably positioned from about 1 to about 50, from about 10 to about 75, from about 30 to about 100, from about 40 to about 150, from about 50 to about 200, from about 60 to about 250, from about 100 to about 300, or from about 150 to about 500 nucleotides downstream of the promoter sequence. In some embodiments, the 5' UTR sequence is operably positioned from about 1, about 2, about 5, about 10, about 15, about 20, about 25, about 30, about 40, about 50, about 60, about 70, about 80, about 90, or 100 nucleotides downstream of the promoter sequence. In some embodiments, the 5' UTR sequence is operably positioned immediately downstream to the promoter sequence. In some embodiments, the 5' UTR sequence is operably positioned downstream to the promoter and upstream to the first nucleic acid sequence.

[0118] In some embodiments, the nucleic acid molecule comprises a 3' unstranslated region (3' UTR) sequence operably positioned downstream of the second nucleic acid sequence. In some embodiments, the 3' UTR sequence is operably positioned from about 1 to about 50 nucleotides, from about 10 to about 75 nucleotides, from about 30 to about 100 nucleotides, from about 40 to about 150 nucleotides, from about 50 to about 200 nucleotides, from about 60 to about 250 nucleotides, from about 100 to about 300 nucleotides, or from about 150 to about 500 nucleotides downstream of the second sequence nucleic acid sequence. In some embodiments, the 3' UTR sequence is operably positioned from about 1, about 2, about 5, about 10, about 15, about 20, about 25, about 30, about 40, about 50, about 60, about 70, about 80, about 90, about 100, about 200, about 300, about 400, about 500, or a range between any two of these values, nucleotides downstream of the second nucleic acid sequence. In some embodiments, the 3' UTR sequence is operably positioned immediately downstream of the second nucleic acid sequence.

[0119] In some embodiments disclosed herein, the coding sequence for the GOI is transcribed into a messenger RNA (mRNA) or part of an mRNA. As used herein, the term "mRNA" or "messenger RNA" refers to a single stranded RNA molecule that is synthesized during transcription, is complementary to one of the strands of double-stranded DNA, and serves to transmit the genetic information contained in DNA to the ribosomes for protein synthesis. The mRNA may be spliced, partially spliced or unspliced, and may be eukaryotic or prokaryotic mRNA. As discussed above, mRNA molecules according to some embodiments of the disclosure can be produced via *de novo* synthesis. In some embodiments disclosed herein, the coding sequence for the GOI encodes a polypeptide. In some embodiments, the polypeptide is a therapeutic polypeptide, a prophylactic polypeptide, a diagnostic polypeptide, a nutraceutical polypeptide, an industrial enzyme, a reporter polypeptide, or any combination thereof. In some embodiments, the polypeptide is an antibody, an antigen, an immune modulator, a cytokine, an enzyme, or any combination thereof.

[0120] In some embodiments, the nucleic acid molecule of the disclosure further includes a coding sequence for an autoprotease peptide (*e.g.*, autocatalytic self-cleaving peptide), where the coding sequence for the autoprotease is optionally operably linked

upstream to the second nucleic acid sequence. Generally, any proteolytic cleavage site known in the art can be incorporated into the nucleic acid molecules of the disclosure and can be, for example, proteolytic cleavage sequences that are cleaved post-production by a protease. Further suitable proteolytic cleavage sites also include proteolytic cleavage sequences that can be cleaved following addition of an external protease. As used herein the term "autoprotease" refers to a "self-cleaving" peptide that possesses autoproteolytic activity and is capable of cleaving itself from a larger polypeptide moiety. First identified in the foot-and-mouth disease virus (FMDV), a member of the picornavirus group, several autoproteases have been subsequently identified such as, for example, "2A like" peptides from equine rhinitis A virus (E2A), porcine teschovirus-1 (P2A) and Thosea asigna virus (T2A), and their activities in proteolytic cleavage have been shown in various *ex vitro* and *in vivo* eukaryotic systems. As such, the concept of autoproteases is available to one of skill in the art with many naturally-occurring autoprotease systems have been identified. Well studied autoprotease systems are *e.g.* viral proteases, developmental proteins (*e.g.* HetR, Hedgehog proteins), RumA autoprotease domain, UmuD, *etc.*). Non-limiting examples of autoprotease peptides suitable for the compositions and methods of the present disclosure include the peptide sequences from porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), or a combination thereof.

[0121] In some embodiments, the coding sequence for an autoprotease peptide is operably linked downstream to the first nucleic acid sequence and upstream to the second nucleic acid sequence. In some embodiments, the autoprotease peptide comprises, or consists of, a peptide sequence selected from the group consisting of porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), and a combination thereof. In some embodiments, the autoprotease peptide includes a peptide sequence of porcine teschovirus-1 2A (P2A).

[0122] One of skill in the art will appreciate that different configurations of the viral capsid enhancer sequence, the sequence encoding the autoprotease peptide, and the sequence encoding the gene of interest can be employed as long as the capsid enhancer sequence enhances expression of the heterologous nucleic acid sequence(s), *e.g.* a coding sequence for a GOI, as compared with the level seen in the absence of the capsid enhancer sequence. These sequences will typically be configured so that the polypeptide encoded by the gene of interest can be released from the protease and any capsid protein sequence after cleavage by the autoprotease.

[0123] A non-limiting list of exemplary combinations of autoprotease peptides described herein (such as P2A, F2A, E2A, T2A, BmCPV2A, and BmIFV2A) with one or more viral capsid enhancer sequences described herein are provided in Tables 1 and 2. Table 1 provides a shorthand name for each viral capsid enhancer (*e.g.*, "CE01") and a shorthand name for each autoprotease peptide (*e.g.*, "AP01"). Each numbered 'X' peptide in Table 2 has a corresponding autoprotease peptide provided in Table 1. Likewise, each numbered 'Y' enhancer in Table 2 has a corresponding viral capsid enhancer provided in Table 1. Therefore, each "X:Y" entry in Table 2 provides an example of a combination of a viral capsid enhancer and an autoprotease peptide that can be used in the molecules, compositions, and methods of the present disclosure. For example, the combination designated as "AP01:CE16" in Table 2 provides a combination of viral capsid enhancer derived from Sindbis virus (SINV) and an autoprotease peptide from porcine teschovirus-1 2A (P2A).

TABLE 1: Exemplary viral capsid enhancers and autoprotease peptides of the disclosure

Viral Capsid Enhancer (Y)		Autoprotease Peptide (X)	
Eastern equine encephalitis virus (EEEV)	(CE01)	porcine teschovirus-1 2A (P2A)	(AP01)
Venezuelan equine encephalitis virus (VEEV)	(CE02)	foot-and-mouth disease virus (FMDV) 2A (F2A)	(AP02)
Everglades virus (EVEV)	(CE03)	Equine Rhinitis A Virus (ERAV) 2A (E2A)	(AP03)
Mucambo virus (MUCV)		Thosea asigna virus 2A (T2A)	(AP04)
Semliki forest virus (SFV)	(CE04)	cytoplasmic polyhedrosis virus 2A (BmCPV2A)	(AP05)
Pixuna virus (PIXV)	(CE05)	Flacherie Virus 2A (BmIFV2A)	(AP06)
Middleburg virus (MIDV)	(CE06)		
Chikungunya virus (CHIKV)	(CE07)		
O'Nyong-Nyong virus (ONNV)	(CE08)		
Ross River virus (RRV)	(CE09)		
Barmah Forest virus (BF)	(CE10)		

Viral Capsid Enhancer (Y)		Autoprotease Peptide (X)
Getah virus (GET)	(CE11)	
Sagiyama virus (SAGV)	(CE12)	
Bebaru virus (BEBV)	(CE13)	
Mayaro virus (MAYV)	(CE14)	
Una virus (UNAV)	(CE15)	
Sindbis virus (SINV)	(CE16)	
Aura virus (AURAV)	(CE17)	
Whataroa virus (WHAV)	(CE18)	
Babanki virus (BABV)	(CE19)	
Kyzylagach virus (KYZV)	(CE20)	
Western equine encephalitis virus (WEEV)	(CE21)	
Highland J virus (HJV)	(CE22)	
Fort Morgan virus (FMV)	(CE23)	
Ndumu (NDUV)	(CE24)	
Salmonid alphavirus (SAV)	(CE25)	
Buggy Creek virus	(CE26)	

TABLE 2:

X : Y	X : Y	X : Y	X : Y	X : Y	X : Y
AP01 : CE01	AP02 : CE01	AP03 : CE01	AP04 : CE01	AP05 : CE01	AP06 : CE01
AP01 : CE02	AP02 : CE02	AP03 : CE02	AP04 : CE02	AP05 : CE02	AP06 : CE02
AP01 : CE03	AP02 : CE03	AP03 : CE03	AP04 : CE03	AP05 : CE03	AP06 : CE03
AP01 : CE04	AP02 : CE04	AP03 : CE04	AP04 : CE04	AP05 : CE04	AP06 : CE04
AP01 : CE05	AP02 : CE05	AP03 : CE05	AP04 : CE05	AP05 : CE05	AP06 : CE05
AP01 : CE06	AP02 : CE06	AP03 : CE06	AP04 : CE06	AP05 : CE06	AP06 : CE06
AP01 : CE07	AP02 : CE07	AP03 : CE07	AP04 : CE07	AP05 : CE07	AP06 : CE07
AP01 : CE08	AP02 : CE08	AP03 : CE08	AP04 : CE08	AP05 : CE08	AP06 : CE08
AP01 : CE09	AP02 : CE09	AP03 : CE09	AP04 : CE09	AP05 : CE09	AP06 : CE09
AP01 : CE10	AP02 : CE10	AP03 : CE10	AP04 : CE10	AP05 : CE10	AP06 : CE10
AP01 : CE11	AP02 : CE11	AP03 : CE11	AP04 : CE11	AP05 : CE11	AP06 : CE11
AP01 : CE12	AP02 : CE12	AP03 : CE12	AP04 : CE12	AP05 : CE12	AP06 : CE12
AP01 : CE13	AP02 : CE13	AP03 : CE13	AP04 : CE13	AP05 : CE13	AP06 : CE13
AP01 : CE14	AP02 : CE14	AP03 : CE14	AP04 : CE14	AP05 : CE14	AP06 : CE14
AP01 : CE15	AP02 : CE15	AP03 : CE15	AP04 : CE15	AP05 : CE15	AP06 : CE15
AP01 : CE16	AP02 : CE16	AP03 : CE16	AP04 : CE16	AP05 : CE16	AP06 : CE16
AP01 : CE17	AP02 : CE17	AP03 : CE17	AP04 : CE17	AP05 : CE17	AP06 : CE17
AP01 : CE18	AP02 : CE18	AP03 : CE18	AP04 : CE18	AP05 : CE18	AP06 : CE18
AP01 : CE19	AP02 : CE19	AP03 : CE19	AP04 : CE19	AP05 : CE19	AP06 : CE19
AP01 : CE20	AP02 : CE20	AP03 : CE20	AP04 : CE20	AP05 : CE20	AP06 : CE20
AP01 : CE21	AP02 : CE21	AP03 : CE21	AP04 : CE21	AP05 : CE21	AP06 : CE21
AP01 : CE22	AP02 : CE22	AP03 : CE22	AP04 : CE22	AP05 : CE22	AP06 : CE22
AP01 : CE23	AP02 : CE23	AP03 : CE23	AP04 : CE23	AP05 : CE23	AP06 : CE23
AP01 : CE24	AP02 : CE24	AP03 : CE24	AP04 : CE24	AP05 : CE24	AP06 : CE24
AP01 : CE25	AP02 : CE25	AP03 : CE25	AP04 : CE25	AP05 : CE25	AP06 : CE25
AP01 : CE26	AP02 : CE26	AP03 : CE26	AP04 : CE26	AP05 : CE26	AP06 : CE26

[0124] In one aspect, disclosed herein are novel nucleic acid molecules which include a nucleic acid sequence encoding a modified viral RNA replicon, wherein the modified viral RNA replicon includes a first nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer (*e.g.*, a DLP motif) or a variant thereof, wherein the viral capsid enhancer is heterologous to the viral RNA replicon, and a second nucleic acid sequence encoding at least one nonstructural viral protein or a portion thereof, wherein the first nucleic acid sequence is operably linked upstream to the second nucleic acid sequence.

[0125] The terms "replicon RNA" and "RNA replicon" used interchangeably herein, refers to RNA which contains all of the genetic information required for directing its own amplification or self-replication within a permissive cell. To direct its own replication, the RNA molecule 1) encodes polymerase, replicase, or other proteins which may interact

with viral or host cell-derived proteins, nucleic acids or ribonucleoproteins to catalyze the RNA amplification process; and 2) contain cis-acting RNA sequences required for replication and transcription of the subgenomic replicon-encoded RNA. These sequences may be bound during the process of replication to its self-encoded proteins, or non-self-encoded cell-derived proteins, nucleic acids or ribonucleoproteins, or complexes between any of these components. In some embodiments of the present disclosure, a modified viral replicon RNA molecule typically contains the following ordered elements: 5′ viral or defective-interfering RNA sequence(s) required in *cis* for replication, sequences coding for biologically active nonstructural proteins, promoter for the subgenomic RNA, 3′ viral sequences required in *cis* for replication, and a polyadenylate tract. Further, the term replicon RNA generally refers to a molecule of positive polarity, or "message" sense, and the replicon RNA may be of length different from that of any known, naturally-occurring RNA viruses. In some embodiments of the present disclosure, the replicon RNA does not contain coding sequences for at least one of the structural viral proteins. In these instances, the sequences encoding structural genes can be substituted with one or more heterologous sequences such as, for example, a coding sequence for a gene of interest (GOI). In those instances where the replicon RNA is to be packaged into a recombinant alphavirus particle, it must contain one or more sequences, so-called packaging signals, which serve to initiate interactions with alphavirus structural proteins that lead to particle formation.

[0126] As used herein, "subgenomic RNA" refers to a RNA molecule of a length or size which is smaller than the genomic RNA from which it was derived. The viral subgenomic RNA should be transcribed from an internal promoter, whose sequences reside within the genomic RNA or its complement. Transcription of a subgenomic RNA may be mediated by viral-encoded polymerase(s) associated with host cell-encoded proteins, ribonucleoprotein(s), or a combination thereof. In some embodiments of the present disclosure, the subgenomic RNA is produced from a modified replicon RNA as disclosed herein and encodes or expresses one or more gene of interest (GOI). Instead of the native subgenomic promoter, the subgenomic RNA can be placed under control of internal ribosome entry site (IRES) derived from encephalomyocarditis viruses (EMCV), Bovine Viral Diarrhea

Viruses (BVDV), polioviruses, Foot-and-mouth disease viruses (FMD), enterovirus 71, or hepatitis C viruses.

[0127] In some embodiments, the second nucleic acid sequence of the modified viral RNA replicon includes the coding sequence for at least one, at least two, at least three, or at least four nonstructural viral proteins. In some embodiments, the second nucleic acid sequence of the modified viral RNA replicon includes the coding sequence for a portion of the at least one nonstructural viral protein. For example, the second nucleic acid sequence of the modified viral RNA replicon can include about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, or a range between any two of these values, of the encoding sequence for the at least one nonstructural viral protein. In some embodiments, the second nucleic acid sequence of the modified viral RNA replicon can include the coding sequence for a substantial portion of the at least one nonstructural viral protein. As used herein, a "substantial portion" of a nucleic acid sequence encoding a nonstructural viral protein comprises enough of the nucleic acid sequence encoding the nonstructural viral protein to afford putative identification of that protein, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (see, for example, in "Basic Local Alignment Search Tool"; Altschul SF *et al.*, J. Mol. Biol. 215:403-410, 1993). In some embodiments, the second nucleic acid sequence of the modified viral RNA replicon can include the entire coding sequence for the at least one nonstructural protein. In some embodiments, the second nucleic acid sequence comprises substantially all the coding sequence for the native viral nonstructural proteins.

[0128] The molecular techniques and methods by which these new nucleic acid molecules were constructed and characterized are described more fully in the Examples herein of the present application. As non-limiting examples, in the Examples section, the Venezuelan equine encephalitis virus (VEEV) and Equine arteritis virus (EAV) have been used to illustrate the compositions and methods disclosed herein.

[0129] In some embodiments, the nucleic acid molecules disclosed herein are recombinant nucleic acid molecules. As used herein, the term recombinant means any molecule (*e.g.* DNA, RNA, *etc.*), that is, or results, however indirect, from human

manipulation of a polynucleotide. As non-limiting examples, a cDNA is a recombinant DNA molecule, as is any nucleic acid molecule that has been generated by *ex vitro* polymerase reaction(s), or to which linkers have been attached, or that has been integrated into a vector, such as a cloning vector or expression vector. As non-limiting examples, a recombinant nucleic acid molecule: 1) has been synthesized or modified *ex vitro*, for example, using chemical or enzymatic techniques (for example, by use of chemical nucleic acid synthesis, or by use of enzymes for the replication, polymerization, exonucleolytic digestion, endonucleolytic digestion, ligation, reverse transcription, transcription, base modification (including, *e.g.*, methylation), or recombination (including homologous and site-specific recombination) of nucleic acid molecules; 2) includes conjoined nucleotide sequences that are not conjoined in nature; 3) has been engineered using molecular cloning techniques such that it lacks one or more nucleotides with respect to the naturally-occurring nucleic acid sequence; and/or 4) has been manipulated using molecular cloning techniques such that it has one or more sequence changes or rearrangements with respect to the naturally-occurring nucleic acid sequence.

[0130] A nucleic acid molecule, including a variant of a naturally-occurring nucleic acid sequence, can be produced using a number of methods known to those skilled in the art. The sequence of a nucleic acid molecule can be modified with respect to a naturally-occurring sequence from which it is derived using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as but not limited to site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations, restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, PCR amplification and/or mutagenesis of selected regions of a nucleic acid sequence, recombinational cloning, and chemical synthesis, including chemical synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules, and combinations thereof. Nucleic acid molecule homologs can be selected from a mixture of modified nucleic acid molecules by screening for the function of the protein or the replicon encoded by the nucleic acid molecule and/or by hybridization with a wild-type gene or fragment thereof, or by PCR using primers having homology to a target or wild-type nucleic acid molecule or sequence.

[0131] In various embodiments disclosed herein, the nucleic acid molecule disclosed herein can include one or more of the following features.

[0132] In some embodiments, the modified viral RNA replicon includes a modified RNA replicon derived from a virus species belonging to the *Alphavirus* genus of the *Togaviridae* family or to the *Arterivirus* genus of the *Arteriviridae* family. Suitable arterivirus species includes Equine arteritis virus (EAV), Porcine respiratory and reproductive syndrome virus (PRRSV), Lactate dehydrogenase elevating virus (LDV), Simian hemorrhagic fever virus (SHFV), and wobbly possum disease virus (WPDV). Virulent and avirulent arterivirus strains are both suitable. Non-limiting examples of preferred arterivirus strains include, but not limited to, EAV-virulent Bucyrus strain (VBS), LDV-Plagemann, LDV-C, PRRSV-type 1, and PRRSV-type 2. Exemplary preferred EAV strains include, but not limited to, EAV VB53, EAV ATCC VR-796, EAV HK25, EAV HK116, EAV ARVAC MLV, EAV Bucyrus strain (Ohio), modified EAV Bucyrus, avirulant strain CA95, Red Mile (Kentucky), 84KY-A1 (Kentucky), Wroclaw-2 (Poland), Bibuna (Switzerland), and Vienna (Australia). Non-limiting preferred examples of PRRSV strains include PRRSV LV4.2.1, PRRSV 16244B, PRRSV HB-1(sh)/2002, PRRSV HB-2(sh)/2002, PRRSV HN1, PRRSV SD 01-08, PRRSV SD0802, PRRSV SD0803, PRRSV, and VR2332. Non-limiting preferred examples of SHFV strains and variants include SHFV variants SHFV-krtg1a and -krtg1b (SHFV-krtg1a/b), SHFVkrtg2a/b (GenBank accession # JX473847 to JX473850), SHFV-LVR, the SHFV prototype variant LVR 42-0/M6941 (NC_003092); SHFV-krc1 and SHFVkrc2 from Kibale red colobus (HQ845737 and HQ845738, respectively). Other non-limiting examples of preferred arteriviruses include PRRSV-Lelystad, the European (type 1) type strain (M96262); PRRSVVR2332, the North American (type 2) type strain (U87392); EAV-Bucyrus (NC_002532); EAV-s3685 (GQ903794); LDV-P, the Plagemann strain (U15146); and LDV-C, the neurovirulent type C strain (L13298).

[0133] In some embodiments, the first nucleic acid sequence is positioned upstream to a nucleic acid sequence encoding a portion or the entire pp1ab nonstructural protein of the modified arterivirus RNA replicon. In some embodiments, the first nucleic acid sequence is operably positioned within a region of about 1 to 1000 nucleotides downstream of the 5'-terminus of the modified viral RNA replicon. In some embodiments,

the first nucleic acid sequence is operably positioned within a region of about 1 to 25, about 1 to 40, about 10 to 25, 10 to 50, about 10 to 100, about 20 to 50, about 20 to 75, about 25 to 100, about 25 to 100 nucleotides downstream of the 5'-terminus of the modified viral RNA replicon. In some embodiments, the first nucleic acid sequence is operably positioned within a region of about 1, 2, 5, 10, 15, 20, 25, 30, 40, 50, 75, 100, 125, 150, 200, 250, 300, or more, or a range between any two of these values, nucleotides downstream of the 5'-terminus of the modified viral RNA replicon. In some embodiments, the first nucleic acid sequence is operably positioned within a region of about 1 to 100, about 1 to 500, about 25 to 800, about 50 to 900, about 50 to 300, about 25 to 200, about 25 to 100, about 50 to 400, about 100 to 500, about 100 to 300, about 100 to 200, about 200 to 500, about 200 to 600, about 200 to 400, about 150 to 700, about 150 to 400, or about 500 to 1000 nucleotides downstream of the 5'-terminus of the modified viral RNA replicon.

[0134] Without being bound by any particular theory, it is believed that translational enhancing activity of a viral DLP motif can depend, in some embodiments, on the distance between the viral DLP motif and the initiation AUGi codon (Toribio *et al.*, 2016 supra). Accordingly, in some embodiments, the first nucleic acid sequence is operably positioned a region of about 10 to 100 nucleotides downstream of the initiation codon AUGi of the modified viral RNA replicon. In some embodiments, the first nucleic acid sequence is operably positioned within a region of about 10 to 75, about 10 to 50, about 10 to 25, 15 to 75, about 15 to 50, about 15 to 25, about 25 to 75, about 25 to 50, about 25 to 100 nucleotides downstream of the initiation codon AUGi of the modified viral RNA replicon. In some embodiments, the first nucleic acid sequence is operably positioned within a region of about 25, 28, 31, 34, 37, 37, 40, 43, 46, 49, 50, or a range between any two of these values, nucleotides downstream of the initiation codon AUGi of the modified viral RNA replicon.

[0135] In some embodiments, the sequence encoding the modified viral RNA replicon further comprising one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a coding sequence for a gene of interest (GOI). As used herein, the term "expression cassette" refers to a construct of genetic material that contains coding sequences and enough regulatory information to direct proper transcription and/or translation of the coding sequences in a recipient cell, *in vivo* and/or *ex*

vivo. The expression cassette may be inserted into a vector for targeting to a desired host cell and/or into a subject. Further, the term expression cassette may be used interchangeably with the term "expression construct". The term "expression cassette" as used herein, refers to a nucleic acid construct that encodes a protein or functional RNA operably linked to expression control elements, such as a promoter, and optionally, any or a combination of other nucleic acid sequences that affect the transcription or translation of the gene.

[0136] The term "operably linked", as used herein, denotes a functional linkage between two or more sequences. For example, an operably linkage between a polynucleotide of interest and a regulatory sequence (for example, a promoter) is functional link that allows for expression of the polynucleotide of interest. In this sense, the term "operably linked" refers to the positioning of a regulatory region and a coding sequence to be transcribed so that the regulatory region is effective for regulating transcription or translation of the coding sequence of interest. In some embodiments disclosed herein, the term "operably linked" denotes a configuration in which a regulatory sequence is placed at an appropriate position relative to a sequence that encodes a polypeptide or functional RNA such that the control sequence directs or regulates the expression or cellular localization of the mRNA encoding the polypeptide, the polypeptide, and/or the functional RNA. Thus, a promoter is in operable linkage with a nucleic acid sequence if it can mediate transcription of the nucleic acid sequence. Operably linked elements may be contiguous or non-contiguous.

[0137] The basic techniques for operably linking two or more sequences of DNA together are familiar to one of ordinary skill in the art, and such methods have been described in many books for standard molecular biological manipulation (see, for example, Maniatis *et al.*, *"Molecular Cloning: A Laboratory Manual"* 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; and Gibson *et al.*, *Nature Methods* 6:343-45, 2009).

[0138] In some embodiments disclosed herein, the nucleic acid molecules disclosed herein can include more than one expression cassette. In principle, the nucleic acid molecules disclosed herein can generally include any number of expression cassettes. In some particular embodiments, the modified viral RNA replicon comprises at least two, three, four, five, or six expression cassettes. In some embodiments, at least one of the one or more expression cassettes is operably positioned downstream to a transcriptional regulatory

sequence (TRS) of the modified arterivirus RNA replicon, wherein the TRS can be TRS1, TRS2, TRS3, TRS4, TRS5, TRS6, TRS7, or a combination thereof. In some particular embodiments, at least one of the one or more expression cassettes is operably positioned downstream of the TRS7 of the modified arterivirus RNA replicon.

[0139] The nucleic acid molecules as provided herein can find use, for example, as an expression or transcription vector that, when operably linked to a heterologous nucleic acid sequence such as, for example, a coding sequence of a gene of interest (GOI), can affect expression of the GOI. In some embodiments, the coding sequence of the GOI is optimized for expression at a level higher than the expression level of a reference coding sequence. In some embodiments, the reference coding sequence is a not codon-optimized. In some embodiments, the GOI coding sequence comprises codon optimization. With respect to codon-optimization of nucleic acid sequences, degeneracy of the genetic code provides the possibility to substitute at least one base of the protein encoding sequence of a gene with a different base without causing the amino acid sequence of the polypeptide produced from the gene to be changed. Hence, the nucleic acid molecules of the present disclosure may also have one or more nucleotide substitutions in accordance with degeneracy of the genetic code. References describing codon usage are readily publicly available. In some further embodiments of the disclosure, polynucleotide sequence variants can be produced for a variety of reasons, *e.g.*, to optimize codon expression for a particular host (*e.g.*, changing codons in the arterivirus mRNA to those preferred by other organisms such as human, hamster, mice, or monkey).

[0140] In some embodiments disclosed herein, the sequence of the GOI encode a polypeptide. The type of the polypeptide can vary depending on specific applications. For example, the polypeptide can be a therapeutic polypeptide, a prophylactic polypeptide, a diagnostic polypeptide, a nutraceutical polypeptide, an industrial enzyme, a reporter polypeptide, or any combination thereof. In some embodiments, the polypeptide is an antibody, an antigen, an immune modulator, a cytokine, an enzyme, or a combination thereof.

[0141] In some embodiments, the nucleic acid molecule as disclosed herein can further comprise a third nucleic acid sequence encoding one or more structural elements of a second viral capsid enhancer (*e.g.*, a DLP motif), wherein the third nucleic acid sequence is

operably linked upstream to the coding sequence for the GOI. The second DLP motif may be the same or may be different from the first DLP motif positioned upstream of the coding sequence for the nonstructural proteins. Accordingly, in some embodiments, the second DLP motif is the same as the first DLP motif positioned upstream of the coding sequence for the nonstructural proteins. In some embodiments, the second DLP motif is different from the first DLP motif positioned upstream of the coding sequence for the nonstructural proteins.

[0142] In some embodiments, the sequence encoding the modified viral RNA replicon further comprising a coding sequence for a proteolytic cleavage site operably linked downstream to the third nucleic acid sequence and upstream to the coding sequence for the GOI. Generally, any proteolytic cleavage site known in the art can be incorporated into the nucleic acid molecules of the disclosure and can be, for example, proteolytic cleavage sequences that are cleaved post-production by a protease. Further suitable proteolytic cleavage sites also include proteolytic cleavage sequences that can be cleaved following addition of an external protease. In some embodiments, the sequence encoding the modified viral RNA replicon further comprising a coding sequence for an autoprotease peptide operably linked downstream to the third nucleic acid sequence and upstream to the coding sequence for the GOI. In some embodiments, the autoprotease peptide includes a peptide sequence selected from the group consisting of porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), and a combination thereof. In some embodiments, the autoprotease peptide includes a peptide sequence from porcine teschovirus-1 2A (P2A).

[0143] One of skill in the art will appreciate that different configurations of the viral capsid enhancer sequence, the coding sequence for the nonstructural proteins, the sequence encoding the autoprotease peptide, and the sequence encoding the gene of interest can be employed as long as the capsid enhancer sequence augments expression of the heterologous nucleic acid sequence(s), as compared with the level seen in the absence of the capsid enhancer sequence. These sequences will typically be configured so that the polypeptide encoded by the gene of interest can be released from the protease and any capsid protein sequence after cleavage by the autoprotease.

[0144] In some embodiments, the sequence of the nucleic acid molecule as disclosed herein includes a modified RNA replicon of an alphavirus virus species. In some embodiments, the modified alphavirus RNA replicon is of an alphavirus belonging to the VEEV/EEEV group, or the SF group, or the SIN group. Non-limiting examples of SF group alphaviruses include Semliki Forest virus, O'Nyong-Nyong virus, Ross River virus, Middelburg virus, Chikungunya virus, Barmah Forest virus, Getah virus, Mayaro virus, Sagiyama virus, Bebaru virus, and Una virus. Non-limiting examples of SIN group alphaviruses include Sindbis virus, Girdwood S.A. virus, South African Arbovirus No. 86, Ockelbo virus, Aura virus, Babanki virus, Whataroa virus, and Kyzylagach virus. Non-limiting examples of VEEV/EEEV group alphaviruses include Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), Everglades virus (EVEV), Mucambo virus (MUCV), Pixuna virus (PIXV), Middleburg virus (MIDV), Chikungunya virus (CHIKV), O'Nyong-Nyong virus (ONNV), Ross River virus (RRV), Barmah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAGV), Bebaru virus (BEBV), Mayaro virus (MAYV), and Una virus (UNAV).

[0145] Non-limiting examples of alphavirus species include Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), Everglades virus (EVEV), Mucambo virus (MUCV), Semliki forest virus (SFV), Pixuna virus (PIXV), Middleburg virus (MIDV), Chikungunya virus (CHIKV), O'Nyong-Nyong virus (ONNV), Ross River virus (RRV), Barmah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAGV), Bebaru virus (BEBV), Mayaro virus (MAYV), Una virus (UNAV), Sindbis virus (SINV), Aura virus (AURAV), Whataroa virus (WHAV), Babanki virus (BABV), Kyzylagach virus (KYZV), Western equine encephalitis virus (WEEV), Highland J virus (HJV), Fort Morgan virus (FMV), Ndumu (NDUV), and Buggy Creek virus. Virulent and avirulent alphavirus strains are both suitable. In some embodiments, the modified alphavirus RNA replicon is of a Sindbis virus (SIN), a Semliki Forest virus (SFV), a Ross River virus (RRV), a Venezuelan equine encephalitis virus (VEEV), or an Eastern equine encephalitis virus (EEEV). In some embodiments, the modified alphavirus RNA replicon is of a Venezuelan equine encephalitis virus (VEEV).

[0146] In some instances where the nucleic acid molecule as disclosed herein includes a modified RNA replicon of an alphavirus virus species, the first nucleic acid sequence is positioned upstream to a nucleic acid sequence encoding one or more nonstructural proteins nsp1-4 or a portion thereof of the modified alphavirus RNA replicon. Accordingly, in some embodiments, the first nucleic acid sequence is positioned upstream to a nucleic acid sequence encoding the nonstructural proteins nsp1, nsp1-2, nsp1-3, nsp1-4, nsp2-4, nsp3-4, nsp2-3, nsp2, nsp3, nsp4, or a portion thereof of the modified alphavirus RNA replicon. In some embodiments, the sequence encoding the modified alphavirus RNA replicon further includes one or more expression cassettes, wherein each of the expression cassettes includes a promoter operably linked to a coding sequence for a gene of interest (GOI). In some embodiments, the modified alphavirus RNA replicon comprises at least two, three, four, five, or six expression cassettes. In some embodiments, at least one of the one or more expression cassettes is operably linked downstream of a nucleic acid sequence encoding one or more nonstructural proteins nsp1-4 or a portion thereof of the modified alphavirus RNA replicon. Accordingly, in some embodiments, at least one of the one or more expression cassettes is operably linked downstream of a nucleic acid sequence encoding the nonstructural proteins nsp1, nsp1-2, nsp1-3, nsp1-4, nsp2-4, nsp3-4, nsp2-3, nsp2, nsp3, nsp4, or a portion thereof, of the modified alphavirus RNA replicon.

[0147] In some embodiments, at least one of the one or more expression cassettes further comprises a third nucleic acid sequence encoding one or more structural elements of a second viral capsid enhancer (*e.g.*, a DLP motif), wherein the third nucleic acid sequence is operably linked upstream to the coding sequence for the GOI. The second DLP motif may be the same or may be different from the first DLP motif positioned upstream of the coding sequence for at least of the nonstructural proteins nsp1-4 or a portion thereof. Accordingly, in some embodiments, the second DLP motif is the same as the first DLP motif positioned upstream of the coding sequence for the nonstructural proteins. In some embodiments, the second DLP motif is different from the first DLP motif positioned upstream of the coding sequence for the nonstructural proteins.

[0148] In some embodiments, the nucleic acid sequence of the present disclosure further comprises a coding sequence for an autoprotease peptide operably linked downstream

to the third nucleic acid sequence and upstream to the coding sequence for the GOI. The autoprotease peptide can generally be any autoprotease peptide known in the art. Non-limiting examples of auprotease peptides include the peptide sequences from porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), and any combinations thereof.

[0149] In a further aspect, some embodiments disclosed herein relate to a nucleic acid molecule including a nucleic acid sequence encoding a modified non-alphavirus RNA replicon, wherein the modified non-alphavirus RNA replicon comprising a first nucleic acid sequence encoding a viral capsid enhancer (*e.g.*, a DLP motif). In some embodiments, the modified non-alphavirus RNA replicon further comprising a second nucleic acid sequence encoding at least one nonstructural viral protein or a portion thereof, wherein the first nucleic acid sequence is operably linked upstream to the second nucleic acid sequence.

[0150] In some embodiments, the modified non-alphavirus RNA replicon further comprising a coding sequence for an autoprotease peptide operably linked downstream to the first nucleic acid sequence and upstream to the second nucleic acid sequence. In some embodiments, the modified non-alphavirus RNA replicon includes a modified RNA replicon of a positive-strand RNA virus. In some embodiments, the modified non-alphavirus RNA replicon includes a modified RNA replicon of a negative-strand RNA virus.

[0151] Non-limiting examples of modified non-alphavirus RNA replicons include modified RNA replicons of virus species belonging to *Togaviridae* family, *Flaviviridae* family, *Orthomyxoviridae* family, *Rhabdoviridae* family, or *Paramyxoviridae* family. Accordingly, in some embodiments, the modified non-alphavirus RNA replicon includes a modified RNA replicon of a negative-strand RNA virus. Suitable negative-strand RNA virus species include, but are not limited to viral species of the families *Orthomyxoviridae*, *Rhabdoviridae,* and *Paramyxoviridae*. In some embodiments, the modified non-alphavirus RNA replicon includes a modified RNA replicon of a positive-strand virus species belonging to the *Togaviridae* family or *Flaviviridae* family. In some embodiments, the modified non-alphavirus RNA replicon includes a modified RNA replicon of a positive-strand virus species

belonging to the *Arterivirus* genus of the *Arteriviridae* family. Suitable arterivirus species include, but are not limited to, species of Equine arteritis virus (EAV), Porcine respiratory and reproductive syndrome virus (PRRSV), Lactate dehydrogenase elevating virus (LDV), Simian hemorrhagic fever virus (SHFV), and wobbly possum disease virus (WPDV).

[0152] In some embodiments, the sequence encoding the non-alphavirus modified RNA replicon further includes one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a coding sequence for a gene of interest (GOI). In some embodiments, the modified non-alphavirus RNA replicon comprises at least two, three, four, five, or six expression cassettes. In some embodiments, at least one of the one or more expression cassettes is operably linked downstream of the second nucleic acid sequence encoding the at least one nonstructural viral protein or a portion thereof. In some embodiments, at least one of the one or more expression cassettes further comprises a third nucleic acid sequence encoding one or more structural elements of a viral capsid enhancer, wherein the third nucleic acid sequence is operably linked upstream to the coding sequence for the GOI. In some embodiments, the modified non-alphavirus RNA replicon further includes a coding sequence for an autoprotease peptide operably linked downstream to the third nucleic acid sequence and upstream to the coding sequence for the GOI.

[0153] Some embodiments of the disclosure relate to a nucleic acid molecule including a nucleic acid sequence encoding a modified viral RNA replicon which includes in 5'−>3'direction a first nucleic acid sequence encoding a capsid enhancer from a Sindbis virus, a second nucleic acid sequence encoding an autoprotease peptide, and a third nucleic acid sequence encoding all of the viral nonstructural proteins. Some embodiments of the disclosure relate to a nucleic acid molecule including a nucleic acid sequence which encodes a modified viral RNA replicon, wherein the modified viral RNA replicon comprises a viral capsid enhancer and wherein the sequence of the modified viral RNA replicon exhibits at least 80% sequence identity to the sequence of at least one of SEQ ID NOs: 15-18 and 27-29.

[0154] Contemplated within the scope of the present disclosure are variants of the polynucleotides provided herein. Such variants may be naturally-occurring, including homologous polynucleotides from the same or a different species, or may be non-natural variants, for example polynucleotides synthesized using chemical synthesis methods, or

generated using recombinant DNA techniques. With respect to nucleic acid sequences, degeneracy of the genetic code provides the possibility to substitute at least one base of the protein encoding sequence of a gene with a different base without causing the amino acid sequence of the polypeptide produced from the gene to be changed. Hence, the nucleic acid molecules of the present disclosure may also have any base sequence that has been changed from any polynucleotide sequence disclosed herein by substitution in accordance with degeneracy of the genetic code. References describing codon usage are readily publicly available. In further embodiments, polynucleotide sequence variants can be produced for a variety of reasons, *e.g.*, to optimize codon expression for a particular host (*e.g.*, changing codons in the viral mRNA to those preferred by other organisms such as mammals or fish species).

[0155] In some embodiments, the nucleic acid molecules of the present disclosure comprises in 5'−>3'direction a nucleic acid sequence encoding a capsid enhancer from a Sindbis virus, a nucleic acid sequence encoding an autoprotease peptide, and a nucleic acid sequence encoding all of the viral nonstructural proteins of a modified viral RNA replicon. In some embodiments, the nucleic acid molecule comprises in 5'−>3'direction a 5-UTR sequence, a first capsid enhancer from a Sindbis virus, an autoprotease peptide, a sequence encoding all of the viral nonstructural proteins of a modified viral RNA replicon, one or more expression cassettes, and a 3'UTR sequence, wherein at least one of the one or more expression cassettes comprises a second capsid enhancer from a Sindbis virus operably linked upstream of a coding sequence for a gene of interest (GOI).

[0156] Accordingly, in some embodiments, the nucleic acid molecule of the present disclosure includes a nucleic acid sequence which encodes a modified viral RNA replicon, wherein the sequence exhibits at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the sequence of at least one of SEQ ID NOs: 15-18 and 27-29.

[0157] In some embodiments, the nucleic acid molecule of the disclosure is an expression vector. In some embodiments, the expression vector further includes one or more additional regulatory sequences, which can be a transcriptional regulatory element or a translational regulatory element. The terms "regulatory element" and "regulatory region", as

used interchangeably in the present disclosure, refer to a nucleic acid sequence that influences transcription or translation initiation and rate, and stability and/or mobility of a transcription or translation product. Such regulatory elements need not be of naturally-occurring sequences. Regulatory sequences include but are not limited to promoter sequences, enhancer sequences, response elements, protein recognition sites, inducible elements, protein binding sequences, 5' and 3' untranslated regions (UTRs), transcriptional start sites, termination sequences, polyadenylation sequences, introns, and combinations thereof. In some embodiments, the expression vector of the disclosure further includes one or more of the following: an origin of replication, one or more sequences for promoting integration of the expression cassette into the host genome, a terminator sequence.

[0158] In some embodiments, the expression vector comprises at least one origin of replication ("ORI") sequence for replication in a cell. The vectors may further optionally comprise one or more selectable markers under the control of one or more eukaryotic promoters, one or more selectable markers under the control of one or more prokaryotic promoters, and/or one or more sequences that mediate recombination of an exogenous nucleic acid sequence into the target cell's genome.

[0159] An ORI is the sequence in a DNA molecule at which replication begins. The ORI serves as a base of assembly for the pre-replication complex. Depending on the ORI, such replication can proceed uni-directionally or bi-directionally. An expression vector as provided herein can include an ORI for replication of the expression vector in a cloning host, such as *E. coli* or yeast, and/or can include an ORI for replication of the expression vector in a target cell, which can be, for example, a mammalian cell. The structural biology of ORIs is widely conserved among prokaryotes, eukaryotes, and viruses. Most ORIs possess simple tri-, tetra-, or higher nucleotide repetition patterns. Most are AT-rich and contain inverted repeats. Those skilled in the art will be familiar with the more common ORIs, such as P15A and the pUC's ORI.

[0160] The expression vector can also, in some embodiments, carry a selectable marker. By way of example, a vector that includes an expression cassette may include, as a selectable marker, a gene conferring resistance to a poisonous substance, such as an antibiotic, a herbicide, or some other toxin, so that transformants can be selected by exposing

the cells to the poison and selecting those cells which survive the encounter. In some embodiments, the selectable marker may be under the control of a promoter. In some embodiments, the promoter regulating expression of the selectable marker may be conditional or inducible. In some embodiments, the promoter regulating expression of the selectable marker may be preferably constitutive, and can be, for example, any promoter described herein or another promoter.

[0161] In some embodiments, the expression vector is a plasmid, a bacteriophage vector, a cosmid, a fosmid, a viral replicon, a shuttle vector, or a combination thereof. In some embodiments, the expression vector is an RNA replicon. In some embodiments, the expression vector is a prokaryotic expression vector. In some embodiments, the expression vector is a eukaryotic expression vector. In some embodiments, the nucleic acid molecule of the disclosure is produced via *de novo* synthesis. In some embodiments of the disclosure, *de novo* synthesis can be used to generate a synthetic mRNA molecule.

Recombinant Cells

[0162] In one aspect, some embodiments disclosed herein relate to a method of transforming a cell that includes introducing into a host cell, such as an animal cell, a nucleic acid molecule as provided herein, and selecting or screening for a transformed cell. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. In some embodiments, the nucleic acid molecule is introduced into a host cell by an electroporation procedure or a biolistic procedure.

[0163] In a related aspect, some embodiments relate to recombinant host cells, for example, recombinant animal cells that include a nucleic acid molecule described herein. The nucleic acid molecule can be stably integrated in the host genome, or can be episomally replicating, or present in the recombinant host cell as a mini-circle expression vector for a stable or transient expression. Accordingly, in some embodiments disclosed herein, the nucleic acid molecule is maintained and replicated in the recombinant host cell as an

episomal unit. In some embodiments, the nucleic acid molecule is stably integrated into the genome of the recombinant cell. Stable integration can be completed using classical random genomic recombination techniques or with more precise genome editing techniques such as using guide RNA directed CRISPR/Cas9, or DNA-guided endonuclease genome editing NgAgo (*Natronobacterium gregoryi* Argonaute), or TALEN genome editing (transcription activator-like effector nucleases). In some embodiments, the nucleic acid molecule present in the recombinant host cell as a mini-circle expression vector for a stable or transient expression.

[0164] In some embodiments, host cells can be genetically engineered (*e.g.* transduced or transformed or transfected) with, for example, a vector construct of the present application that can be, for example, a vector for homologous recombination that includes nucleic acid sequences homologous to a portion of the genome of the host cell, or can be an expression vector for the expression of any or a combination of the genes of interest. The vector can be, for example, in the form of a plasmid, a viral particle, a phage, *etc.* In some embodiments, a vector for expression of a polypeptide of interest can also be designed for integration into the host, *e.g.*, by homologous recombination. The vector containing a polynucleotide sequence as described herein, *e.g.*, nucleic acid molecule comprising a modified alphavirus genome or replicon RNA, as well as, optionally, a selectable marker or reporter gene, can be employed to transform an appropriate host cell.

[0165] The methods and compositions disclosed herein may be deployed for genetic engineering of any species, including, but not limited to, prokaryotic and eukaryotic species. Suitable host cells to be modified using the compositions and methods according to the present disclosure can include, but not limited to, algal cells, bacterial cells, heterokonts, fungal cells, chytrid cells, microfungi, microalgae, and animal cells. In some embodiments, the animal cells are invertebrate animal cells. In some embodiments, the vertebrate animal cells are mammalians cells. Host cells can be either untransformed cells or cells that have already been transfected with at least one nucleic acid molecule.

[0166] The methods and compositions disclosed herein can be used, for example, with subject and/or host cells that are important or interesting for aquaculture, agriculture, animal husbandry, and/or for therapeutic and medical applications, including production of

polypeptides used in the manufacturing of vaccines, pharmaceutical products, industrial products, chemicals, and the like. In some embodiments, the compositions and methods disclosed herein can be used with host cells from species that are natural hosts of alphaviruses, such as rodents, mice, fish, birds, and larger mammals such as humans, horses, pig, monkey, and apes as well as invertebrates. Particularly preferred species, in some embodiments of the application, are vertebrate animal species and invertebrate animal species. In principle, any animal species can be generally used and can be, for example, human, dog, bird, fish, horse, pig, primate, mouse, cotton rat, ferret, cattle, swine, sheep, rabbit, cat, goat, donkey, hamster, or buffalo. Non-limiting examples of suitable bird species include chicken, duck, goose, turkey, ostrich, emu, swan, peafowl, pheasant, partridge, and guinea fowl. In some particular embodiments, the fish is any species in the Salmonidae family. Primary mammalian cells and continuous/immortalized cells types are also suitable. Non-limiting examples of suitable animal host cells include, but not limited to, pulmonary equine artery endothelial cell, equine dermis cell, baby hamster kidney (BHK) cell, rabbit kidney cell, mouse muscle cell, mouse connective tissue cell, human cervix cell, human epidermoid larynx cell, Chinese hamster ovary cell (CHO), human HEK-293 cell, mouse 3T3 cell, Vero cell, Madin-Darby Canine Kidney Epithelial Cell (MDCK), primary chicken fibroblast cell, a HuT78 cell, A549 lung cell, HeLa cell, PER.C6® cell, WI-38 cell, MRC-5 cell, FRhL-2, and CEM T-cell. In some embodiments, the host cell is baby hamster kidney cell. In some embodiments, the baby hamster kidney cell is a BHK-21 cell.

[0167] Techniques for transforming a wide variety of the above-mentioned host cells and species are known in the art and described in the technical and scientific literature. Accordingly, cell cultures including at least one recombinant cell as disclosed herein are also within the scope of this application. Methods and systems suitable for generating and maintaining cell cultures are known in the art.

Heterologous Nucleic Acid Sequences

[0168] In accordance of some embodiments of the present disclosure, a wide variety of nucleic acid sequences can be carried by the nucleic acid molecules of the present disclosure. In some embodiments, nucleic acid molecules as described herein does not contain any additional heterologous nucleic acid sequence. In some embodiments, the

nucleic acid molecules of the present disclosure contains one or more additional heterologous or foreign nucleic acid sequences. In some embodiments, the one or more additional heterologous or foreign nucleic acid sequences include a coding sequence for a gene of interest (GOI). In some embodiments disclosed herein, the coding sequence for the GOI encodes a polypeptide or a functional RNA. In some embodiments, the coding sequence for the GOI encodes a functional RNA selected from a ribosomal RNA, a tRNA, a ribozyme, a transactivating (tr) RNA of a CRISPR system, a crispr (cr) RNA of a CRISPR system, a chimeric guide RNA of a CRISPR system, a micro RNA, an interfering RNA (RNAi) molecule, a short hairpin (sh) RNA, or an antisense RNA molecule. In some embodiments, the coding sequence for the GOI encodes a polypeptide selected from the group consisting of a therapeutic polypeptide, a prophylactic polypeptide, a diagnostic polypeptide, a nutraceutical polypeptide, an industrial enzyme, a reporter polypeptide, or any combination thereof. In some embodiments, the coding sequence for the GOI encodes a polypeptide is selected from the group consisting of an antibody, an antigen, an immune modulator, and a cytokine.

[0169] In some embodiments, the heterologous nucleic acid sequence comprises a heterologous nucleic acid sequence of at least about 100 bases, 2 kb, 3.5 kb, 5 kb, 7 kb, or 8 kb. The heterologous RNA or heterologous nucleic acid sequence can be chosen from a wide variety of sequences derived from viruses, prokaryotes or eukaryotes. Examples of categories of heterologous sequences include, but are not limited to, immunogens (including native, modified or synthetic antigenic proteins, peptides, epitopes or immunogenic fragments), cytokines, toxins, therapeutic proteins, enzymes, antisense sequences, and immune response modulators.

[0170] A wide variety of GOI can be included in the nucleic acid molecules of the present disclosure to express a polypeptide of the GOI, including but not limited to, cytokines, toxins, prodrugs, antigens which stimulate an immune response, ribozymes, and proteins which assist or inhibit an immune response, as well as antisense sequences (or sense sequences for "antisense applications"). As noted above, within various embodiments of the disclosure the modified RNA replicon provided herein may contain the coding region of (and express, in some embodiments) two or more polypeptides of interest.

1) Cytokines

[0171] In some embodiments disclosed herein, the GOI encodes a cytokine. Generally, cytokines act to proliferate, activate, and/or differentiate immune effectors cells. Examples of cytokines include, but are not limited to macrophages, B lymphocytes, T lymphocytes, endothelial cells, fibroblasts, lymphokines likes gamma interferon, tumor necrosis factor, interleukin, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL- 1, IL-12, IL-13, IL-14, IL-15, GM-CSF, CSF-1 and G-CSF.

[0172] In some related embodiments, the GOI encodes an immunomodulatory cofactor. As utilized within the context of the present disclosure, "immunomodulatory cofactor" refers to factors which, when manufactured by one or more of the cells involved in an immune response, or when added exogenously to the cells, cause the immune response to be different in quality or potency from that which would have occurred in the absence of the cofactor. The quality or potency of a response may be measured by a variety of assays known to one of skill in the art including, for example, *ex vitro* assays which measure cellular proliferation (*e.g.*, 3 H thymidine uptake), and *ex vitro* cytotoxic assays (*e.g.*, which measure 51 Cr release) (see *Warner et al.*, AIDS Res. and Human Retroviruses 7:645-655, 1991).

[0173] Examples of immunomodulatory co-factors include, but are not limited, alpha interferon, gamma interferons, G-CSF, GM-CSF, TNFs, Interleukin-2 (IL-2), IL-4, IL-6, IL-12, IL-15, ICAM-1, ICAM-2, LFA-1, LFA-3, MHC class I molecules, MHC class II molecules, 2 -microglobulin, chaperones, CD3, B7/BB 1, MHC linked transporter proteins, and analogues thereof.

[0174] The choice of which immunomodulatory cofactor to include within the nucleic acid molecules of the present disclosure may be based upon known therapeutic effects of the cofactor, or experimentally determined. For example, in chronic hepatitis B infections alpha interferon has been found to be efficacious in compensating a patient's immunological deficit and thereby assisting recovery from the disease. In some situations, a suitable immunomodulatory cofactor may be experimentally determined. Briefly, blood samples are first taken from patients with a hepatic disease. Peripheral blood lymphocytes (PBLs) are restimulated *ex vitro* with autologous or HLA-matched cells (*e.g.*, EBV transformed cells), and transduced with modified arterivirus genome or replicon RNA of the

present disclosure which directs the expression of an immunogenic portion of a hepatitis antigen and the immunomodulatory cofactor. Stimulated PBLs are used as effectors in a CTL assay with the BLA-matched transduced cells as targets. An increase in CTL response over that seen in the same assay performed using HLA-matched stimulator and target cells transduced with a vector encoding the antigen alone, indicates a useful immunomodulatory cofactor. In some embodiments, the immunomodulatory cofactor gamma interferon is particularly preferred.

[0175] Another non-limiting example of an immunomodulatory cofactor is the B7/BB1 costimulatory factor. Activation of the full functional activity of T cells requires two signals. One signal is provided by interaction of the antigen-specific T cell receptor with peptides which are bound to major histocompatibility complex (MHC) molecules, and the second signal, referred to as costimulation, is delivered to the T cell by antigen-presenting cells. The second signal is required for interleukin-2 (IL-2) production by T cells and appears to involve interaction of the B7/BB 1 molecule on antigen-presenting cells with CD28 and CTLA-4 receptors on T lymphocytes. In some embodiments, B7/BB 1 may be introduced into tumor cells in order to cause costimulation of CD8+T cells, such that the CD8+T cells produce enough IL-2 to expand and become fully activated. These CD8+T cells can kill tumor cells that are not expressing B7 because costimulation is no longer required for further CTL function. Vectors that express both the costimulatory B7/BB1 factor and, for example, an immunogenic HBV core protein, may be constructed utilizing methods which are described herein. Cells transduced with these vectors will become more effective antigen-presenting cells. The HBV core-specific CTL response will be augmented from the fully activated CD8+T cell via the costimulatory ligand B7/BB 1.

2) Toxins

[0176] In some embodiments disclosed herein, the GOI encodes a toxin. In some embodiments, toxins act to directly inhibit the growth of a cell. Examples of toxins include, but are not limited to, ricin, abrin, diphtheria toxin, cholera toxin, gelonin, pokeweed, antiviral protein, tritin, Shigella toxin, Pseudomonas exotoxin A, herpes simplex virus thymidine kinase (HSVTK), and *E. coli*. guanine phosphoribosyl transferase.

3) Pro-drugs

[0177] In some embodiments disclosed herein, the GOI encodes a "pro-drug". As utilized within the context of the present disclosure, "pro-drug" refers to a gene product that activates a compound with little or no cytotoxicity into a toxic product. Representative examples of such gene products include HSVTK and VZVTK (as well as analogues and derivatives thereof), which selectively monophosphorylate certain purine arabinosides and substituted pyrimidine compounds, converting them to cytotoxic or cytostatic metabolites. More specifically, exposure of the drugs ganciclovir, acyclovir, or any of their analogues (*e.g.*, FIAU, FIAC, and DHPG) to HSVTK phosphorylates the drug into its corresponding active nucleotide triphosphate form.

[0178] Non-limiting examples of pro-drugs which may be utilized within the context of the present disclosure include: *E. coli* guanine phosphoribosyl transferase which converts thioxanthine into toxic thioxanthine monophosphate; alkaline phosphatase, which will convert inactive phosphorylated compounds such as mitomycin phosphate and doxorubicin-phosphate to toxic dephosphorylated compounds; fungal (*e.g.*, *Fusarium oxysporum*) and bacterial cytosine deaminase, which can convert 5-fluorocytosine to the toxic compound 5-fluorouracil; carboxypeptidase G2, which will cleave the glutamic acid from para-N-bis (2-chloroethyl) aminobenzoyl glutamic acid, thereby creating a toxic benzoic acid mustard; and Penicillin-V amidase, which will convert phenoxyacetabide derivatives of doxorubicin and melphalan to toxic compounds.

4) Antisense Sequence

[0179] In some embodiments disclosed herein, the coding sequence for the GOI is an antisense sequence. Antisense sequences are designed to bind to RNA transcripts, and thereby prevent cellular synthesis of a particular protein or prevent use of that RNA sequence by the cell. Non-limiting examples of such sequences include antisense thymidine kinase, antisense dihydrofolate reductase, antisense HER2, antisense ABL, antisense Myc, antisense ras, as well as antisense sequences which block any of the enzymes in the nucleotide biosynthetic pathway. In addition, in accordance with some embodiments disclosed herein, antisense sequences to interferon and 2 microglobulin may be utilized in order to decrease immune response.

[0180] In some embodiments, antisense RNA may be utilized as an anti-tumor agent in order to induce a potent Class I restricted response. In addition to binding RNA and thereby preventing translation of a specific mRNA, high levels of specific antisense sequences are believed to induce the increased expression of interferons (including gamma-interferon) due to the formation of large quantities of double-stranded RNA. The increased expression of gamma interferon, in turn, boosts the expression of MHC Class I antigens. Preferred antisense sequences for use in this regard include actin RNA, myosin RNA, and histone RNA. Antisense RNA which forms a mismatch with actin RNA is particularly preferred.

5) Ribozymes

[0181] In some embodiments disclosed herein, nucleic acid molecules comprising one or more RNA stem-loop structures are provided which produce ribozymes upon infection of a host cell. Ribozymes are used to cleave specific RNAs and are designed such that it can only affect one specific RNA sequence. Generally, the substrate binding sequence of a ribozyme is between 10 and 20 nucleotides long. The length of this sequence is sufficient to allow a hybridization with target RNA and disassociation of the ribozyme from the cleaved RNA. Representative examples for creating ribozymes include those described in U.S. Pat. Nos. 5,116,742; 5,225,337 and 5,246,921.

6) Proteins and Other Cellular Constituents

[0182] In some embodiments disclosed herein, a wide variety of proteins or other cellular constituents can be carried by the nucleic acid molecules of the present disclosure. Non-limiting examples of such proteins include native or altered cellular components, as well as foreign proteins or cellular constituents, found in for example, viruses, bacteria, parasites, fungus or animal such as mammalian.

Methods for Producing Polypeptides

[0183] The host cells of the present disclosure, such as a prokaryotic or eukaryotic host cell, can be used to produce (*e.g.*, express) a molecule of interest such as, *e.g.*, a polypeptide, encoded in an open reading frame of a gene of interest (GOI) as disclosed herein. Thus, the present application further provides methods for producing a molecule of

interest such as, *e.g.*, a polypeptide, using the host cells and/or the nucleic acid molecules of the present disclosure. The host cells can be, for example, isolated cells, cells in cell culture, cells in a living body, or a combination thereof.

[0184] Some embodiments disclosed herein provides methods for producing a polypeptide of interest. The method can include the introduction of a nucleic acid molecule according to any one of the aspects and embodiments of the present disclosure into a host cell, thereby producing a polypeptide encoded by the GOI in the host cell. In some embodiments where the introduced nucleic acid molecule is a RNA molecule, for example an mRNA molecule or a RNA replicon. The RNA molecule can be generated by any method known in the art, for example by *de novo* synthesis in whole or in part. For example, the RNA molecules, including but not limited to mRNA molecules and RNA replicons, can be produced using chemical methods, enzymatic techniques, or any combination thereof, for example, by chemical synthesis through *de novo* assembly (such as with oligonucleotides) or *in vitro* transcription reactions (using appropriate enzymes, buffers, nucleotides, etc.). In some instances where the introduced nucleic acid molecule is an mRNA, the mRNA can be directly delivered to cells *in vivo* for producing a polypepide of interest (e.g., drug, antigen, *etc.*) in cells. The cells can be isolated cells; cells in cell cultures; cells in an tissue, an organ, and/or a subject; or any combination thereof. In some embodiments, no new mRNA copies are made in the cells, As disclosed herein, the incorporation of one or more RNA stem-loops from a viral capsid enhancer (e.g., DLP motifs) into the chemically synthesized RNA can confer the intended enhancement of gene expression once the DLP-containing mRNA is introduced into the cells.

[0185] In some embodiments where the introduced nucleic acid molecule is a vector such as, for example, an RNA replicon, new mRNA copies may be generated which includes coding sequence for a gene of interest operably linked to one or more DLP motifs. The incorporation the one or more DLP motifs into the vector, e.g., RNA replicon, can then confer the intended enhancementof gene expression once the DLP-containing vector or replicon is introduced into the cells

[0186] Some embodiments disclosed herein provides methods for producing a polypeptide of interest in a host cell. Such method includes the cultivation of a recombinant

host cell, including a nucleic acid molecule according to any one of the aspects and embodiments of the present disclosure. In some embodiments, the methods include culturing the host cell of present disclosure (into which a recombinant expression vector encoding the molecule of interest has been introduced) in a suitable medium such that the molecule of interest is produced. In some embodiments, the methods further include isolating the molecule of interest from the medium or the host cell.

[0187] Also disclosed are methods for producing a polypeptide of interest in a subject, including administering to the subject a nucleic acid molecule according to any one of the aspects and embodiments.

[0188] Suitable host cells and/or subjects for use in the methods and compositions disclosed herein include, but are not limited to, prokaryotic and eukaryotic species. Suitable host cells to be modified using the compositions and methods according to the present disclosure can include, but not limited to, algal cells, bacterial cells, heterokonts, fungal cells, chytrid cells, microfungi, microalgae, and animal cells. In some embodiments, the animal cells are invertebrate animal cells. In some embodiments, the vertebrate animal cells are mammalians cells. Host cells can be either untransformed cells or cells that have already been transfected with at least one nucleic acid molecule. Accordingly, biological samples, biomass, and progeny of a recombinant cell according to any one of the aspects and embodiments are also within the scope of the present application. Thus, as discussed in more detail below, polypeptides produced by a method according to this aspect of the application are also within the scope of this application.

[0189] In some embodiments, the recombinant cell is an animal cell. Therapeutic protein production in small and large scale is important field of development in pharmaceutical industry, because proteins produced in animal cells are believe to generally have proper processing, post-translational modification and therefore have adequate activity for treatment of the physiological condition. In principle, any animal species can be generally used and can be, for example, human, dog, bird, fish, horse, pig, primate, mouse, cotton rat, ferret, cattle, swine, sheep, rabbit, cat, goat, donkey, hamster, or buffalo. Non-limiting examples of suitable bird species include chicken, duck, goose, turkey, ostrich, emu, swan, peafowl, pheasant, partridge, and guinea fowl. In some particular embodiments, the fish is

any species in the Salmonidae family. Primary mammalian cells and continuous/immortalized cells types are also suitable. Non-limiting examples of suitable animal host cells include, but not limited to, pulmonary equine artery endothelial cell, equine dermis cell, baby hamster kidney (BHK) cell, rabbit kidney cell, mouse muscle cell, mouse connective tissue cell, human cervix cell, human epidermoid larynx cell, Chinese hamster ovary cell (CHO), human HEK-293 cell, mouse 3T3 cell, Vero cell, Madin-Darby Canine Kidney Epithelial Cell (MDCK), primary chicken fibroblast cell, a HuT78 cell, A549 lung cell, HeLa cell, PER.C6® cell, WI-38 cell, MRC-5 cell, FRhL-2, and CEM T-cell. In some embodiments, the host cell is baby hamster kidney cell. In some embodiments, the baby hamster kidney cell is a BHK-21 cell.

Recombinant Polypeptides

[0190] Some embodiments disclosed herein relate to recombinant polypeptides produced by a method in accordance with one or more embodiments described herein. The recombinant polypeptides of the present application generally can be any recombinant polypeptides and can be, for example, one or more of therapeutic polypeptides, prophylactic polypeptides, diagnostic polypeptides, nutraceutical polypeptides, industrial enzymes, and reporter polypeptides. In some embodiments, the recombinant polypeptides can be one or more of antibodies, antigens, immune modulators, and cytokines. In some embodiments, the polypeptide of interest may have therapeutic or prophylactic activity.

Compositions and Formulations

[0191] Some embodiments disclosed herein relate to a composition comprising any of the recombinant polypeptides described herein. The composition can be, for example, a nutraceutical composition, a prophylactic composition, a pharmaceutical composition comprising a pharmaceutically acceptable carrier, or a mixture thereof. In some embodiments, the compositions of the present application can be used as a vaccine.

[0192] Some embodiments disclosed herein relate to a composition including any of the nucleic acid molecules (*e.g.*, expression vectors) described herein. The composition can be, for example, a nutraceutical composition, a prophylactic composition, a pharmaceutical composition comprising a pharmaceutically acceptable carrier, or a mixture

thereof. In some embodiments, the compositions of the present application can be used as a vaccine.

[0193] Some embodiments disclosed herein relate to a composition including any of the recombinant cells described herein. The composition can be, for example, a nutraceutical composition, a prophylactic composition, a pharmaceutical composition comprising a pharmaceutically acceptable carrier, or a mixture thereof. In some embodiments, the compositions of the present application can be used as a vaccine.

[0194] As used herein, the term "pharmaceutically-acceptable carrier" means a carrier that is useful in preparing a pharmaceutical composition or formulation that is generally safe, non-toxic, and neither biologically nor otherwise undesirable, and includes a carrier that is acceptable for veterinary use as well as human pharmaceutical use. In some embodiments, a pharmaceutically acceptable carrier is as simple as water, but it can also include, for example, a solution of physiological salt concentration. In some embodiments, a pharmaceutically acceptable carrier can be, or may include, stabilizers, diluents and buffers. Suitable stabilizers are for example SPGA, carbohydrates (such as dried milk, serum albumin or casein) or degradation products thereof. Suitable buffers are for example alkali metal phosphates. Diluents include water, aqueous buffers (such as buffered saline), alcohols and polyols (such as glycerol). For administration to animals or humans, the composition according to the present application can be given by any enteral or parenteral route, which includes *inter alia* intranasally, by spraying, intradermally, subcutaneously, orally, by aerosol, intramuscularly, or any combination thereof.

[0195] In some embodiments, the nucleic acid molecules (*e.g.*, mRNAs and/or expression vectors), protein molecules, and/or compositions of the disclosure are in suitable formulations, for example pharmaceutical formulations. Provided herein include pharmaceutical formulations containing one or more of the molecules and/or compositions disclosed herein in a pharmaceutically acceptable vehicle. Some embodiments of the disclosure relate to pharmaceutical formulations comprising one or more of the expression vectors disclosed herein. Some embodiments of the disclosure relate to pharmaceutical formulations containing one or more of the nucleic acid molecules disclosed herein. Some embodiments of the disclosure relate to pharmaceutical formulations containing one or more

of the polypeptides disclosed herein. Some embodiments of the disclosure relate to pharmaceutical formulations containing one or more of the recombinant cells disclosed herein.

[0196] The molecules (*e.g.*, protein and nucleic acid molecules) and compositions disclosed herein can be in various formulations, for example pharmaceutical formulations. For example, the nucleic acid molecules (*e.g.*, replicons, mRNAs and expression vectors), protein molecules, and/or compositions of the disclosure can be formulated, for example into a pharmaceutical formulation, with one or more covalent compounds (*e.g.*, via direct linkage), non-covalent compounds (*e.g.*, via charged based associations from LNPs or cationic nano-emulsions), physical compositions (*e.g.*, vault proteins, non-charged lipid encapsulations), pharmaceutically acceptable buffers (*e.g.*, saline, lactated Ringer's), and any combinations thereof. Many methods, reagents, and systems suitable for generating the foregoing pharmaceutical formulations are known in the art.

[0197] In some embodiments, molecules and/or compositions disclosed herein is formulated in a saline or a lipid formulation. The lipid formulation can be selected from, but is not limited to, liposomes, lipoplexes, copolymers such as PLGA, and lipid nanoparticles.

Particles and Nanoparticles

[0198] In some embodiments, one or more of the nucleic acid molecules, polypeptide molecules, and/or compositions disclosed herein can be incorporated into particles or nanoparticles. Particles comprising one or more of the molecules and compositions disclosed herein can be polymeric particles, lipid particles, solid lipid particles, self-assembled particles, composite nanoparticles of conjugate phospholipids, surfactants, proteins, polyaminoacids, inorganic particles, or combinations thereof (*e.g.*, lipid stabilized polymeric particles). In some embodiments, the molecules and/or compositions disclosed herein are substantially encapsulated or partially encapsulated in the particles. In some embodiments, the molecules and/or compositions disclosed herein are deposited and/or absorbed on the surface of the particles. In some embodiments, the molecules and/or compositions disclosed herein are incorporated in the particles. In some embodiments, the molecules and/or compositions disclosed herein are part of or a component of the particle. The molecules and/or compositions of the disclosure can be, in some embodiments, attached

to the surface of the particles with covalent bonds, or non-covalent interactions. In some embodiments, the molecules and/or compositions of the disclosure self-assemble into a particle.

[0199] As used herein, the term "encapsulate" means to enclose, surround or encase. As it relates to the formulation of the molecules and/or compositions of the present disclosure, encapsulation may be substantial, complete or partial. The term "substantially encapsulated" means that at least greater than 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, or 99.999% of the molecules and/or compositions of the present disclosure may be enclosed, surrounded or encased within the particle. "Partially encapsulation" means that less than 10%, 15%, 20%, 30%, 40%, 50% of the molecules and/or compositions of the present disclosure may be enclosed, surrounded or encased within the particle. For example, at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, or 99.999% of the molecules and/or compositions of the present disclosure are encapsulated in the particle. Encapsulation may be determined by any known method.

[0200] In some embodiments, the particles are polymeric particles or contain a polymeric matrix. The particles can generally contain any of the polymers known in the art. The particles will generally contain one or more biocompatible polymers. The polymers can be biodegradable polymers. The polymers can be hydrophobic polymers, hydrophilic polymers, or amphiphilic polymers. In some embodiments, the particles contain one or more polymers having an additional targeting moiety attached thereto. In some embodiments, the particles are inorganic particles, such as but not limited to, gold nanoparticles and iron oxide nanoparticles.

[0201] The size of the particles can be adjusted for the intended application. The particles can be nanoparticles or microparticles. The particle can have a diameter of about 10 nm to about 10 microns, about 10 nm to about 1 micron, about 10 nm to about 500 nm, about 20 nm to about 500 nm, or about 25 nm to about 250 nm. In some embodiments the particle is a nanoparticle having a diameter from about 25 nm to about 250 nm. In some embodiments, the particle is a nanoparticle having a diameter from about 50 nm to about 150 nm. In some embodiments, the particle is a nanoparticle having a diameter from about 70 nm

to about 130 nm. In some embodiments, the particle is a nanoparticle having a diameter of about 100 nm. It is understood by those in the art that a plurality of particles will have a range of sizes and the diameter is understood to be the median diameter of the particle size distribution.

[0202] In some embodiments, the molecules and/or compositions disclosed herein may be incorporated into particles that are responsive to temperature, pH, and ionic conditions. For example, the particles may comprise an ionizable network of covalently cross-linked homopolymeric ionizable monomers wherein the ionizable network is covalently attached to a single terminal region of an amphiphilic copolymer to form a plurality of "dangling chains" and wherein the "dangling chains" of amphiphilic copolymer form immobile intra-network aggregates in aqueous solution, as disclosed in U.S. Pat. No. 7,204,997.

Liposomes, Lipoplexes, and Lipid Nanoparticles (LNPs)

[0203] The molecules and/or compositions of the disclosure can be formulated using one or more liposomes, lipoplexes, and/or lipid nanoparticles. In one embodiment, pharmaceutical formulations of the molecules and/or compositions of the disclosure include liposomes. Liposomes are artificially-prepared vesicles which may primarily be composed of a lipid bilayer and may be used as a delivery vehicle for the administration of nutrients and pharmaceutical formulations. Liposomes can be of different sizes such as, but not limited to, a multilamellar vesicle (MLV) which may be hundreds of nanometers in diameter and may contain a series of concentric bilayers separated by narrow aqueous compartments, a small unicellular vesicle (SUV) which may be smaller than 50 nm in diameter, and a large unilamellar vesicle (LUV) which may be between 50 and 500 nm in diameter. Liposome design may include, but is not limited to, opsonins or ligands in order to improve the attachment of liposomes to unhealthy tissue or to activate events such as, but not limited to, endocytosis. Liposomes may contain a low or a high pH in order to improve the delivery of the pharmaceutical formulations.

[0204] The formation of liposomes may depend on the physicochemical characteristics such as, but not limited to, the pharmaceutical formulation entrapped and the liposomal ingredients, the nature of the medium in which the lipid vesicles are dispersed, the

effective concentration of the entrapped substance and its potential toxicity, any additional processes involved during the application and/or delivery of the vesicles, the optimization size, polydispersity and the shelf-life of the vesicles for the intended application, and the batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products.

[0205] In some embodiments, the molecules and/or compositions of the disclosure may be formulated in a lipid vesicle which may have crosslinks between functionalized lipid bilayers. In some embodiments, the molecules and/or compositions of the disclosure may be formulated in a lipid-polycation complex. The formation of the lipid-polycation complex may be accomplished by methods known in the art. As a non-limiting example, the polycation may include a cationic peptide or a polypeptide such as, but not limited to, polylysine, polyornithine and/or polyarginine and the cationic peptides. In some embodiments, the nucleic acid molecules and/or compositions disclosed herein may be formulated in a lipid-polycation complex which may further include a neutral lipid such as, but not limited to, cholesterol or dioleoyl phosphatidylethanolamine (DOPE). The liposome formulation may be influenced by, but not limited to, the selection of the cationic lipid component, the degree of cationic lipid saturation, the nature of the PEGylation, ratio of all components and biophysical parameters such as size.

[0206] In some embodiments, the ratio of PEG in the lipid nanoparticle (LNP) formulations may be increased or decreased and/or the carbon chain length of the PEG lipid may be modified from C14 to C18 to alter the pharmacokinetics and/or biodistribution of the LNP formulations. As a non-limiting example, LNP formulations may contain 1-5% of the lipid molar ratio of PEG-c-DOMG as compared to the cationic lipid, DSPC and cholesterol. In another embodiment, the PEG-c-DOMG may be replaced with a PEG lipid such as, but not limited to, PEG-DSG (1,2-Distearoyl-sn-glycerol, methoxypolyethylene glycol) or PEG-DPG (1,2-Dipalmitoyl-sn-glycerol, methoxypolyethylene glycol). The cationic lipid may be selected from any lipid known in the art such as, but not limited to, DLin-MC3-DMA, DLin-DMA, C12-200, and DLin-KC2-DMA.

[0207] In some embodiments, LNP formulations described herein may comprise a polycationic composition. In some embodiments, the LNP formulations comprising a

polycationic composition may be used for the delivery of the modified RNA described herein *in vivo* and/or *ex vitro*. In some embodiments, the LNP formulations described herein may additionally comprise a permeability enhancer molecule. The nanoparticle formulations may be a carbohydrate nanoparticle comprising a carbohydrate carrier and a modified nucleic acid molecule (*e.g.*, mRNA). As a non-limiting example, the carbohydrate carrier may include, but is not limited to, an anhydride-modified phytoglycogen or glycogen-type material, phtoglycogen octenyl succinate, phytoglycogen beta-dextrin, and anhydride-modified phytoglycogen beta-dextrin.

[0208] Lipid nanoparticle formulations may be improved by replacing the cationic lipid with a biodegradable cationic lipid which is known as a rapidly eliminated lipid nanoparticle (reLNP). Ionizable cationic lipids, such as, but not limited to, DLinDMA, DLin-KC2-DMA, and DLin-MC3-DMA, have been shown to accumulate in plasma and tissues over time and may be a potential source of toxicity. The rapid metabolism of the rapidly eliminated lipids can improve the tolerability and therapeutic index of the lipid nanoparticles by an order of magnitude from a 1 mg/kg dose to a 10 mg/kg dose in rat. Inclusion of an enzymatically degraded ester linkage can improve the degradation and metabolism profile of the cationic component, while still maintaining the activity of the reLNP formulation. The ester linkage can be internally located within the lipid chain or it may be terminally located at the terminal end of the lipid chain. The internal ester linkage may replace any carbon in the lipid chain.

[0209] Additional information regarding cationic lipids suitable for LNP formulations can be found in, for example, U.S. Publication No. US2017/0151339, which is herein incorporated by reference in its entirety.

[0210] The molecules and/or compositions of the disclosure can also be formulated as a nanoparticle using a combination of polymers, lipids, and/or other biodegradable agents, such as, but not limited to, calcium phosphate. Components may be combined in a core-shell, hybrid, and/or layer-by-layer architecture, to allow for fine-tuning of the nanoparticle so that delivery of the molecules and/or compositions of the disclosure may be enhanced.

[0211] Pharmaceutical formulations of the present disclosure may additionally comprise one or more pharmaceutically acceptable excipients, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening and emulsifying agents, preservatives, solid binders, lubricants, and the like, as suited to the particular dosage form desired. More information in this regard can be found in Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, Md., 2006) which discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this disclosure.

EXAMPLES

[0212] Additional alternatives are disclosed in further detail in the following examples, which are not in any way intended to limit the scope of the claims.

EXAMPLE 1

General Experimental Procedure

DNA Template Preparation

[0213] Plasmid DNA templates were purified (Qiagen Cat. no. 12163) from 300 mL of saturated *E. coli* TransforMax Epi300 (Epicentre Cat. no. EC300105) cultures grown in LB broth media (Teknova Cat. no. L8000 06) supplemented with 50ng/ml carbamicilin (Teknova Cat. no. NC9730116). Plasmid DNA was linearized by *Not*-I digestion (New England Biolabs NEB cat. no. R3189S) for one hour at 37°C. Linearized template DNA was then re-purified (Zymo Cat. no. D4003), and analyzed by 0.8% agarose gel (Life Technologies Cat. no. G5018-08) against a commercial 2-log DNA ladder (New England Biolabs, NEB Cat. no. N3200S). The presence of a single band was confirmed in each sample, corresponding to the expected fragment size of the linear DNA template, prior to proceeding with *ex vitro* transcription.

Ex vitro transcription

[0214] *Ex vitro* transcription (IVT) reactions were performed using 1μg of DNA template prepared as described above, in a 20 μl reaction over a one hour incubation at 37°C (NEB cat. no. E2065S). 1 Unit of DNase I, provided by the supplier was then added directly to the IVT reaction, and incubated at 37°C for an additional 15 mins. Reactions were then placed on ice, and purified using the manufactures suggested method (Qiagen Cat. no. 74104). Purified RNA was then quantified using a NanoDrop 2000c UV-Vis Spectrophotometer. RNA was visualized by electrophoresis through 0.8% Agarose gels (Life Technologies Cat. no. G5018-08) and compared with Millennium RNA Marker (Ambion Cat. No. AM7150), prior to proceeding with electroporation.

Transfection and analysis

[0215] In a typical cell transfection experiment, replicon RNA was introduced into BHK-21 cells by electroporation using the SF Cell Line Nucleofector™ kit for the 4D-Nucleofector™ System (Lonza). BHK-21 cells were harvested using 0.25% trypsin and washed once with cold PBS. Cells were resuspended in SF Buffer at a cell density of 1×10^6 cells per 20 μL electroporation reaction. Three micrograms of RNA was electroporated into cells in triplicate in a 16-well cuvette strip and incubated at room temperature for 10 minutes. Electroporated cells were recovered into plates containing Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum, followed by incubation for 16 – 18 h at standard cell culture conditions.

[0216] Intracellular analyses of replicon transfection efficiency and protein production were performed by flow cytometry. In these assays, transfected BHK-21 cells were fixed and permeabilized using fix/perm concentrate and permeabilization buffer (eBioscience). Cells were then incubated with antibodies for double-stranded RNA production (J2 anti-dsRNA IgG2A monoclonal antibody, English & Scientific Company) conjugated with R-Phycoerythrin (Innova Biosciences). Antigen production was assessed by additional incubation with antigen-specific antibodies conjugated with PE-Cy5 (Innova Biosciences) (*e.g.* antibodies for red Firefly, green Renilla, HA, or RSV-F0 (Abcam)). Cells were then washed once and analyzed using a FACSAria™ Fusion Cell Sorter (BD Biosciences) or FACSAria™ II Cell Sorter (BD Biosciences). Transfected BHK-21 cells

stained with single colors for compensation controls were run prior to sample collection. Data was collected using FACSDiva (BD Biosciences) and further analyzed using FlowJo software. Initial gating was performed to exclude dead cells and debris using forward and side scatter plots. Further gating was conducted to identify cell populations that were positive for both dsRNA (R-PE-positive) and protein expression (PE-Cy5-positive or FITC-positive for GFP expression). Frequencies and mean fluorescence intensities were collected and utilized for construct comparison and optimization.

EXAMPLE 2

Construction of DLP-containing EAV Replicon Designs

[0217] This Example describes the generation of a number of arterivirus RNA replicon-based expression vectors with a DLP motif operably positioned upstream of the polyprotein/non-structural protein genes and/or a reporter gene. These arterivirus RNA replicon-based expression vectors were subsequently characterized and analyzed in the flow cytometry analysis and bulk luciferase analyses described in EXAMPLE 4.

A. Design

[0218] The respective design features of four EAV-based DLP replicon constructs are described below.

(1) rEX-DLP-rFF

[0219] In this construct, a DLP motif as placed immediately upstream of rFF and downstream of the TRS7 driving the transcription of rFF.

(2) rEX-DLP-pp1ab-rFF

[0220] In this construct, a DLP motif was placed immediately upstream of the pp1ab genes with a few careful design modifications described below to maintain the stem loop structure in the 5'UTR of the replicon known to be essential for replication and subgenomic mRNA transcription.

[0221] (i) The first 79 nucleotides of the nonstructural viral gene 1a is duplicated with its start codon mutated from ATG to TAG, denoted as "ATG-shifting region" (bold in the sequence of SEQ ID NO: 2 below).

[0222] (ii) The corresponding nucleotides, located upstream of the 1a gene, base-pairing with its start codon ATG and forming the stem, were also changed accordingly from CAT to CTA (underlined in the sequence of SEQ ID NO: 2 below).

[0223] (iii) DLP (italicized in the sequence below) was placed immediately downstream of the "ATG-shifting region" and upstream of the polyprotein 1ab genes (start codon ATG shown in the sequence of SEQ ID NO: 2 below).

SEQ ID NO: 2 (partial sequence)

CGAAGTGTGTATGGTGCCATATACGGCTCACCACCATATACACTGCAAGAATTACTATTC
TTGTGGGCCCCTCTCGGTAAATCCTAGAGGGCTTTCCTCTCGTTATTGCGAGATTCGTCG
TTAGATAACGGCAAGTTCCCTTTCTTACTATCCTATTTTCATCTTGTGGCTTGACGGGTCA
CTGCCTACGTCGTCGATCTCTATCAACTACCCTTGCGACTTAGGCAACCTTCTCCGCTA
CTGGATTTGGAGGGAGTTTTGTTAGGGACTGGTCCCTGGACTTACCCGACGCTTGT
GAGC*ATAGTCAGCATAGTACATTTCATCTGACTAATACTACAACACCACCACCATGAATAGAGG*
ATTCTTTAACATGCTCGGCCGCCGCCCCTTCCCGGCCCCCACTGCCATGTGGAGGCCGCGGA
*GAAGGAGGCAGGCGGCCCCGATG*ATGGCAACCTTCTCCGCTACTGGATTTGGAGG...

[0224] This construct was essentially identical to the second construct, where DLP was placed following the same three design modifications, except that a 2A protease sequence (SEQ ID NO: 3) was added immediately at the 3' end of DLP such that, when translated, the polyproteins could be released from the DLP-derived peptide through a selective cleavage by the protease. A comparative analysis of performances by replicon Construct 2 (described above) and Construct 3 would provide information on whether the 2A protease was needed for a functional replicon (see EXAMPLE 4 below).

SEQ ID NO: 3

GGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACC
CTGGACCT

(4) rEX-DLP-2A-pp1ab-DLP-rFF

[0225] This construct was essentially identical to the third construct described above, except that another DLP was placed immediately upstream of the reporter rFF gene (the same way as a DLP motif was placed in construct 1). A comparative analysis of performances by replicon Construct 3 (described above) and Construct 4 would provide

information on whether the additional DLP placed upstream of the reporter gene has an added value to the expression of the reporter gene.

B. Construction

[0226] rEx-DLP-rFF was built according to a 3-piece Gibson Assembly® procedure described in Gibson *et al.* (Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat. Methods* 6, 343–345, 2009) with rEx-rFF (c4; SEQ ID NO: 34) digested with SphI and EcoRI as a vector and a DLP-containing g-block as an insert. The nucleic acid sequence of the g-block used for construction of rEx-DLP-rFF is set forth at SEQ ID NO: 4 in the Sequence Listing.

[0227] The following primers were designed to amplify the corresponding fragments required to build the 3 new EAV-based DLP replicon constructs described above.

TABLE 3

Primer	Primers designed for construction DLP-(2A)-pp1ab-rFF/DLP-rFF replicons	
RP114	pp1a-DLP-F	GCCATGTGGAGGCCGCGGAGAAGGAGGCAGGCG GCCCCGATGATGGCAACCTTCTCCGCTACTGGAT (SEQ ID NO: 5)
RP115	pBR322-3'SrfI-R	ACAATGTTGCCTCCCACATCTGCAA (SEQ ID NO: 6)
RP116	pBR322-3'SrfI-F	GGGTCACAAGGTAGTCGCCGTGGTT (SEQ ID NO: 7)
RP117	pBR322-bla-R	ACGTCAGGTGGCACTTTTCGGGGAA (SEQ ID NO: 8)
RP118	pp1a-DLP-2A-F	AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAG AACCCTGGACCTATGGCAACCTTCTCCGCTACTGG AT (SEQ ID NO: 9)

Construction of rEx-DLP-pp1ab-rFF

[0228] For the construction of the rEx-DLP-pp1ab-rFF vector, three nucleic acid fragments were generated by using a 3-piece Gibson Assembly® procedure, as follows.

[0229] Fragment 1 was generated with primers RP114 and RP115 and the template backbone rEx-rFF.

[0230] Fragment 2 was generated with primers RP116 and RP117 and template backbone rEx-rFF.

[0231] Fragment 3 was a g-block for rEx-DLP-pp1ab-rFF with the nucleic acid sequence set forth at SEQ ID NO: 10 in the Sequence Listing.

Construction of rEx-DLP-2A-pp1ab-rFF

[0232] For the construction of rEx-DLP-2A-pp1ab-rFF vector, three nucleic acid fragments were generated by using a 3-piece Gibson Assembly® procedure, as follows.

[0233] Fragment 4 was generated with primers RP118 and RP115 and the template backbone rEx-rFF.

[0234] Fragment 5 was generated with primers RP116 and RP117 and template backbone rEx-rFF.

[0235] Fragment 6 was a g-block for rEx-DLP-2A-pp1ab-rFF with the nucleic acid sequence set forth at SEQ ID NO: 11 in the Sequence Listing.

Construction of rEx-DLP-2A-pp1ab-DLP-rFF

[0236] For the construction of rEx-DLP-2A-pp1ab-DLP-rFF vector, three nucleic acid fragments were generated by using a 3-piece Gibson Assembly® procedure, as follows.

[0237] Fragment 7 was generated with primers RP118 and RP115 and the template backbone rEx-DLP-rFF.

[0238] Fragment 8 was generated with primers RP116 and RP117 and template backbone rEx-DLP-rFF.

[0239] Fragment 9 was a g-block for rEx-DLP-2A-pp1ab-DLP-rFF with the nucleic acid sequence set forth at SEQ ID NO: 12 in the Sequence Listing.

[0240] Construct assembly was performed according to a 3-piece Gibson Assembly® procedure described in Gibson *et al.* (2009, supra). In particular, the rEx-DLP-pp1ab-rFF construct was built using fragments 1, 2, and 3; the rEx-DLP-2A-pp1ab-rFF construct was built using fragments 4, 5, and 6; and the rEx-DLP-2A-pp1ab-DLP-rFF construct was built using fragments 7, 8, and 9. Assembled products were subsequently transformed into EPI300 cells from Epicenter. A total of 144 colonies were screened using the primers RP126 (SEQ ID NO: 13) and RP127 (SEQ ID NO: 14) for each transformation, resulting in 4 PCR-positive clones for rEx-DLP-pp1ab-rFF, 3 PCR-positive clones for rEx-DLP-2A-pp1ab-rFF, and 2 PCR-positive clones for rEx-DLP-2A-pp1ab-DLP-rFF. Subsequent MiSeq results revealed that clone 4, clones 3 and 15, and clones 18 and 20 were completely sequence-correct for rEx-DLP-pp1ab-rFF, rEx-DLP-2A-pp1ab-rFF, and rEx-DLP-2A-pp1ab-DLP-rFF, respectively.

TABLE 4

Primer	Primers designed for colony screening of the DLP-(2A)-pp1ab replicons	
RP126	DLP-pp1ab-screen-F	CAGCATCTTTTACTTTCACCAGCGTTTCTG (SEQ ID NO: 13)
RP127	DLP-pp1ab-screen-R	GGAACTGGCGAAGCCAGTTTTAACA (SEQ ID NO: 14)

[0241] The maps of rEx-DLP-rFF, rEx-DLP-pp1ab-rFF, rEx-DLP-2A-pp1ab-rFF, and rEx-DLP-2A-pp1ab-DLP-rFF are also shown in FIGUREs 2A-2D.

[0242] The sequences of the resulting replicons are disclosed in the Sequence Listing with a T7 promoter and a polyA tail of 65 A's, as follows: rEx-DLP-rFF (SEQ ID NO: 15), rEx-DLP-pp1ab-rFF (SEQ ID NO: 16), rEx-DLP-2A-pp1ab-rFF (SEQ ID NO: 17), and rEx-DLP-2A-pp1ab-DLP-rFF (SEQ ID NO: 18).

EXAMPLE 3

Construction of DLP-containing Alphavirus Replicon Designs

[0243] This Example describes the generation of a number of Alphavirus RNA replicon-based expression vectors with a DLP motif positioned upstream of the polyprotein/non-structural protein genes and/or a reporter gene. These Alphavirus RNA replicon-based expression vectors were subsequently characterized and analyzed in the flow cytometry analysis and bulk luciferase analyses described in EXAMPLE 5.

A. Design

[0244] The respective design features of three Alphavirus-based DLP replicon constructs are described below.

(1) Alpha-R-DLP-rFF

[0245] In this construct, In this construct, DLP was placed immediately upstream of the start codon of the reporter gene rFF.

(2) Alpha-R-DLP-2A-nsp-rFF

[0246] In this construct, the sequence encoding the DLP motif and the 2A peptide sequence (which was the same sequence used in the rEx-DLP-2A-pp1ab-rFF replicon described in Example 2 above) was placed within the 5' end of the replicon with a few careful design modifications described below, to potentially maintain the sequence-structure requirement for replication and subgenomic mRNA transcription.

[0247] (i) The first 195 nucleotides of the nsp1 gene was duplicated with its start codon mutated from ATG to TAG (bold in the sequence of SEQ ID NO: 19 below).

[0248] (ii) This 195-nucleotide duplicated sequence was placed immediately following the 5' UTR of the wild-type Alphavirus (underlined in the sequence of SEQ ID NO: 19 below) and is followed by the DLP-2A sequence (italicized in the sequence below).

[0249] (iii) The start codon of the nsp1 gene following the DLP-2A sequence was removed (strike-through in the sequence of SEQ ID NO: 19 below).

SEQ ID NO: 19 (partial sequence)

GATAGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAA**TAGGAGAAAGTTC**
ACGTTGACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGGAGCTTCCCG
CAGTTTGAGGTAGAAGCCAAGCAGGTCACTGATAATGACCATGCTAATGCCAGAGC
GTTTTCGCATCTGGCTTCAAAACTGATCGAAACGGAGGTGGACCCATCCGACACGA
TCCTTGACATTGGA*ATAGTCAGCATAGTACATTTCATCTGACTAATACTACAACACCACCACC*
ATGAATAGAGGATTCTTTAACATGCTCGGCCGCCGCCCCTTCCCGGCCCCCACTGCCATGTGG
AGGCCGCGGAGAAGGAGGCAGGCGGCCCCGGGAAGCGGAGCTACTAACTTCAGCCTGCTGA
AGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCT~~ATG~~GAGAAAGTTCACG...

(3) Alpha-R-DLP-2A-nsp-DLP-rFF

[0250] This construct is essentially identical to Construct 2 following the same three design modifications, except that another DLP motif was placed immediately upstream of the reporter rFF gene (the same way as a DLP motif was placed in Construct 1). A comparative analysis of performances by replicon Constructs 2 and 3 would provide information on whether the additional DLP placed upstream of the reporter gene has an added value to the expression of the reporter gene (see EXAMPLE 5 below).

B. Construction

Construction of Alpha-R-DLP-rFF

[0251] Alpha-R-DLP-rFF was built via Gibson Assembly® procedure, using Alpha-R-eGFP (c6; SEQ ID NO: 35) digested with EcoRI/SapI as a vector and DLP-rFF as an insert PCR-amplified from the template rEx-DLP-rFF (c2, SEQ ID NO: 15) using the primers RP112 (SEQ ID NO: 20) and RP113 (SEQ ID NO: 21) to replace eGFP with DLP-rFF. Clones 2 and 3 were sequence-confirmed to be completely correct via MiSeq sequencing.

TABLE 5

Primer	Primers used to clone DLP-rFF into Alpha-R-GFP (EcoRV/SapI)	
RP112	DLP-rFF-F	CCTGAATGGACTACGACATAGTCTAGTCCGCCAAGAT ATCGCACCATAGTCAGCATAGTACATTTCATCTGAC TAATACT (SEQ ID NO: 20)
RP113	DLP-rFF-R	GCAGCTTGCCAATTGCTGCTGTATCGATCAATT AATCACATCTTGGCCACGGGTTTCTTC (SEQ ID NO: 21)

Construction of Alpha-R-DLP-2A-nsp-rFF and Alpha-R-DLP-2A-nsp-DLP-rFF

[0252] Alpha-R-DLP-2A-nsp-rFF (Construct 2) and Alpha-R-DLP-2A-nsp-DLP-rFF (Construct 3) were built via Gibson Assembly® procedure, using the respective g-blocks as inserts and the vectors that had been PCR-amplified from the respective templates, Alpha-R-rFF (c6; SEQ ID NO: 35) and Alpha-R-DLP-rFF (c2; SEQ ID NO: 26), using the primers RP124 (SEQ ID NO: 22) and RP125 (SEQ ID NO: 23). Clones 1 and 3 of Alpha-R-DLP-2A-nsp-rFF and clones 8 and 32 of Alpha-R-DLP-2A-nsp-DLP-rFF were sequence-confirmed to be completely correct via MiSeq.

TABLE 6

Primer	Primers used for construction of Alpha-DLP-nsp-rFF/DLP-rFF	
RP124	5'Alpha-P2A-F	GAAGCAGGCTGGAGACGTGGAGGAGAACCCT GGACCTGAGAAAGTTCACGTTGACATCGAGGA AGAC (SEQ ID NO: 22)
RP125	5'ScaI-R	CACCAGTCACAGAAAAGCATCTTACGGATG (SEQ ID NO: 23)

[0253] The sequence of g-block used for the construction of Alpha-R-DLP-2A-nsp-rFF is provided in the Sequence Listing as SEQ ID NO: 24. The sequence of g-block

used for the construction of Alpha-R-DLP-2A-nsp-DLP-rFF is also provided in the Sequence Listing as SEQ ID NO: 25.

[0254] The maps of Alpha-R-rFF, Alpha-R-DLP-rFF, Alpha-R-DLP-2A-nsp-rFF, and Alpha-R-DLP-2A-nsp-DLP-rFF are shown in **FIGS. 3A-3D**.

[0255] The sequences of the resulting replicons are also provided in the Sequence Listing with a T7 promoter and a polyA tail of 40 A's, as follows: Alpha-R-rFF (SEQ ID NO: 26), Alpha-R-DLP-rFF (SEQ ID NO: 27), Alpha-R-DLP-2A-nsp-rFF (SEQ ID NO: 28), and Alpha-R-DLP-2A-nsp-DLP-rFF (SEQ ID NO: 29).

Construction of Alpha-R-DLP-2A-rFF and Alpha-R-DLP-2A-nsp-DLP-2A-rFF

[0256] Without being bound by any particular theory, it is believed that placing a DLP motif immediately upstream of the reporter gene rFF without the inclusion of the 2A protease in between them may negatively impact protein expression of the GOI; this negative impact could be due to the fact that rFF now became a "fusion" protein, resulting from the presence of the DLP sequence translated into a peptide at the 5' end of rFF. Therefore, 2 new constructs were designed and built, including the 2A protease sequence between the DLP motif and the rFF gene for the two Alphavirus-replicon constructs, Alpha-R-DLP-rFF and Alpha-R-DLP-2A-nsp-DLP-rFF, to generate Alpha-R-DLP-2A-rFF and Alpha-R-DLP-2A-nsp-DLP-2A-rFF, respectively. The inclusion of the 2A protease peptide sequence would enable cleavage of the peptide encoded by the DLP sequence from rFF (see Example 5 below).

[0257] For this purpose, two g-block fragments were synthesized (SEQ ID NOS: 30 and 31) and cloned into their respective vectors digested with EcoRV/SbfI via Gibson Assembly. Clone 1 of Alpha-R-DLP-2A-rFF and clones 8 and 9 of Alpha-R-DLP-2A-nsp-DLP-2A-rFF were sequence-confirmed to be completely correct via Sanger sequencing using RP123 (SEQ ID NO: 32) and RP96 (P89; SEQ ID NO: 96).

TABLE 7

Primer	Primers used to sequence Alpha-R-(DLP-2A-nsp)-DLP-2A-rFF constructs	
RP123	Alpha-3'nsp4-F	GGCTGTTTAAGCTTGGCAAACCTCT (SEQ ID NO: 32)
RP96	rFF-seq1	AGCGAGAACTGCGAGGAATTCTT (SEQ ID NO: 33)

[0258] Schematic maps of Alpha-R-DLP-2A-rFF and Alpha-R-DLP-2A-nsp-DLP-2A-rFF are provided in **FIGS. 4A-4B**.

EXAMPLE 4

Expression Analysis of EAV-based DLP containing replicons

[0259] As presented in Examples 2 and 3 above, a number of EAV-based DLP containing replicons were constructed to determine the impact of engineering a DLP motif positioned upstream of either the replicon nonstructural protein genes or the GOI gene on a subgenomic mRNA (TABLE 8).

TABLE 8: Listing of DLP-containing EAV Replicons and DLP-containing VEEV replicons.

EAV DLP Replicons
rEx-DLP-rFF
rEx-DLP-2A-rFF
rEx-DLP-pp1ab-rFF
rEx-DLP-2A-pp1ab-rFF
rEx-DLP-2A-pp1ab-DLP-rFF
rEx-DLP-2A-pp1ab-DLP-2A-rFF
VEEV DLP replicons
alpha-R-DLP-rFF
alpha-R-DLP-2A-rFF
alpha-R-DLP-2A-nsp-rFF
alpha-R-DLP-2A-nsp-DLP-rFF
alpha-R-DLP-2A-nsp-DLP-2A-rFF

[0260] Initial characterization of the DLP replicon constructs was carried out *ex vitro*. RNA was produced and used to electroporate BHK cells as described in EXAMPLE 1 above. After electroporation cells were analyzed for protein expression by FACs analysis, Western blot or bulk luciferase assay.

[0261] A graphical summary of the results of experiments performed to measure the expression level of an exemplary gene of interest (GOI), rFF luciferase reporter, from EAV-based DLP replicons is shown in **FIG. 5**. Both FACs analysis and bulk luciferase data

are presented. In these experiments, four different EAV DLP replicons were analyzed as follows:

[0262] 1) rEx-DLP-rFF: an EAV-based replicon with a DLP motif positioned upstream to the subgenomic mRNA rFF transcript);

[0263] 2) rEx-DLP-pp1ab-rFF: an EAV-based replicon with DLP positioned upstream to the non-structural pp1ab genes);

[0264] 3) rEx-DLP-2A-pp1ab-rFF: an EAV-based replicon with a DLP motif positioned upstream to the nonstructural proteins and a 2A protease peptide positioned between the DLP and the pp1ab region); and

[0265] 4) rEx-DLP-2A-pp1ab-DLP-rFF: an EAV-based replicon with a first DLP motif positioned upstream to the nonstructural proteins and a 2A protease peptide positioned between the DLP and the pp1ab region as well as a second DLP motif positioned upstream to the rFF subgenomic mRNA transcript).

[0266] The results presented in **FIGS. 5A-5B** demonstrated that engineering a DLP motif upstream to either the EAV nonstructural protein genes (*e.g.*, rEx-DLP-pp1ab-rFF, rEx-DLP-2A-pp1ab-rFF or rEx-DLP-2A-pp1ab-DLP-rFF) or the rFF reporter gene subgenomic RNA (*e.g.*, rEx-DLP-rFF and rEx-DLP-2A-pp1ab-DLP-rFF) did not negatively impact genomic RNA replication as all four constructs demonstrated nearly identical electroporation efficiencies (**FIG. 5A**). Interestingly, bulk luciferase activity analysis demonstrated that the rEx-DLP-pp1ab-rFF replicon expressed significantly less luciferase than the other three replicon designs (**FIG. 5B**). As stated above, incorporation of a DLP motif upstream of any GOI would result in an N terminal fusion of Sindbis capsid amino acids encoded in the in-frame codons found in the DLP sequence. The fusion protein generated with the amino acids encoding DLP and the EAV nsP1 protein is believed to impact the EAV replication complex from efficiently producing subgenomic RNAs and result in the reduced rFF GOI expression levels noted. One of the most remarkable results from this study was that EAV replicon constructs with a DLP controlling translation of the nonstructural protein genes (rEx-DLP-pp1ab-rFF, rEx-DLP-2A-pp1ab-rFF and rEx-DLP-2A-pp1ab-DLP-rFF) were as efficiently translated as the replicon RNA that did not have a DLP in this position (rEx-DLP-rFF). This result would not be predicted based on work conducted

by other researchers. It has been previously reported that incorporation 5' Sindbis virus subgenomic RNA sequences (including the DLP region) were only efficiently translated in cells infected with the virus. Stated differently, mRNA that contains a DLP motif associated with a reporter gene was reported to be poorly translated in cells that were not infected with Sindbis virus. The absence of innate immune activation in these cells rendered the DLP modified mRNA at a distinct translation disadvantage relative to translation of mRNAs that lack the DLP modification (all cellular mRNAs). The innate immune system was not activated in these cells at the time the DLP-containing replicon vectors were introduced so these DLP-containing mRNAs (capable of self-amplification) should be very inefficiently translated. Unexpectedly, that was not borne out in the experiments presented herein.

[0267] Subsequently, the rEx-DLP-2A-pp1ab-rFF EAV replicon was examined in cells that had been treated with IFN to induce the cellular innate immune system. IFN treatment of BHK cells will induce PKR activation and phosphorylation of eIF2α which in-turn results in shut-down of global cellular mRNA translation. It has been reported previously that arteriviruses are sensitive to IFN treatment (Luo *et al. Antiviral Res.* Aug;91(2):99-101, 2011), therefore the IFN treatment of BHK cells, which are capable of responding to IFN exposure and induce the innate immune system, would result in shut-down of arterivirus replication. A representative example of the expression capacity of the DLP modified EAV replicon in the presence of innate immune system activation is shown in **FIG. 6**. The rEx-DLP-2A-pp1ab-rFF replicon demonstrated significant resistance to innate immune system activation when compared to an EAV replicon that was not modified to contain the DLP motif, *i.e.* rEx-rFF. Both replication (**FIG. 6A**) and expression (**FIG. 6B**) of the rEx-DLP-2A-pp1ab-rFF replicon were significantly higher in IFN treated cells when compared to the control rEx-rFF replicon. These data demonstrate that DLP modified EAV replicons are capable of overcoming innate immune system shut-down and that this replicon vector represents a significant advance in self-amplifying RNA technology.

EXAMPLE 5

Expression Analysis of DLP-containing VEEV replicons

[0268] As presented in Examples 2 and 3 above, a number of VEEV-based DLP containing replicons were constructed to determine the impact of engineering a DLP motif

positioned upstream of either the replicon nonstructural protein genes or the GOI gene on a subgenomic mRNA.

[0269] VEEV alphavirus replicon vectors were engineered to contain one or more DLP motifs by using a strategy similar to the construction of EAV-based replicon vectors. Importantly, unlike other members of the Alphavirus genus (mostly Old World virus members), the genome of VEEV does not contain a DLP motif associated with translation of its subgenomic mRNA. Initial analysis of the VEEV DLP replicons was carried out in BHK-21 cells as described in EXAMPLE 1 above. BHK-21 cells do not secrete IFN in response to RNA replication but these cells are able to respond to exogenous IFN to induce innate immune activation. In this experiment, four different alphavirus replicon constructs were tested. The experimental data presented in **FIG.** 7 shows DLP-containing alphavirus replicon replication and expression of the rFF luciferase gene in BHK cells that had been treated either at the time of electroporation (0 hr) or at 3 hr post electroporation with 1000 U/ml of exogenous IFN. The replicon RNAs tested were:

[0270] 1) Alpha-R-rFF: a control VEEV-based replicon with no DLP present;

[0271] 2) Alpha-R-DLP-rFF: a VEEV-based replicon with a DLP motif positioned upstream to the subgenomic mRNA rFF transcript;

[0272] 3) Alpha-R-DLP-2A-nsp-rFF: a VEEV-based replicon with a DLP motif positioned upstream to the nonstructural proteins with a 2A protease between the DLP and the nsp region; and

[0273] 4) Alpha-R-DLP-2A-nsp-DLP-rFF: VEEV-based replicon with a first DLP motif positioned upstream to the nonstructural proteins with a 2A protease between the DLP and the nsp region as well as with a second DLP motif positioned upstream to the rFF subgenomic mRNA transcript.

[0274] The results of luciferase expression normalized to the number of positive cells detected by FACs analysis are shown in **FIG.** 7. It was observed that the presence of a DLP motif controlling the translation of the VEEV non-structural protein genes resulted in higher reporter gene expression both in the absence and the presence of IFN treatment post electroporation (**FIG.** 7A-7C). Although the increase in rFF expression may have been considered statistically insignificant, the trend in all conditions was for increased protein

expression. As stated above in EXAMPLE 4 with respect to DLP-containing EAV replicons, one may have expected that a DLP motif would have a negative impact on mRNA translation in cells that are not in an innate immune response activated state. In direct contrast to that expectation, the BHK cells that had not been treated with IFN (**FIG. 7A**) in these experiments represent the sample with the largest benefit to incorporation of a DLP motif.

[0275] Subsequently, the two RNA replicons alpha-R-rFF and alpha-DLP-2A-nsp-rFF were tested *in vivo* in Balb/c mice. In this experiment, mice were tested in groups of 10 animals. In these experiments, equal doses of RNA were injected intramuscularly into mice and whole body IVIS (*In vivo* Imaging System) analysis was carried out over course of one week. Whole body imaging was performed at day 1, day 3 and day 7 post injection. The total flux measured at the injection site is shown in FIG. 8. Although only modest increases in protein expression were noted *ex vitro* (**FIG. 8**) from the DLP modified VEEV replicon, statistically significantly higher protein expression was detected at all time points measured from the DLP modified VEEV replicon RNA (**FIG. 8**). This observation represents a significant advantage, because as unmodified VEEV replicon vectors are capable of very high protein expression that can reach up to 20% of the total cellular protein (Pushko et al 1997). The DLP modified VEEV replicon surpassed even this expression potential and demonstrated superior protein expression; for this reason, the DLP modified alphavirus replicon vector represents a significant advance over existing alphavirus replicon RNA technology.

[0276] There are at least three unexpected results that can be drawn from the experimental data presented in the Examples above. First, the DLP motif has been shown to negatively impact translation of mRNAs when a cell is not in an innate immune system activated state. The DLP-containing replicon RNAs disclosed herein were found to have not been negatively impacted in cells at a basal state of innate activation. Second, expression levels, especially for the DLP-containing VEEV replicons, were found to have been even higher than unmodified replicons *in vivo*; this observation demonstrated that expression levels even from an alphavirus replicon can be increased from previously high historic expression levels. Third, all positive strand RNA viruses have considerable sequence conservation in both the 5' and 3' ends of their genomes. The fact that both the VEEV

replicon and the EAV replicon are flexible enough to accept incorporation of a stem loop structure (the DLP) in the 5' end of their RNAs is unexpected.

EXAMPLE 6

In vivo Immunogenicity Response Using DLP Replicon Expression Systems

[0277] Alphavirus replicon vectors were engineered to contain one or more DLP motifs, as described above. The RNA replicon, Alpha-R-gDLP-HA, containing the DLP sequence was further analyzed *in vivo* in Balb/c mice. In this experiment, 15 µg, 1.5 µg, or 0.15 µg of RNA encoding Hemagglutinin from Influenza A/Vietnam/1203/2004 (H5N1) was injected into mice at intervals 6 weeks apart. Fourteen days following the final boost, spleens and serum were collected to analyze the immune responses to HA. A summary of the results of these experiments is presented in **FIGs. 12A-12C**. In **FIG. 12A**, a significant increase in memory precursor effector cells (MPECs) was observed in constructs containing the DLP motif compared with each comparable dose of an unmodified replicon. HA-specific MPECs were detected using dextramers (H-2 Kd (IYSTVASSL; SEQ ID NO: 44)) along with other population-specific markers ($CD8^+CD44^+CD62L^{Lo}KLRG\text{-}1^{Lo}IL\text{-}7Ra^{Hi}CXCR3^{Hi}$). Of note, this benefit was also observable at low doses. In **FIGs 12B** and **12C**, effector T cell responses were measured by the number of antigen-specific HA cells that were secreting IFN-γ following stimulation with a $CD4^+$ T cell or $CD8^+$ T cell peptide. Animals immunized with replicons containing the DLP motif had a significantly higher frequency of cytokine-expressing $CD4^+$ and $CD8^+$ T cells are the 15ug and 1.5ug doses. Taken together, these data indicate a significant increase in both effector and memory T cell responses in response to immunization with antigen expressed by replicons containing the DLP motif as compared to the unmodified version.

[0278] The above DLP-containing replicons were further analyzed *in vivo* in Balb/c mice for compatibility with LNP formulations. In this experiment, 2 µg or 0.2 µg of RNA encoding Hemagglutinin from Influenza A/Vietnam/1203/2004 (H5N1) was injected into mice at intervals 4 weeks apart. Fourteen days following the final boost, spleens and serum were collected to analyze the immune response to HA. A summary of these experiments is presented in **FIG. 14A-14C**. In figures **14A-14C**, an increase in T-cell and B-cell responses was observed using constructs containing the DLP motif when combined with

LNP (cationic lipid nanoparticles) formulations. In figure **14A**, HA-specific total IgG titers were significantly higher in all dose groups using LNP formulations compared to the group with replicon administered in saline. Furthermore, in figure **14B** and **14C**, it was observed that HA-specific CD8+ and CD4+ T cells were also significantly higher in all dose groups using LNP formulations compared to the group with replicon administered in saline. Taken together, this data demonstrates that replicon constructs containing the DLP motif are compatible with representative formulations.

EXAMPLE 7

Preventing Suppression of Immune Response Using DLP-containing Replicons

[0279] DLP-containing replicons constructed as described above were further evaluated *in vivo* for the ability to prevent suppression of immune response in Balb/c mice. In these experiments, 1.5 μg of mRNA, with or without DLP motif, and carrying a coding sequence for Hemagglutinin derived from Influenza A/Vietnam/1203/2004 (H5N1) is injected into mice at intervals 4 weeks apart. Approximately 24 hours prior to injection, 6-8 week old BALB/c mice are pre-treated with 20 μg of Poly(I:C) or saline by hydrodynamic tail vein injection to simulate a viral infection. Fourteen days following the final boost, serum from these mice are collected to analyze the immune response to Hemagglutinin (HA). A summary of these experiments is presented in **FIG. 13**. In Figure 13, a significant decrease is observed in the serum concentration of HA-specific antibodies in mice who were pre-treated with Poly(I:C) and received a doses of unmodified replicons. The levels in the Poly(I:C) group were not significantly above background. In contrast, animals pre-treated with Poly(I:C) and dosed with a construct containing the DLP motif showed no significant reductions in serum antigen-specific total IgG concentration. Taken together, these data show that the DLP motif protects against suppression of serum antibody levels in response to vaccination following a simulated viral infection compared to the unmodified version.

EXAMPLE 8

Construction of DLP-containing Expression Cassettes

[0280] This Example describes the generation of a plasmid vector for *ex vitro* transcription of an mRNA containing a Sindbis virus DLP element upstream of a gene of

interest, *e.g.*, a reporter gene, in accordance with some embodiments of the disclosure. The 5' and 3' untranslated regions (UTR) used in these experiments (SEQ ID NO: 36 and SEQ ID NO: 41, respectively) were derived from the human beta globin gene. The 5' UTR sequence was placed immediately downstream of a T7 promoter (SEQ ID NO: 37) and upstream of the Sindbis virus DLP sequence (SEQ ID NO: 38). In some experiments, the coding sequence for a gene of interest (GOI) was linked to the DLP via a P2A signal, which is an autocatalytic self-cleaving peptide (*e.g.*, autoprotease peptide) derived from the porcine teschovirus-1. In some experiments, a coding sequence for a destabilized form of EGFP reporter gene (dsGFP) which, in this case used as a GOI, was operably linked to the proteolytic PEST degradation signal derived from a mouse ornithine decarboxylase gene (MODC). In some other experiments, a coding sequence of the Red firefly luciferase reported gene was used as the gene of interest (also see, Example 9 below). However, it is contemplated that coding sequences for any gene of interest could be deployed in this configuration. In addition, as illustrated in **FIG. 15**, a 3' UTR sequence derived from human beta globin, a polyA tail consisting of 120 adenine residues, and a T7 terminator were inserted downstream and adjacent to the stop codon of dsGFP. The nucleic acid sequences of each of the components described above are as follows:

TABLE 9

Components of DLP dsGFP mRNAs	
5' human beta globin UTR	5'- ACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAA ACAGACACCGCCGCCACC-3' (SEQ ID NO 36)
T7 Promoter	5'-TAATACGACTCACTATAG-3' (SEQ ID NO 37)
DLP Motif	5'-ATAGTCAGCATAGTACATTTCATCTGACTAATACTACAACAC CACCACCATGAATAGAGGATTCTTTAACATGCTCGGCCGCCGC CCCTTCCCGGCCCCCACTGCCATGTGGAGGCCGCGGAGAAGGA GGCAGGCGGCCCCG -3' (SEQ ID NO 38)
P2A peptide	5'-GGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGG CTGGAGACGTGGAGGAGAACCCTGGACCT-3' (SEQ ID NO 39)
DsGFP	5'- ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGT GGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAAC GGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCG ATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGC ACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGT GACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCT ACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCC GCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTT

	CTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAG CTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCT GGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAG GTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAG CGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCA TCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTAC CTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGA GAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCG CCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAA GAAGCTTAGCCATGGCTTCCCGCCGGAGGTGGAGGAG CAGGATGATGGCACGCTGCCCATGTCTTGTGCCCAGGA GAGCGGGATGGACCGTCACCCTGCAGCCTGTGCTTCTG CTAGGATCAATGTGTAG -3' (SEQ ID NO 40)
3' Human beta globin UTR	5'-GCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTT GTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGG GCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTT TCATTGCAA -3' (SEQ ID NO: 41)
T7 Terminator	5'- AACCCCTCTCTAAACGGAGGGGTTTTTTT-3' (SEQ ID NO: 42)
Sequence of DLP dsGFP Mrna	5'-TAATACGACTCACTATAGACATTTGCTTCTGACAC AACTGTGTTCACTAGCAACCTCAAACAGACACCGC CGCCACCATAGTCAGCATAGTACATTTCATCTGAC TAATACTACAACACCACCACCATGAATAGAGGATT CTTTAACATGCTCGGCCGCCGCCCCTTCCCGGCCCC CACTGCCATGTGGAGGCCGCGGAGAAGGAGGCAGG CGGCCCCGGGAAGCGGAGCTACTAACTTCAGCCTG CTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGG ACCTATGGTGAGCAAGGGCGAGGAGCTGTTCACCG GGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACG TAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGC GAGGGCGATGCCACCTACGGCAAGCTGACCCTGAA GTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTG GCCCACCCTCGTGACCACCCTGACCTACGGCGTGCA GTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCA CGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTC CAGGAGCGCACCATCTTCTTCAAGGACGACGGCAAC TACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGAC ACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGAC TTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTG GAGTACAACTACAACAGCCACAACGTCTATATCATGG CCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCA AGATCCGCCACAACATCGAGGACGGCAGCGTGCAGC TCGCCGACCACTACCAGCAGAACACCCCCATCGGCG ACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAG CACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAA GCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCC

	GCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGA AGCTTAGCCATGGCTTCCCGCCGGAGGTGGAGGAGCA GGATGATGGCACGCTGCCCATGTCTTGTGCCCAGGAG AGCGGGATGGACCGTCACCCTGCAGCCTGTGCTTCTG CTAGGATCAATGTGTAGGCTCGCTTTCTTGCTGTCCAA TTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTA CTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTG GATTCTGCCTAATAAAAAACATTTATTTTCATTGCAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAACCCCTCTCTAAACGGAGGGGTTTT TTT -3' (SEQ ID NO: 43)

[0281] In the above experiments, a DLP sequence from Sindbis virus was used. Additional experiments are performed to incorporate DLP sequences from other Old World alphavirus members such as SV, SFV, BEBV, RRV, SAG, GETV, MIDV, CHIKV, and ONNV, into the nucleic acid molecules of the present disclosure. The linkage of the DLP to the gene of interest can be configured with or without a self-cleaving peptide such as P2A. Without bound to any particular theory, it is believed that the requirement for a 2A sequence or other self-cleaving peptide is dependent on the individual gene being inserted into the gene cassette and on whether the additional amino acids added by the inclusion of DLP would affect the translated proteins function. It is further contemplated that the 5' and 3' UTR sequences used here may also be changed for any other set of functional UTRs regardless of origin.

EXAMPLE 9

Ex vivo Evaluation of Gene Expression in DLP-containing Expression Cassettes

[0282] mRNAs derived from DLP-containing expression cassettes engineered to contain one or more DLP motifs, as described above, were evaluated *ex vivo* for the ability to enhance expression of the gene of interest in BHK-21 cells. As control, mRNA samples lacking the DLP sequence but otherwise identical to the DLP-containing mRNAs described above were assayed in parallel under the same conditions. In these experiments, BHK-21 cells were pre-treated with 300, 600 or 1000 U/mL of universal type I interferon or vehicle control for 2 hours. Following pre-treatment the cells were electroporated, in triplicate, with 2.5 μg of mRNA containing or lacking DLP motifs. The cells were placed back into media

containing the same concentrations of interferon used during the pretreatment. The frequency of GFP positive cells and Mean Fluorescence Intensity (MFI) was assayed at 2, 4 and 24 hours post electroporation by flow cytometry. It was observed that DLP-containing mRNA yields significantly higher frequency of GFP positive cells compared to the non-DLP mRNA in the presence of interferon (**FIG. 16A**).

[0283] Furthermore, when the MFI of GFP was normalized to the frequency of GFP positive cells and plotted versus time, it was observed that the unmodified mRNA was sensitive to interferon treatment as exhibited by a statistically significant reduction of 30% in overall protein produced during the 24-hour time course (**FIG. 16B**). In contrast, the DLP-containing modified mRNA demonstrated resistance to interferon treatment as exhibited by a statistically significant increase of 30% in overall protein production over the control unmodified mRNA during the same 24-hour time course (**FIG. 16C**). The resistance to interferon treatment conferred by the presence of the DLP motifs was further strengthened by the finding that cells treated with interferon and electroporated with a DLP-containing mRNA produced as much protein as untreated cells electroporated with an unmodified mRNA (**FIG. 16C**).

EXAMPLE 10

In vivo Evaluation of Gene Expression in DLP-containing Expression Cassettes

[0284] mRNAs derived from DLP-containing expression cassettes engineered to contain one or more DLP motifs, as described above, are further evaluated *in vivo* for the ability to enhance expression of the gene of interest in Balb/c mice. In this experiment, 30 µg, 15 µg, or 1.5 µg of DLP-containing mRNA encoding red firefly luciferase is injected into mice at interval of 6 weeks apart. Red firefly luciferase expression is subsequently monitored by IVIS (*In vivo* Imaging System) analysis at 1, 3, 7, 10, 14, 21 and 28 days post injection. A significant increase in luciferase expression is observed in mice that receive DLP-containing mRNAs when compared to control animals that receive mRNA lacking the DLP motif.

EXAMPLE 11

Preventing Suppression of Immune Response Using DLP-containing mRNAs

[0285] DLP-containing mRNAs as described above are further evaluated *in vivo* for the ability to enhance expression of the gene of interest in Balb/c mice. In this experiment, 30 μg, 15 μg, or 1.5 μg of mRNA, with or without DLP motif, and carrying a coding sequence for Hemagglutinin derived from Influenza A/Vietnam/1203/2004 (H5N1) is injected into mice at intervals 4 weeks apart. Approximately 24 hours prior to injection, mice are pre-treated with 20 μg of Poly(I:C) or saline by hydrodynamic tail vein injection to simulate a viral infection. Fourteen days following the final boost, serum from these mice are collected to analyze the immune response to Hemagglutinin (HA). A significant decrease in the serum concentration of HA-specific antibodies is expected to be observed in mice that are pre-treated with Poly(I:C) and receive a dose of mRNA lacking the DLP sequence. In contrast, animals pre-treated with Poly(I:C) and dosed with mRNA containing the DLP motif are expected to not show significant reductions in serum antigen-specific total IgG concentration.

[0286] While particular alternatives of the present disclosure have been disclosed, it is to be understood that various modifications and combinations are possible and are contemplated within the true spirit and scope of the appended claims. There is no intention, therefore, of limitations to the exact abstract and disclosure herein presented.

[0287] All of the references disclosed herein, including but not limited to journal articles, textbooks, publications, patents and patent applications are hereby incorporated by reference in their entireties to the same extent as if each reference was specifically and individually indicated to be incorporated by reference.

[0288] No admission is made that any reference cited herein constitutes prior art. The discussion of the references states what their authors assert, and the inventors reserve the right to challenge the accuracy and pertinence of the cited documents. It will be clearly understood that, although a number of information sources, including scientific journal articles, patent documents, and textbooks, are referred to herein; any discussion and comment in a specific information source should no way be considered as an admission that such comment was widely accepted as the general opinion in the field.

[0289] The discussion of the general compositions and methods given herein is intended for illustrative purposes only. It is not intended to be exhaustive or to limit the disclosure. Individual aspects or features of a particular embodiment are generally not limited to that particular embodiment, but, where applicable, are interchangeable and can be used in a selected embodiment, even if not specifically shown or described. It is expressly contemplated that any aspect or feature of the present disclosure can be combined with any other aspect, features, or combination of aspects and features disclosed herein. Other alternative compositions, methods, and embodiments will be apparent to those of skill in the art upon review of this disclosure, and are to be included within the spirit and purview of this application.

CLAIMS

1. A nucleic acid molecule, comprising a modified viral RNA replicon, wherein the modified viral RNA replicon comprises:

a first nucleic acid sequence encoding a viral capsid enhancer; and

a second nucleic acid sequence encoding at least one nonstructural viral protein encoding a replicase, wherein the first nucleic acid sequence is operably linked upstream to the second nucleic acid sequence,

wherein the modified viral RNA replicon is derived from a virus species belonging to the Togaviridae family or from a virus species belonging to the Arterivirus genus of the Arteriviridae family; and the viral capsid enhancer comprises a nucleotide sequence having a sequence identity of at least 80% to RNA corresponding to any one of SEQ ID NOs: 1 and 46-52.

2. The nucleic acid molecule of claim 1, wherein the modified viral RNA replicon further comprises a coding sequence for an autoprotease peptide operably linked upstream to the second nucleic acid sequence.

3. The nucleic acid molecule of claim 2, wherein the coding sequence for the autoprotease peptide is operably linked downstream to the first nucleic acid sequence and upstream to the second nucleic acid sequence.

4. The nucleic acid molecule of claim 2 or claim 3, wherein the autoprotease peptide comprises a peptide sequence selected from the group consisting of porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), and a combination thereof.

5. The nucleic acid molecule of any one of claims 1-4, wherein the viral capsid enhancer is heterologous to the viral RNA replicon.

6. The nucleic acid molecule of any one of claims 1 to 5, wherein the second nucleic acid sequence comprises substantially all the coding sequence for the native viral nonstructural proteins of the corresponding unmodified viral RNA replicon.

7. The nucleic acid molecule of any one of claims 1-6, wherein the second nucleic acid sequence comprises the coding sequence for the native viral nonstructural proteins of the corresponding unmodified viral RNA replicon from an Venezuelan equine encephalitis virus (VEEV) or from an Equine arteritis virus (EAV).

8. The nucleic acid molecule of any one of claims 1-6, wherein the modified viral RNA replicon comprises a modified RNA replicon derived from a virus species belonging to Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), Everglades virus (EVEV), Mucambo virus (MUCV), Semiiki forest virus (SFV), Pixuna virus (PIXV), Middleburg virus (MH3V), Chikungunya virus (CHIKV), O'Nyong-Nyong virus (O'NNV), Ross River virus (RRV), Barm ah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAGV), 13eba.ru virus (BEBV), Mayaro virus (MAYV), Una virus (UNAV), Sindbis virus (SINV), Aura virus (AURAV), Whataroa virus (WHAV), Babanki virus (BABV), Kyzyl agach virus (KYZV), Western equine encephalitis virus (WEEV), Highland J virus (HJV), Fort Morgan virus (FMV), Ndumu (NDUV), Salmonid alphavirus (SAV), or Buggy Creek virus, or the modified viral RNA replicon comprises a modified RNA replicon derived Equine arteritis virus (EAV), Porcine respiratory and reproductive syndrome virus (PRRSV), Lactate dehydrogenase elevating virus (LDV), or Simian hemorrhagic fever virus (SHFV).

9. The nucleic acid of claim 8, wherein the modified viral RNA replicon is derived from Venezuelan equine encephalitis virus (VEEV).

10. The nucleic acid molecule of any one of claims 1-6, wherein the modified viral RNA replicon is derived from a virus species belonging to the *Arterivirus* genus of the Arteriviridae family, and wherein the second nucleic acid sequence encoding the nonstructural protein is a

portion of or the entire pp1ab nonstructural protein of the virus species belonging to the *Arterivirus* genus.

11. The nucleic acid of claim 10, wherein the second nucleic acid sequence encodes the nonstructural of pp1ab nonstructural protein of the virus species belonging to the *Arterivirus* genus.

12. The nucleic acid molecule of any one of claims 1 to 11, wherein the viral capsid enhancer comprises a nucleic acid sequence exhibiting at least 90% sequence identity to RNA corresponding to at least one of SEQ ID Nos: 1 and 46-52.

13. The nucleic acid molecule of claim 12, wherein the viral capsid enhancer comprises a nucleic acid sequence exhibiting at least 95% sequence identity to RNA corresponding to at least one of SEQ ID Nos: 1 and 46-52.

14. The nucleic acid molecule of claim 13, wherein the viral capsid enhancer comprises a nucleic acid sequence of RNA corresponding to SEQ ID Nos: 1 or 46-52.

15. The nucleic acid molecule of any one of claims 1 to 14, wherein the modified viral RNA replicon further comprises one or more expression cassettes, wherein at least one of the one or more expression cassettes comprises a promoter operably linked to a sequence for a first gene of interest (GOI).

16. The nucleic acid molecule of claim 15, wherein the modified viral RNA replicon further comprises:

a third nucleic acid sequence encoding one or more RNA stem-loops of a second viral capsid enhancer or a variant thereof; and

a fourth nucleic acid sequence operably linked to the third nucleic acid sequence, wherein the fourth nucleic acid sequence comprises a sequence for a second gene of interest (GOI).

17. The nucleic acid molecule of claim 15 or claim 16, wherein the coding sequence for the first GOI encodes a polypeptide.

18. The nucleic molecule of claim 17, wherein said polypeptide is selected from the group consisting of an antibody, an antigen, an immune modulator, a cytokine, an enzyme, and any combination thereof.

19. A nucleic acid molecule comprising a modified viral RNA replicon, wherein the modified viral RNA replicon comprises, ordered from the 5'- to 3'-end,

(1) a 5' untranslated region (5'-UTR),

(2) a nucleotide sequence encoding an amino-terminal fragment of the nsp1 of the VEEV,

(3) a downstream loop (DLP) motif derived from Sindbis virus (SINV),

(4) a nucleotide sequence encoding a 2A protease sequence (P2A), and

(5) a nucleotide sequence encoding a polyprotein comprising the sequences of at least one of the non-structural proteins nsp1, nsp2, nsp3 and nsp4 of the VEEV.

20. The nucleic acid molecule of claim 19, wherein the modified viral RNA replicon comprises, ordered from the 5'- to 3'-end,

(1) a 5'-UTR comprising nucleotides 1 to 45 of SEQ ID NO: 19,

(2) a nucleotide sequence consisting of nucleotides 46-240 of SEQ ID NO: 19,

(3) a DLP motif comprising the nucleotide sequence of SEQ ID NO: 38,

(4) a nucleotide sequence encoding a P2A having the nucleotide sequence of SEQ ID NO: 3, and

(5) a nucleotide sequence encoding a polyprotein comprising the sequences of the non-structural proteins nsp1, nsp2, nsp3 and nsp4 of the VEEV.

21. The nucleic acid molecule of claim 19, wherein the nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO: 19.

22. A nucleic acid molecule comprising a nucleic acid sequence encoding the modified viral RNA replicon defined in any one of claims 1-21.

23. A method for producing a polypeptide of interest in a cell, comprising introducing the nucleic acid molecule of any one of claims 17-22 into the cell, thereby producing the polypeptide encoded by at least the first GOI in the cell.

24. The method of claim 23, wherein the cell is present in a tissue, an organ, or a subject, and wherein the subject is a vertebrate or invertebrate.

25. A composition, comprising the nucleic acid molecule of any one of claims 15-24 and a pharmaceutically acceptable carrier.

26. A method for producing a polypeptide of interest in a subject, comprising administering to the subject the nucleic acid molecule of any one of claims 17-22.

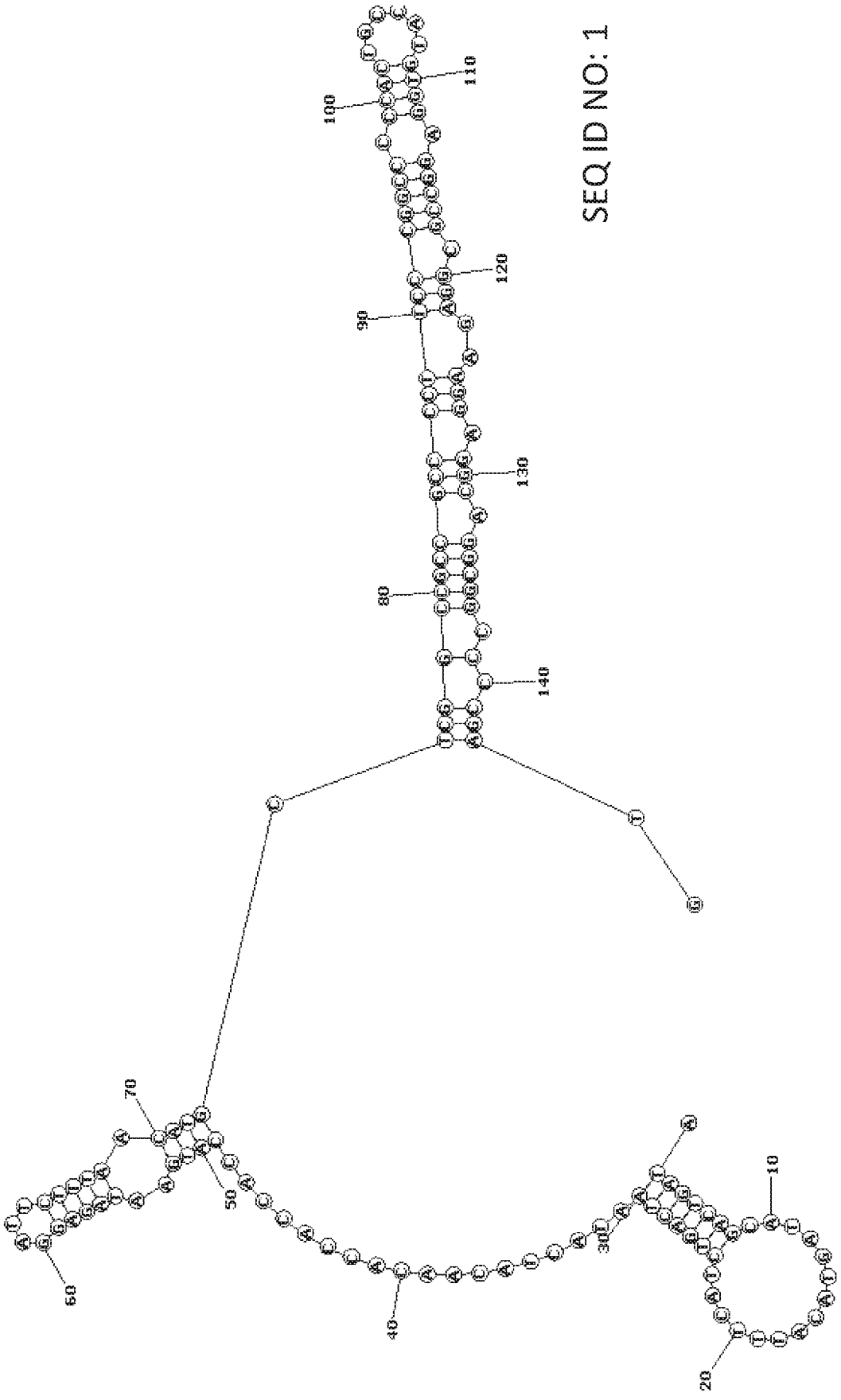


FIG 1

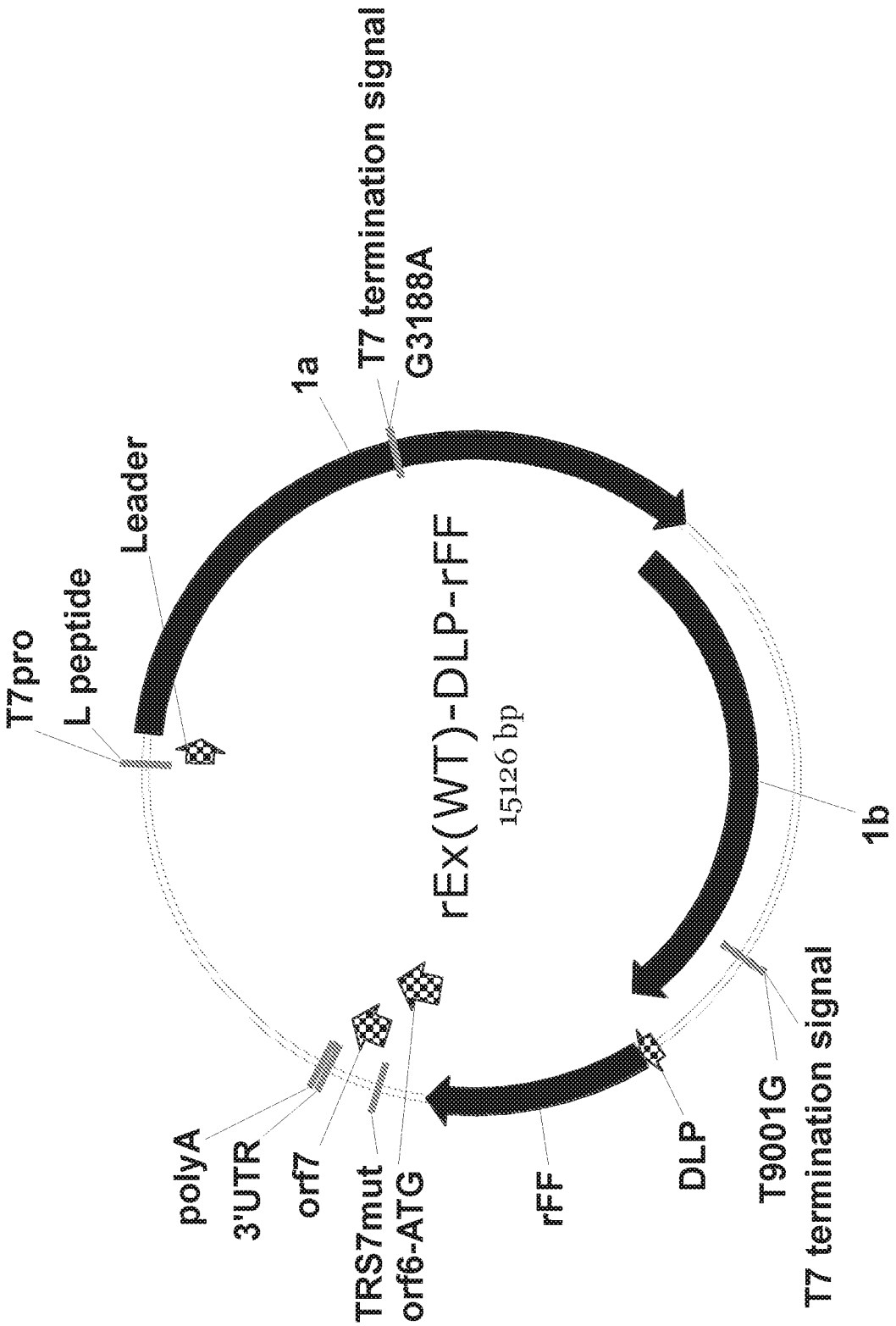

rEx(WT)-DLP-rFF
15126 bp
T7pro
L peptide
Leader
1a
T7 termination signal
G3188A
1b
T7 termination signal
T9001G
DLP
rFF
polyA
3'UTR
orf7
TRS7mut
orf6-ATG

FIG. 2A

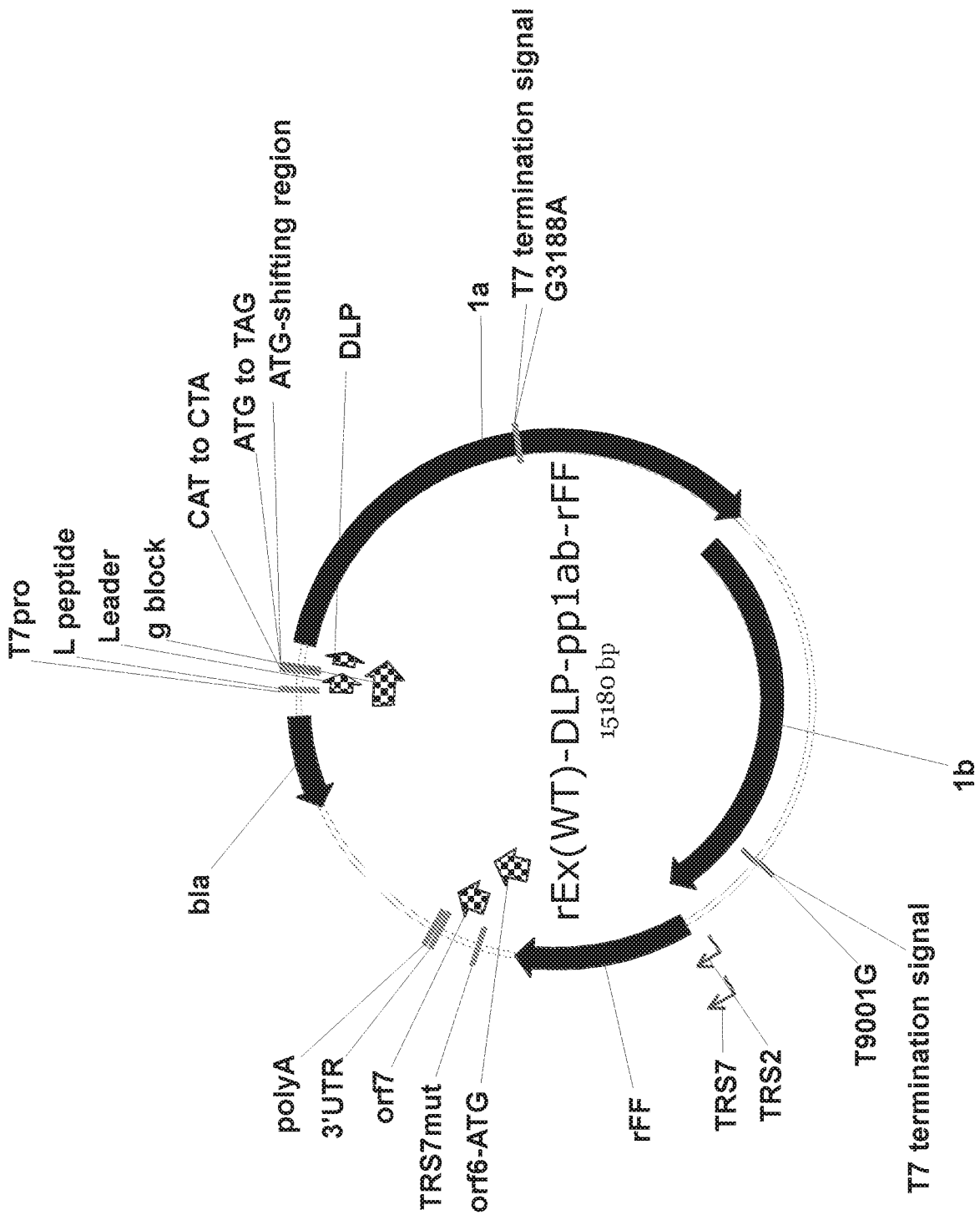

rEx(WT)-DLP-pp1ab-rFF
15180 bp
T7pro
L peptide
Leader
g block
CAT to CTA
ATG to TAG
ATG-shifting region
DLP
1a
T7 termination signal
G3188A
1b
T9001G
T7 termination signal
TRS2
TRS7
rFF
orf6-ATG
TRS7mut
orf7
3'UTR
polyA
bla

FIG. 2B

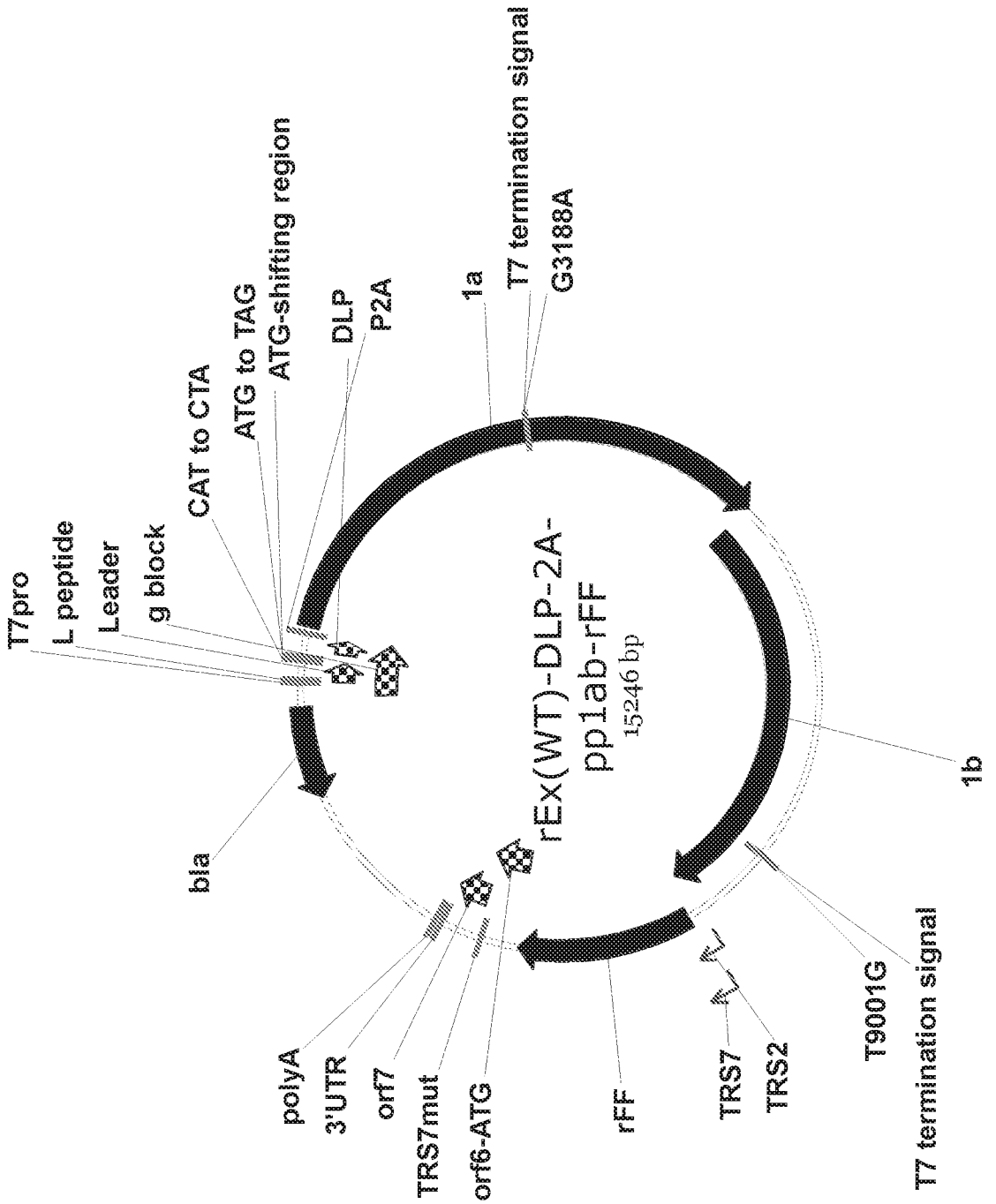

T7pro
L peptide
Leader
g block
CAT to CTA
ATG to TAG
ATG-shifting region
DLP
P2A
1a
T7 termination signal
G3188A
rEx(WT)-DLP-2A-pp1ab-rFF
15246 bp
1b
T7 termination signal
T9001G
TRS2
TRS7
rFF
orf6-ATG
TRS7mut
orf7
3'UTR
polyA
bla

FIG. 2C

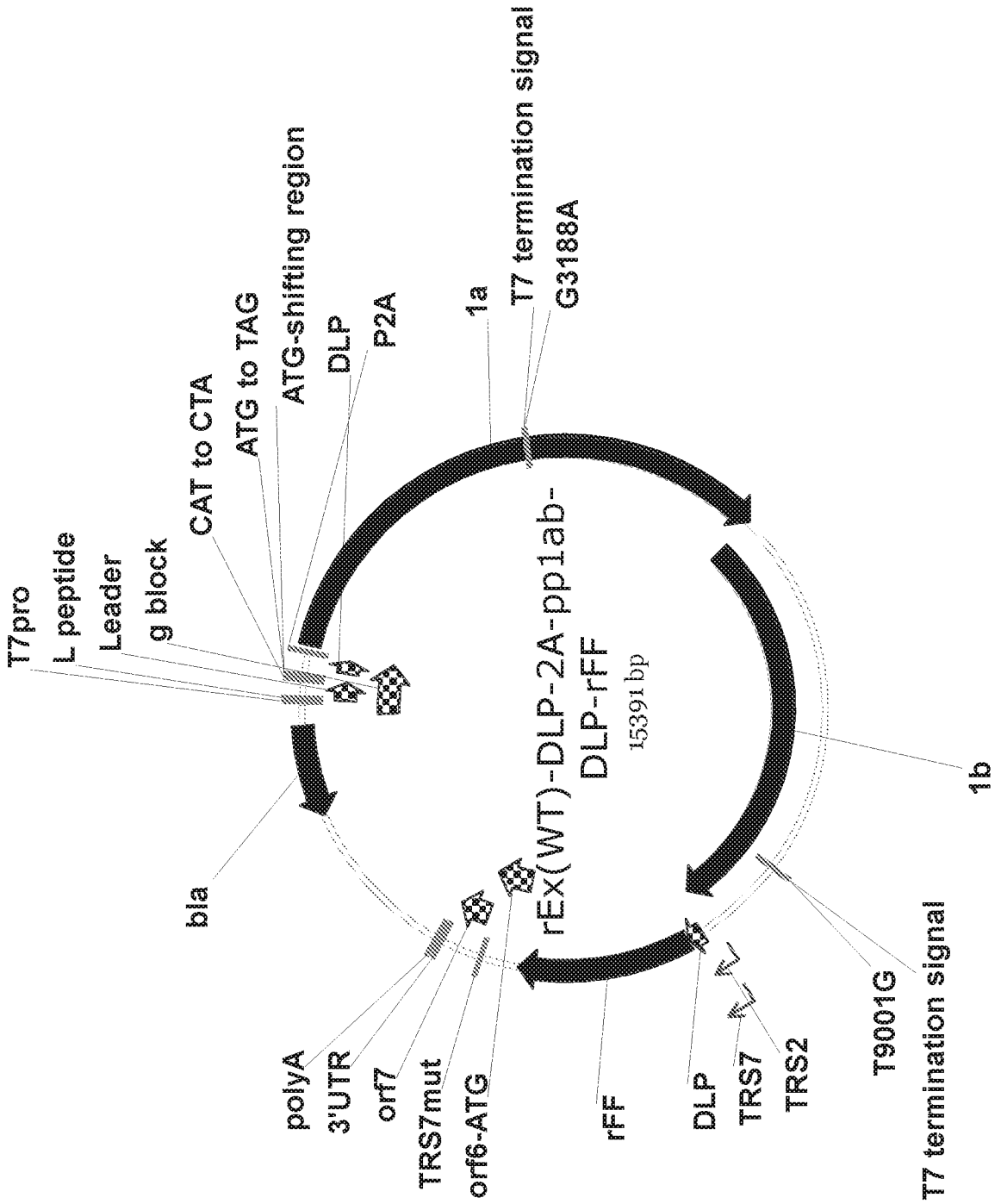

T7pro
L peptide
Leader
g block
CAT to CTA
ATG to TAG
ATG-shifting region
DLP
P2A
1a
T7 termination signal
G3188A
rEx(WT)-DLP-2A-pp1ab-DLP-rFF
15391 bp
1b
bla
polyA
3'UTR
orf7
TRS7mut
orf6-ATG
rFF
DLP
TRS7
TRS2
T9001G
T7 termination signal

FIG. 2D

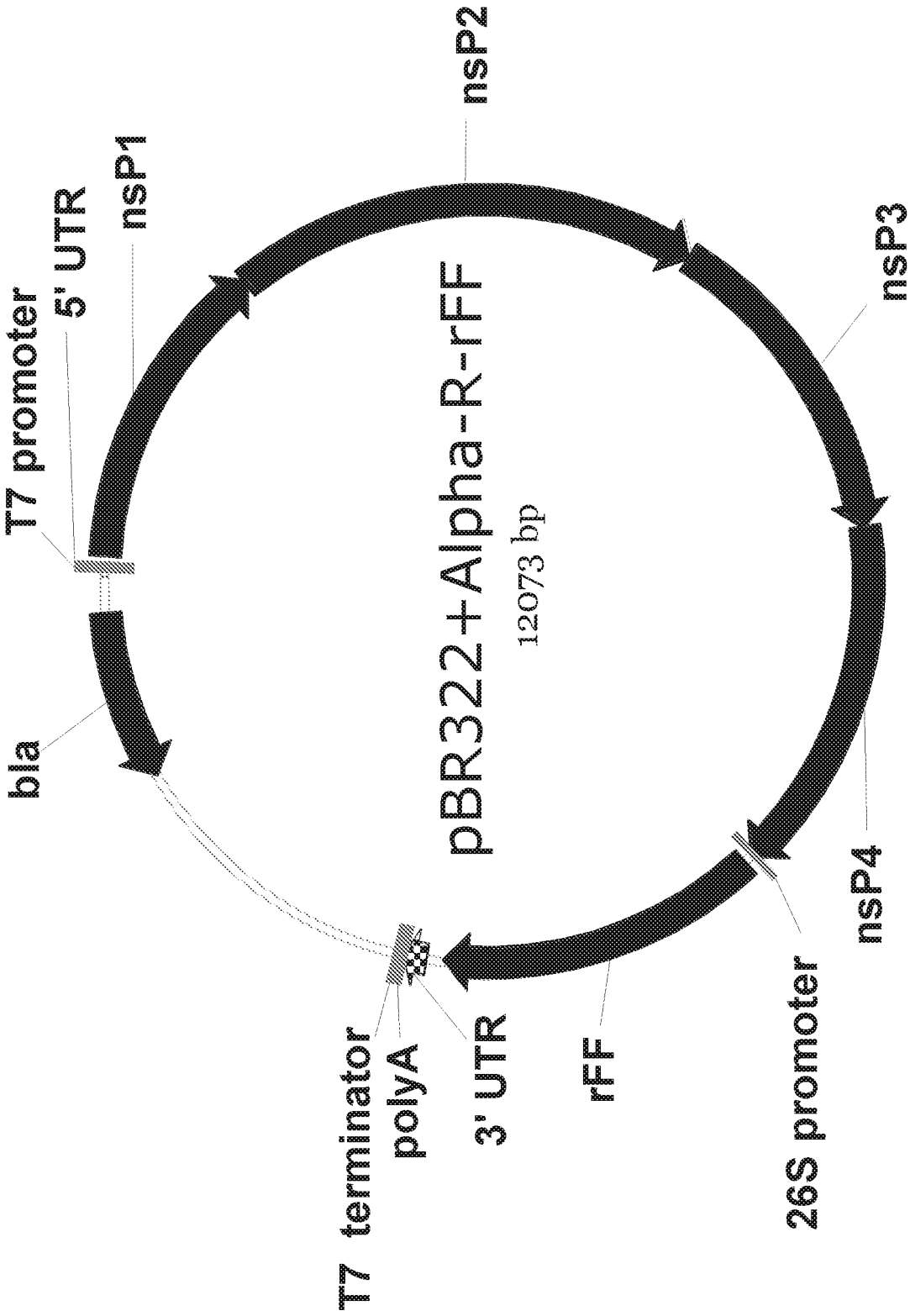

pBR322+Alpha-R-rFF
12073 bp
T7 promoter
5' UTR
nsP1
nsP2
nsP3
nsP4
26S promoter
rFF
3' UTR
polyA
T7 terminator
bla

FIG. 3A

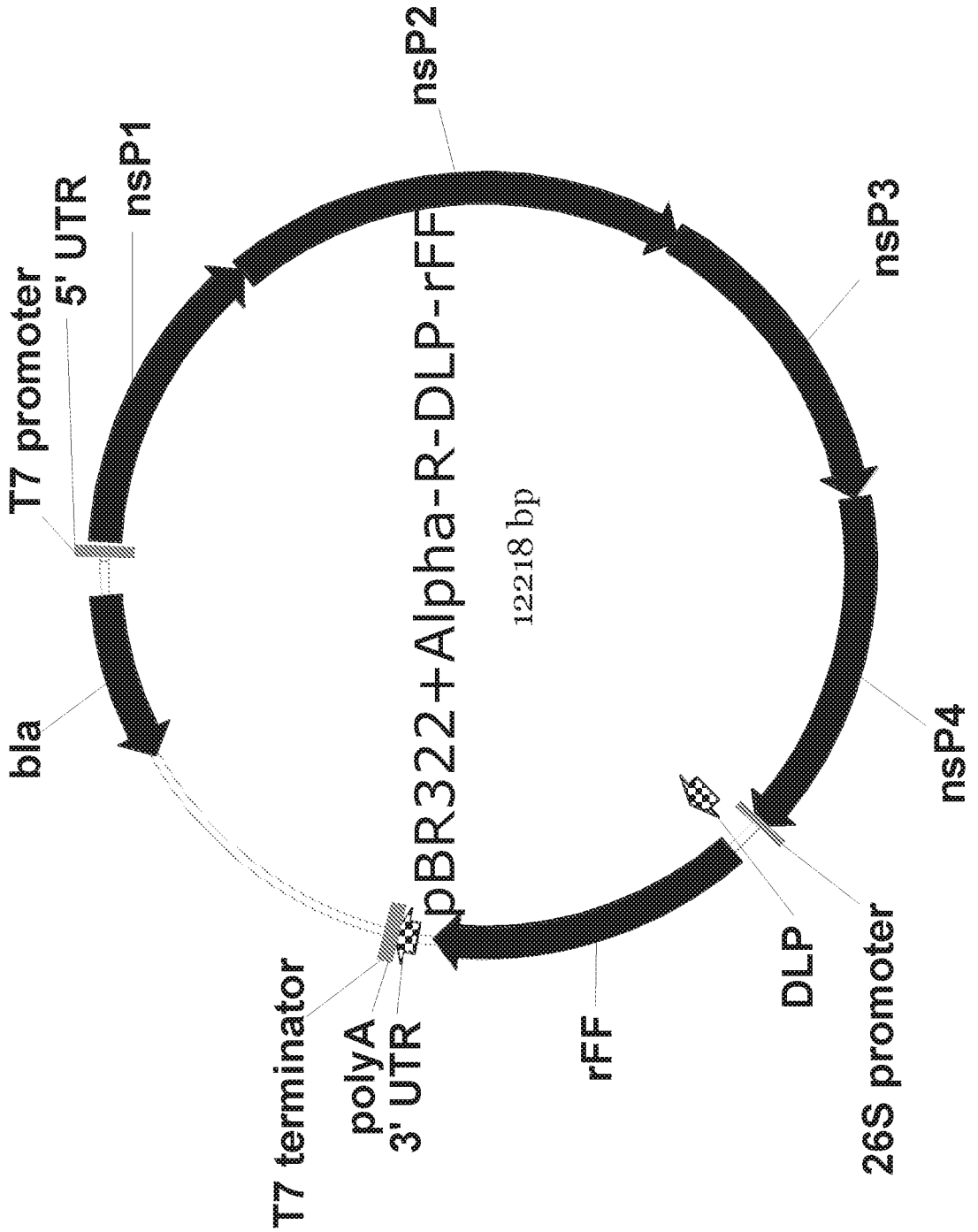

pBR322+Alpha-R-DLP-rFF
12218 bp
T7 promoter
5' UTR
nsP1
nsP2
nsP3
nsP4
26S promoter
DLP
rFF
polyA
3' UTR
T7 terminator
bla

FIG. 3B

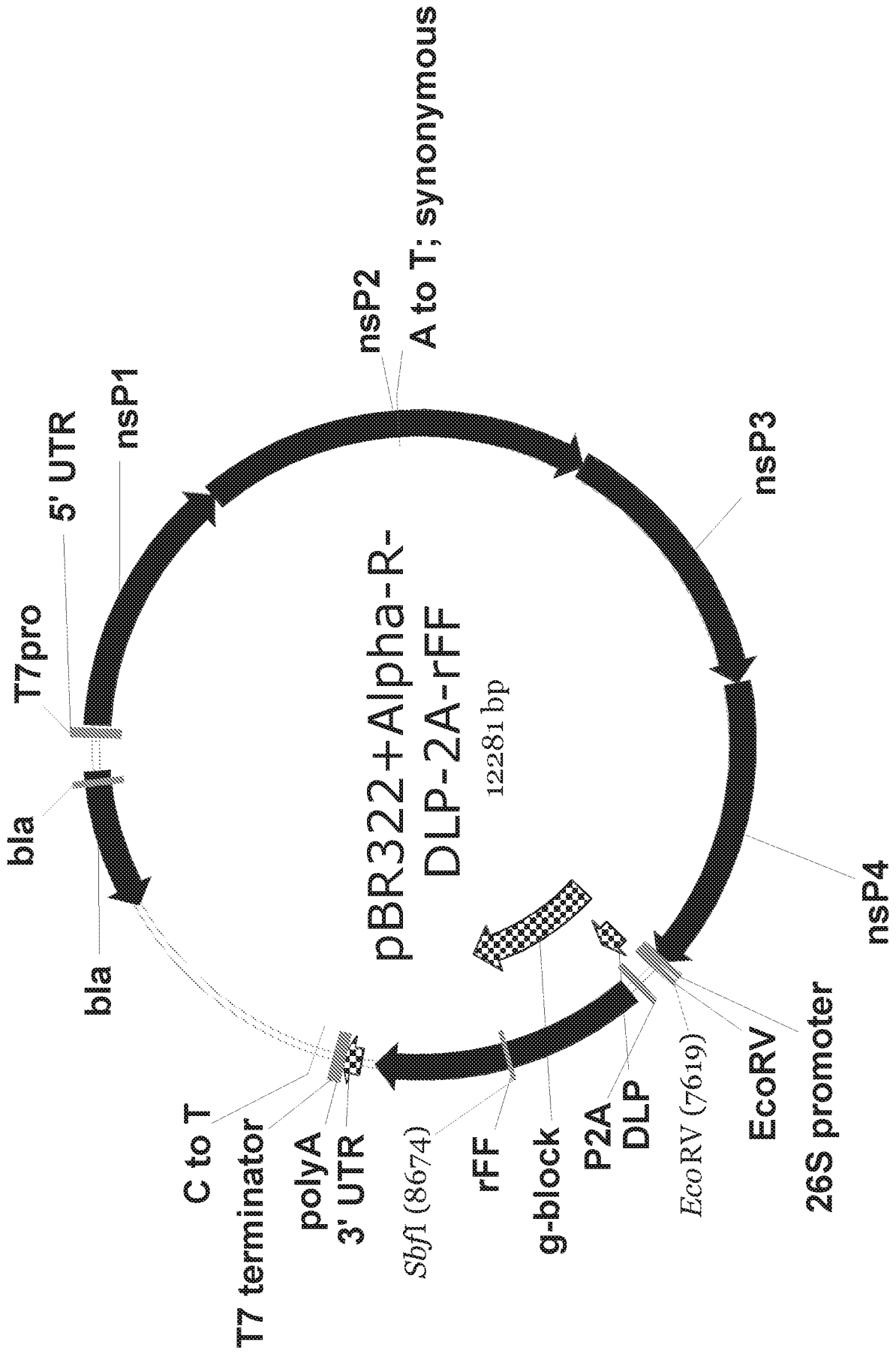

pBR322+Alpha-R-DLP-2A-rFF
12281 bp
T7pro
5' UTR
nsP1
nsP2
A to T; synonymous
nsP3
nsP4
26S promoter
EcoRV
EcoRV (7619)
DLP
P2A
g-block
rFF
SbfI (8674)
3' UTR
polyA
T7 terminator
C to T
bla
bla

FIG. 3C

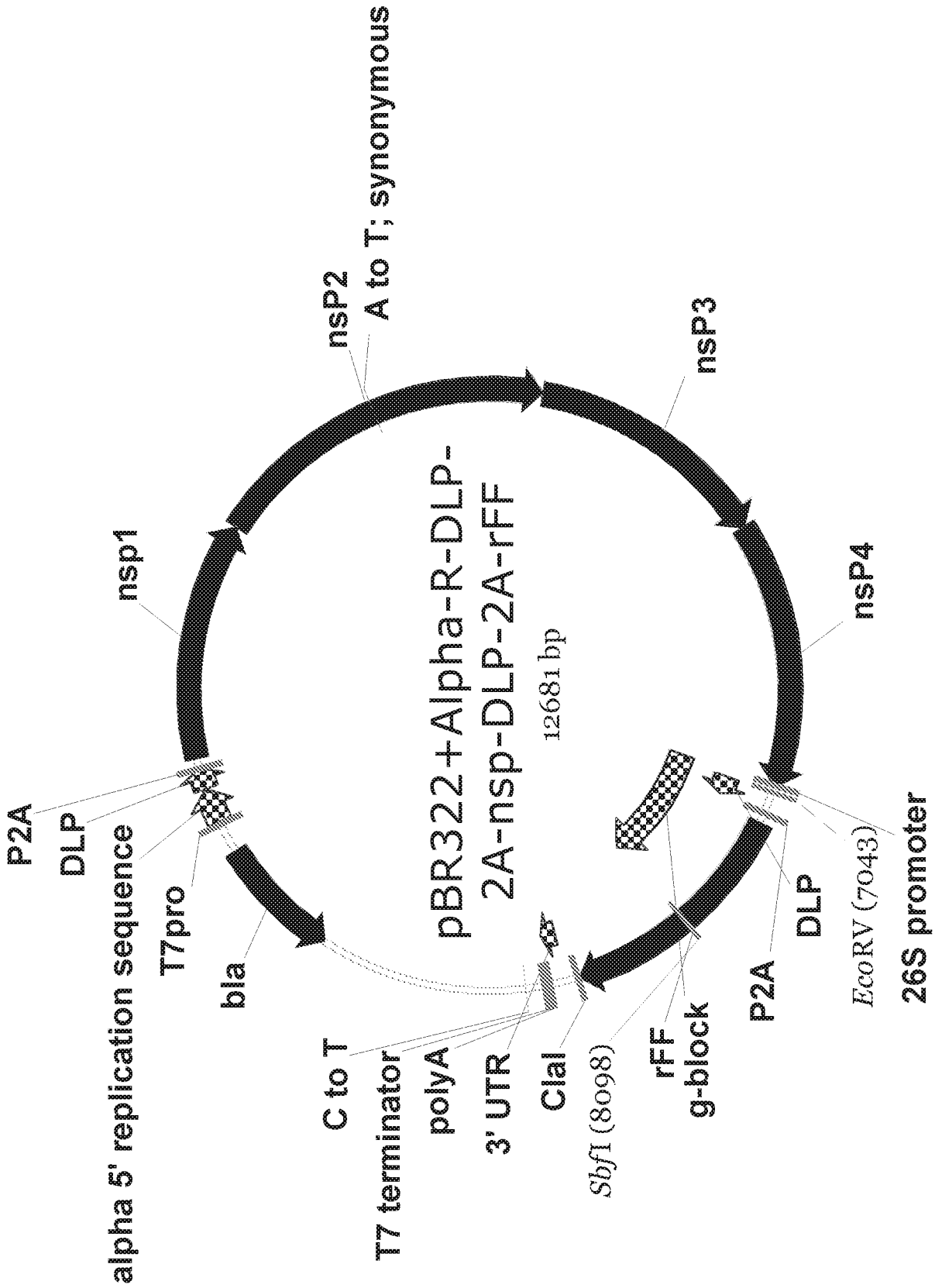

pBR322+Alpha-R-DLP-2A-nsp-DLP-2A-rFF
12681 bp
nsP2
A to T; synonymous
nsP3
nsP4
nsp1
P2A
DLP
alpha 5' replication sequence
T7pro
bla
C to T
T7 terminator
polyA
3' UTR
ClaI
SbfI (8098)
rFF
g-block
P2A
DLP
EcoRV (7043)
26S promoter

FIG. 3D

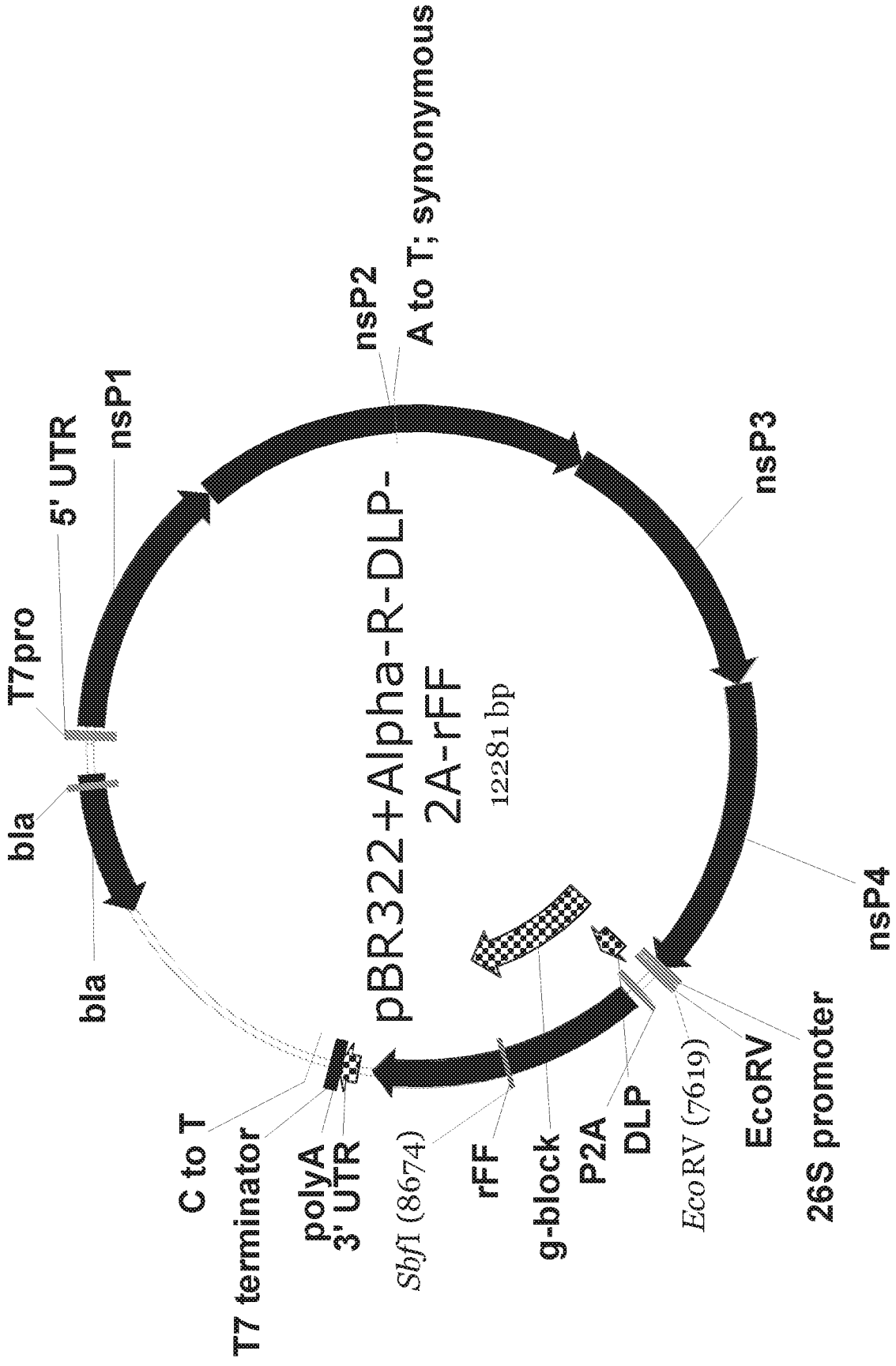

pBR322+Alpha-R-DLP-2A-rFF
12281 bp
T7pro
5' UTR
nsP1
nsP2
A to T; synonymous
nsP3
nsP4
26S promoter
EcoRV
EcoRV (7619)
DLP
P2A
g-block
rFF
SbfI (8674)
polyA 3' UTR
T7 terminator
C to T
bla
bla

FIG. 4A

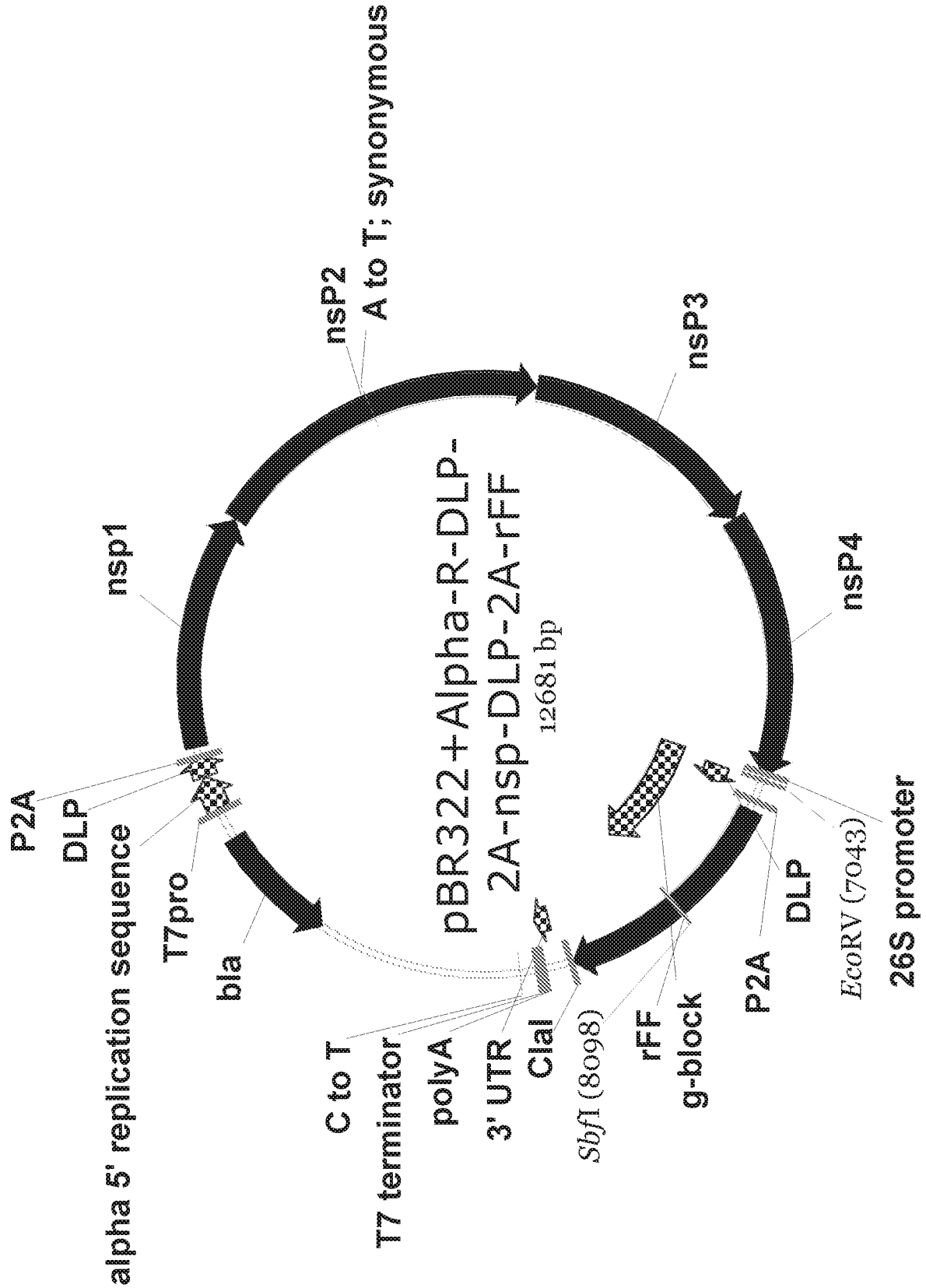

pBR322+Alpha-R-DLP-2A-nsp-DLP-2A-rFF
12681 bp
nsP2
A to T; synonymous
nsP3
nsP4
nsp1
P2A
DLP
alpha 5' replication sequence
T7pro
bla
C to T
T7 terminator
polyA
3' UTR
ClaI
SbfI (8098)
rFF
g-block
P2A
DLP
EcoRV (7043)
26S promoter

FIG. 4B

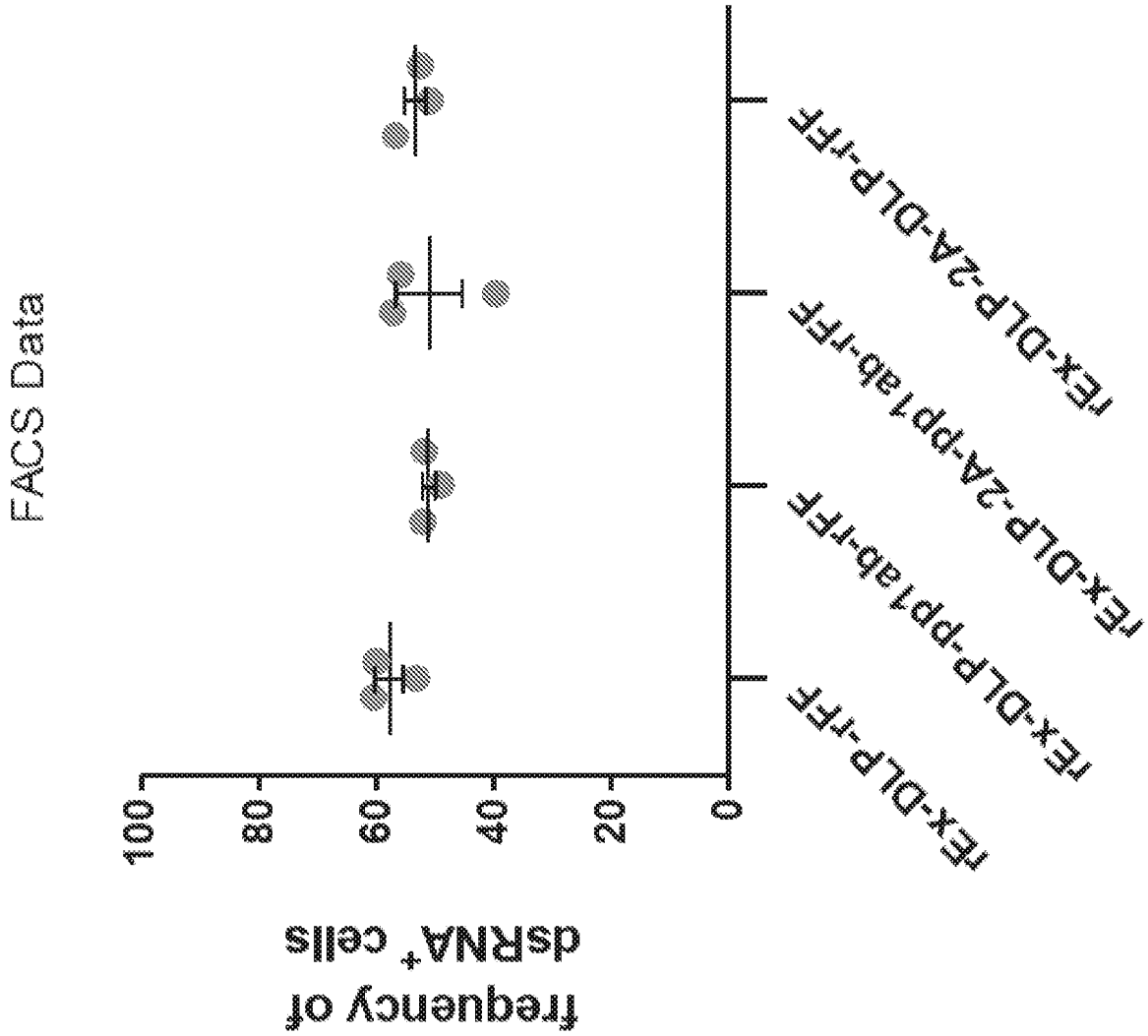

FACS Data
Frequency of dsRNA+ cells
100
80
60
40
20
0
rEx-DLP-rFF
rEx-DLP-pp1ab-rFF
rEx-DLP-2A-pp1ab-rFF
rEx-DLP-2A-DLP-rFF

FIG. 5A

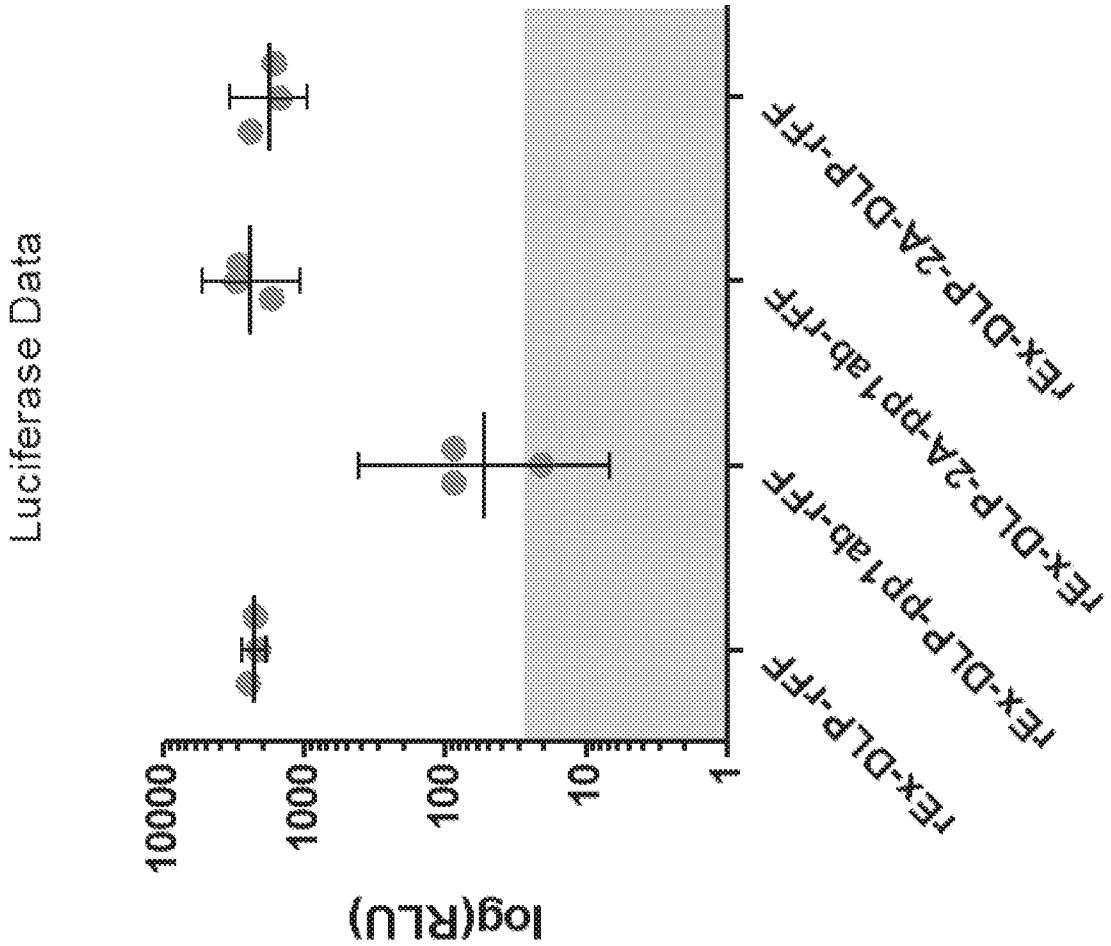

Luciferase Data
log(RLU)
10000
1000
100
10
1
rEx-DLP-rFF
rEx-DLP-PP1ab-rFF
rEx-DLP-2A-PP1ab-rFF
rEx-DLP-2A-DLP-rFF

FIG. 5B

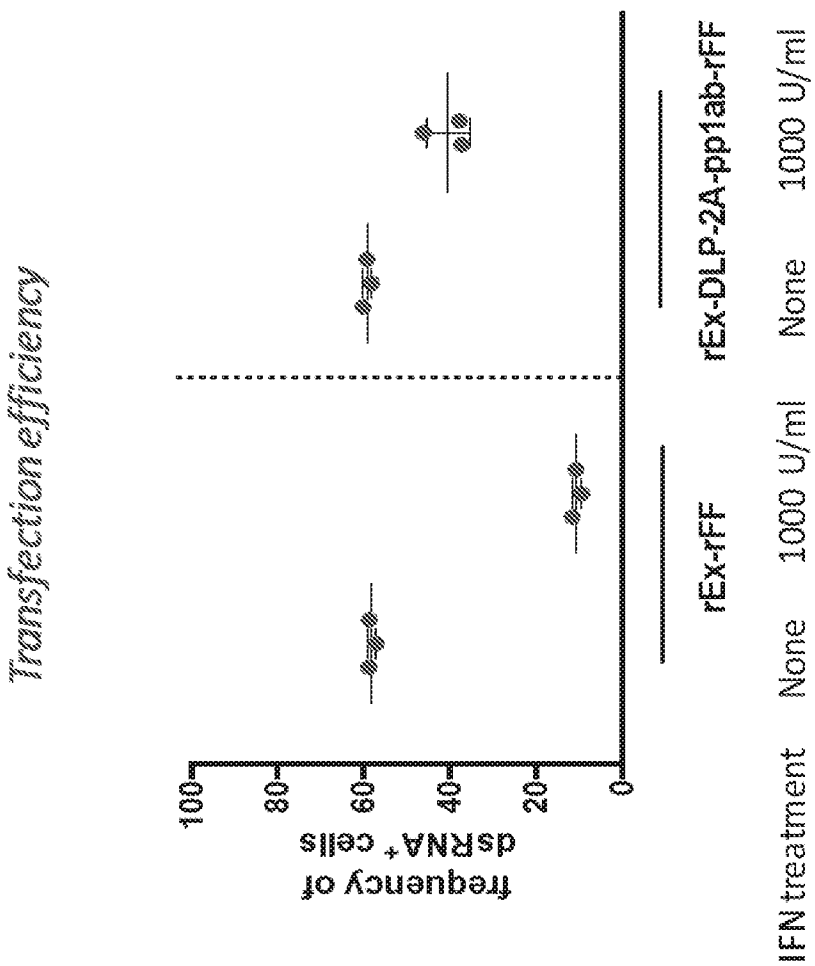

Transfection efficiency
Frequency of dsRNA+ cells
100
80
60
40
20
0
rEx-rFF
rEx-DLP-2A-pp1ab-rFF
IFN treatment
None
1000 U/ml
None
1000 U/ml

FIG. 6A

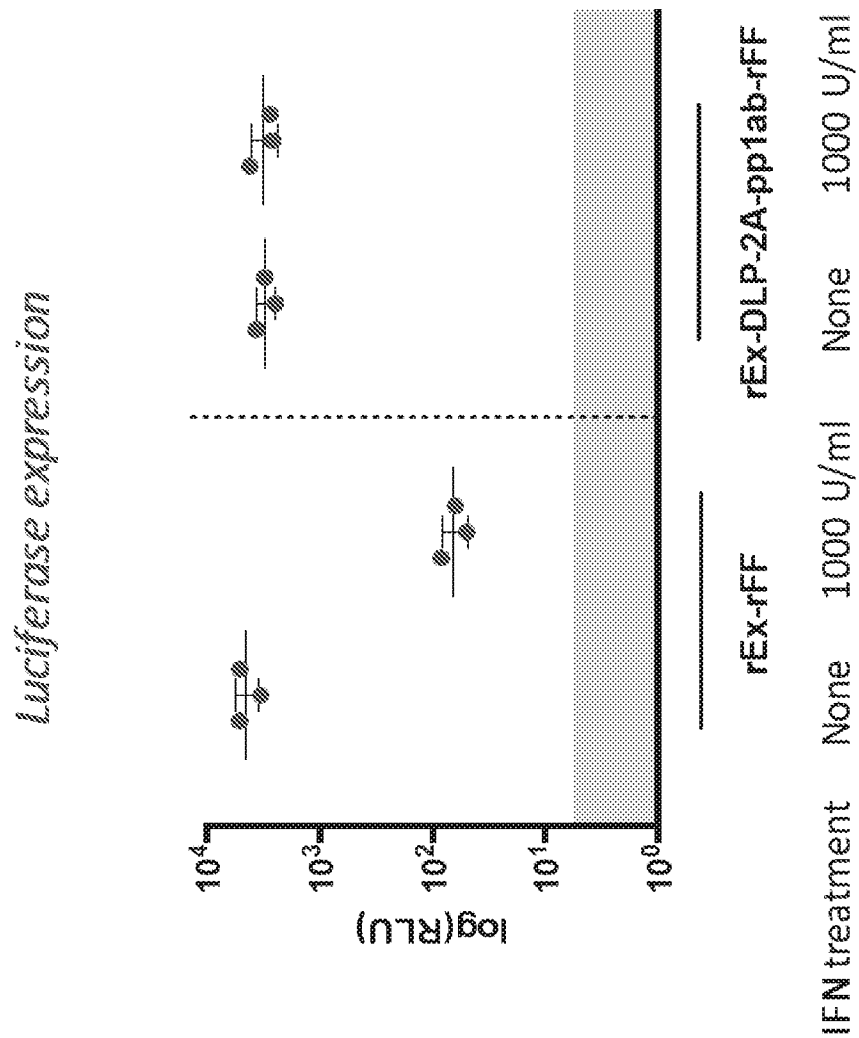

Luciferase expression
log(RLU)
10^4
10^3
10^2
10^1
10^0
IFN treatment
None
1000 U/ml
None
1000 U/ml
rEx-rFF
rEx-DLP-2A-pp1ab-rFF

FIG. 6B

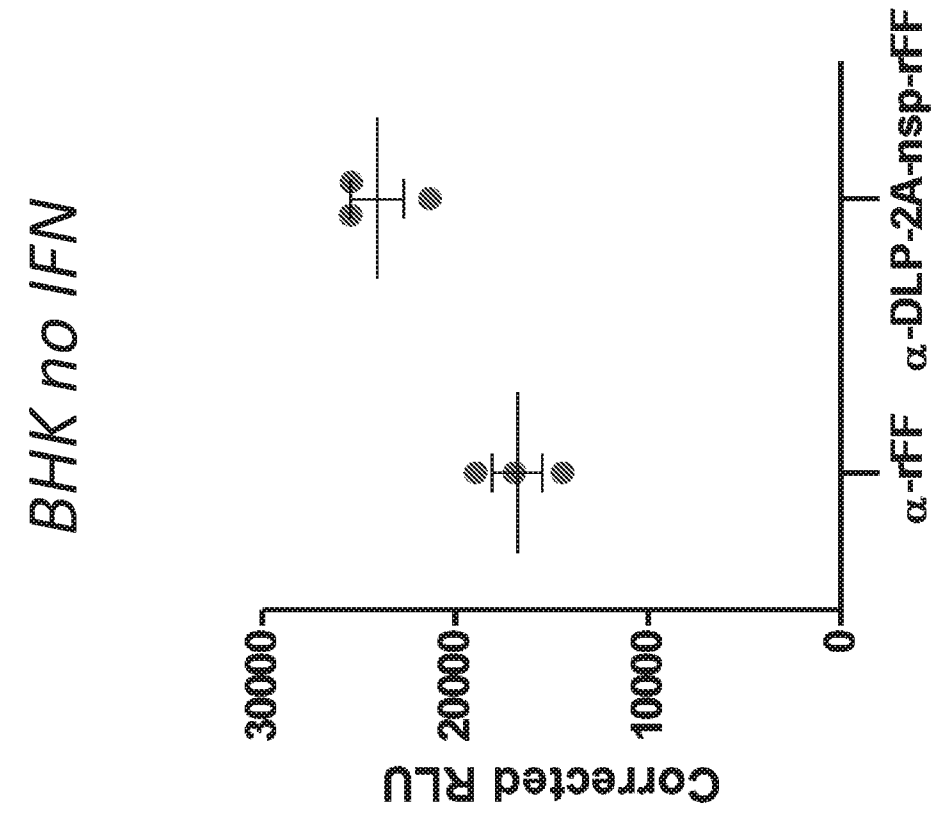

BHK no IFN
Corrected RLU
30000
20000
10000
0
α-rFF
α-DLP-2A-nsp-rFF

FIG. 7A

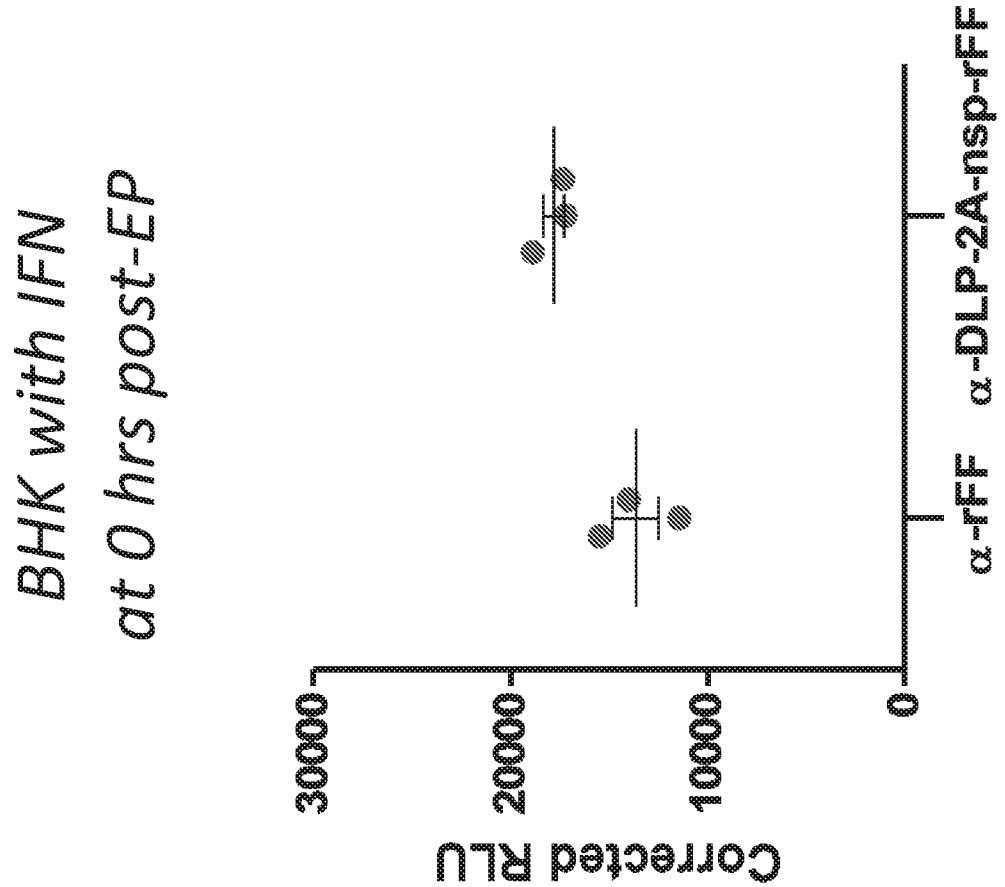

BHK with IFN
at 0 hrs post-EP
Corrected RLU
30000
20000
10000
0
α-rFF
α-DLP-2A-nsp-rFF

FIG. 7B

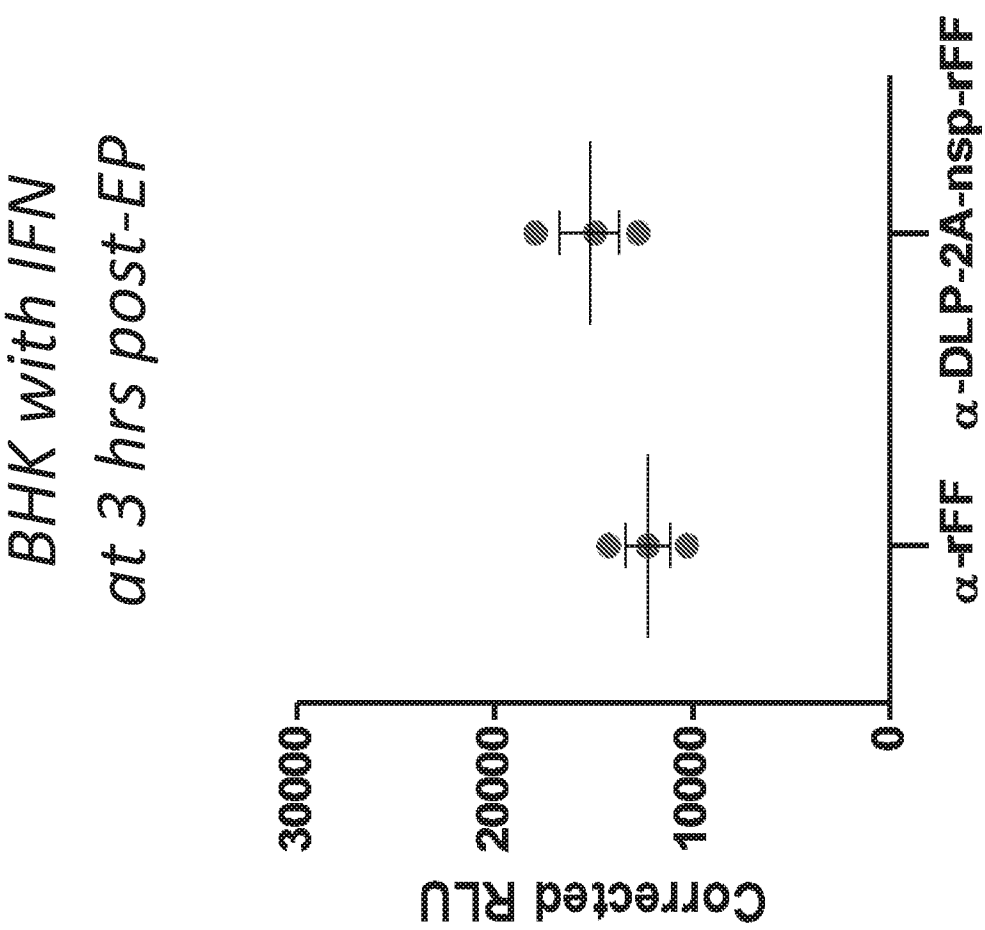

BHK with IFN
at 3 hrs post-EP
Corrected RLU
30000
20000
10000
0
α-rFF
α-DLP-2A-nsp-rFF

FIG. 7C

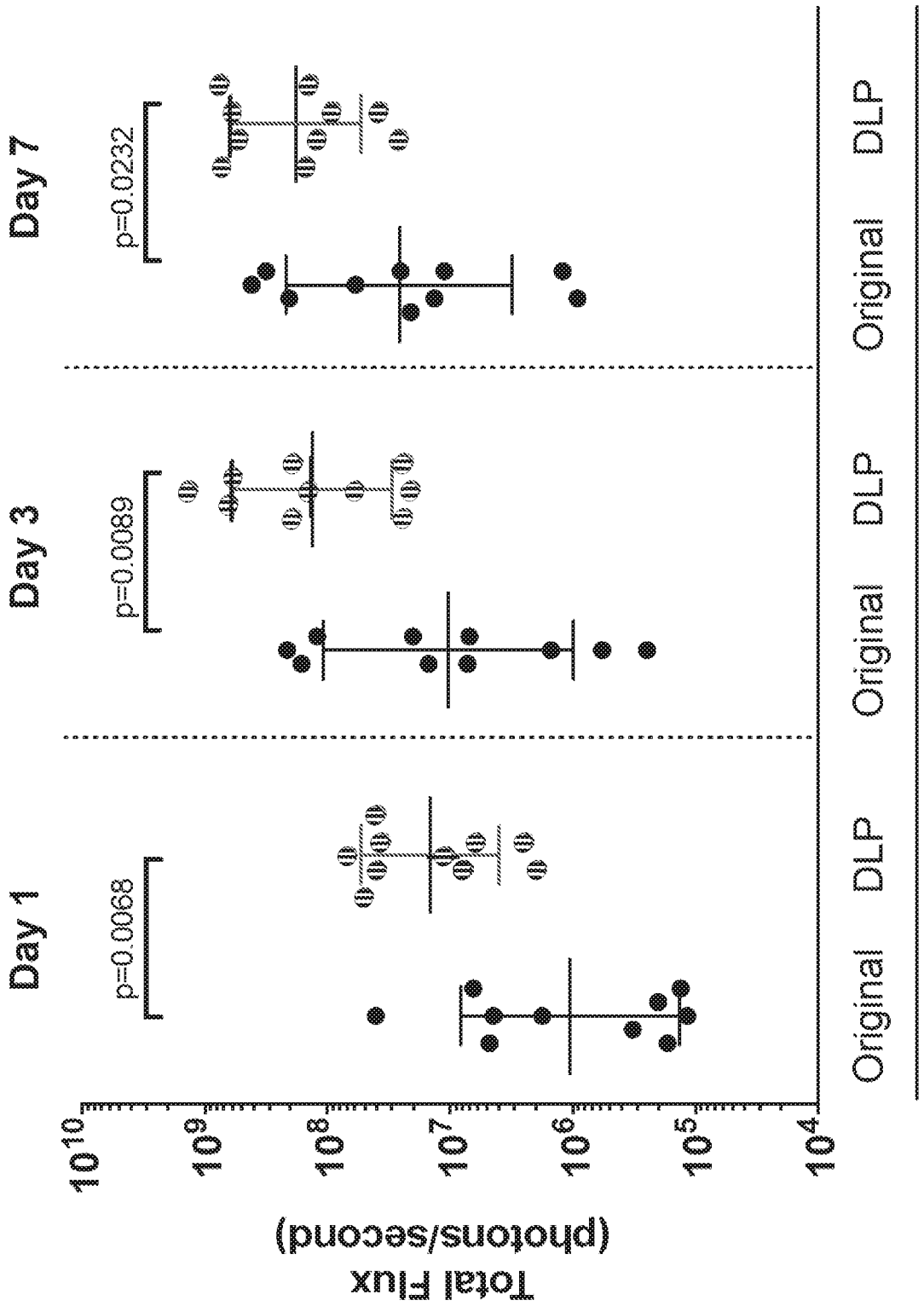

Day 1
Day 3
Day 7
p=0.0068
p=0.0089
p=0.0232
Total Flux
(photons/second)
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
Original
DLP
Original
DLP
Original
DLP
Alphavirus replicon

FIG. 8

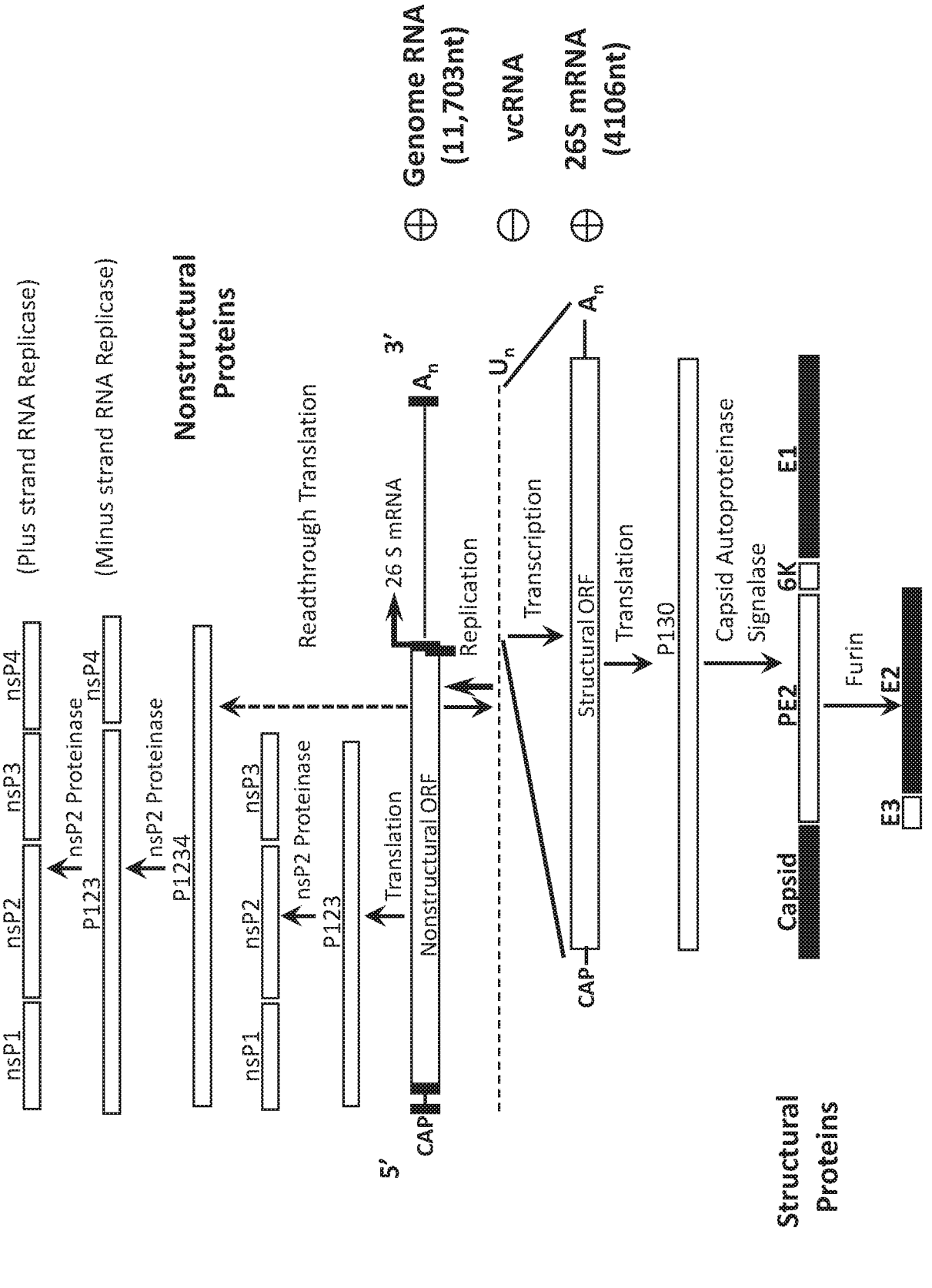


FIG. 9

Adapted from Strauss *et al.*, 1994

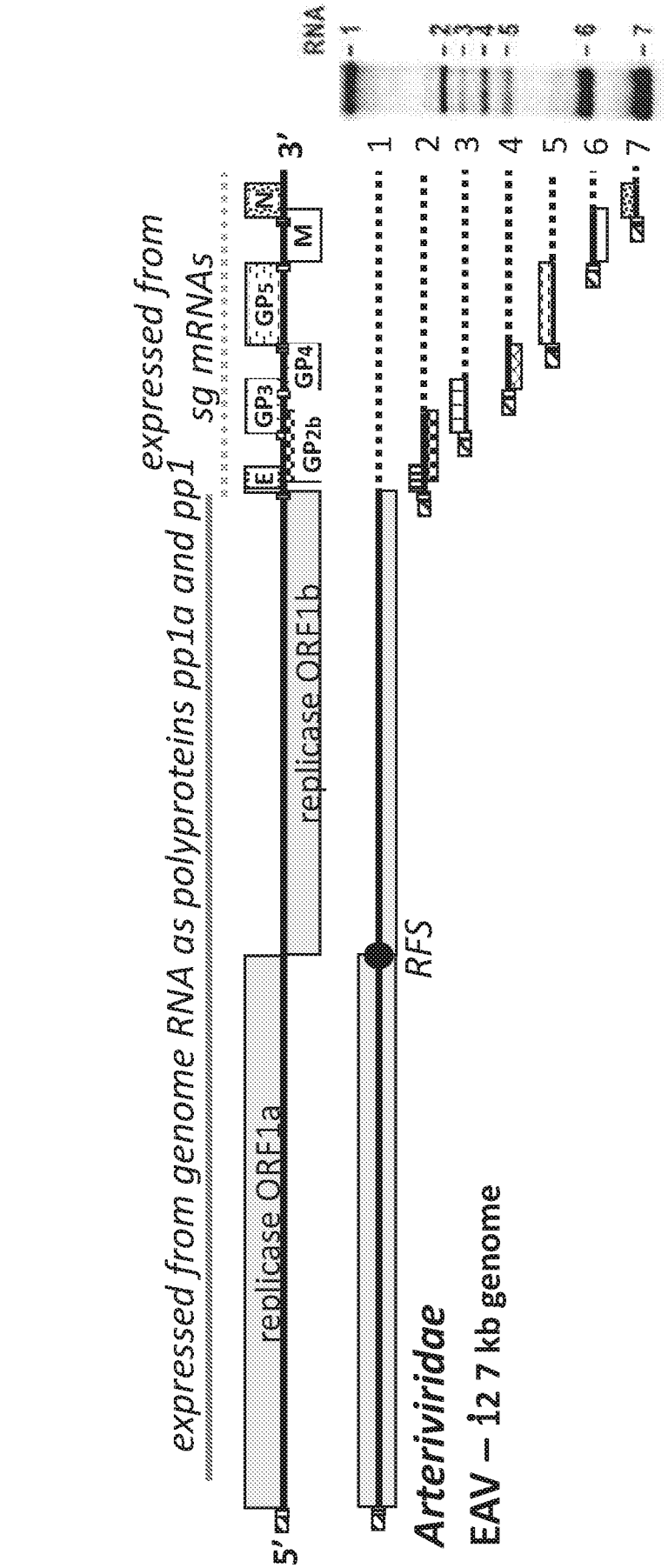
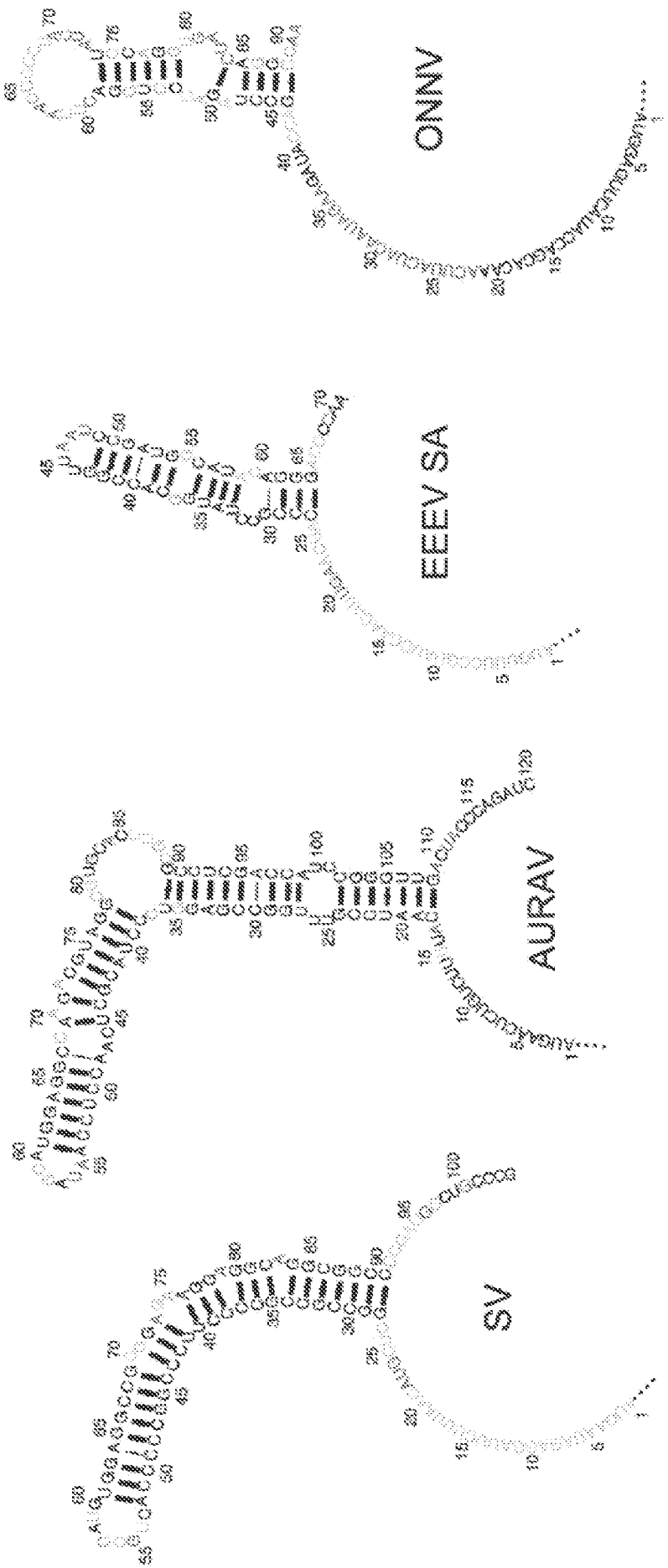

expressed from genome RNA as polyproteins pp1a and pp1
expressed from sg mRNAs
5'
3'
replicase ORF1a
replicase ORF1b
E
GP3
GP2b
GP4
GP5
M
N
RFS
Arteriviridae
EAV – 12.7 kb genome
RNA
1
2
3
4
5
6
7

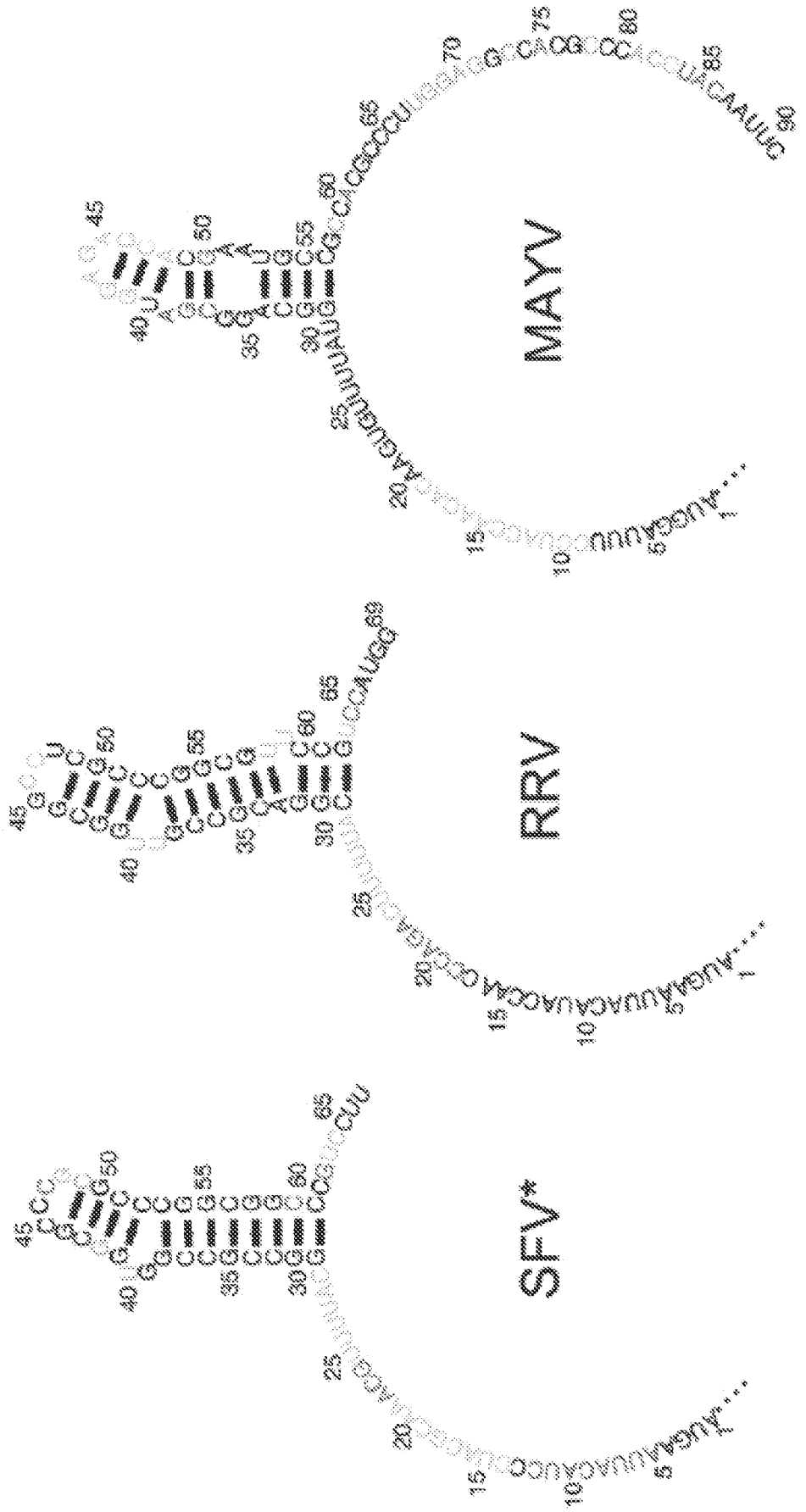
FIG. 10



SEQ ID NO: 46 SEQ ID NO: 47 SEQ ID NO: 48 SEQ ID NO: 49

Adopted from Toribio *et al.* 2016

FIG. 11A



SEQ ID NO: 52

SEQ ID NO: 51

SEQ ID NO: 50

Adopted from Toribio *et al.* 2016

FIG. 11B

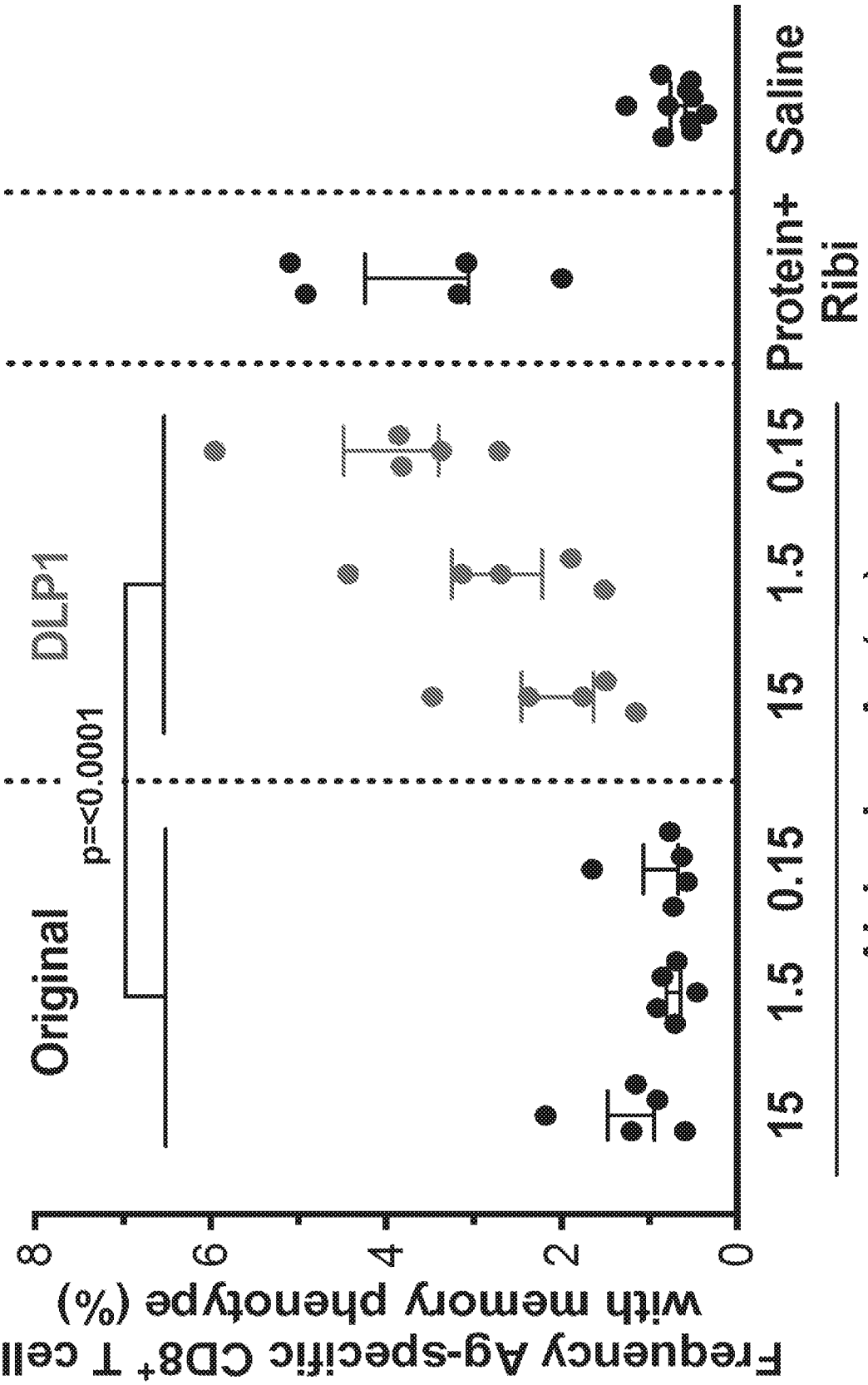

Frequency Ag-specific CD8+ T cells with memory phenotype (%)
0
2
4
6
8
Original
DLP1
p=<0.0001
15
1.5
0.15
15
1.5
0.15
Alphavirus.Ag (μg)
Protein+ Ribi
Saline

FIG. 12A

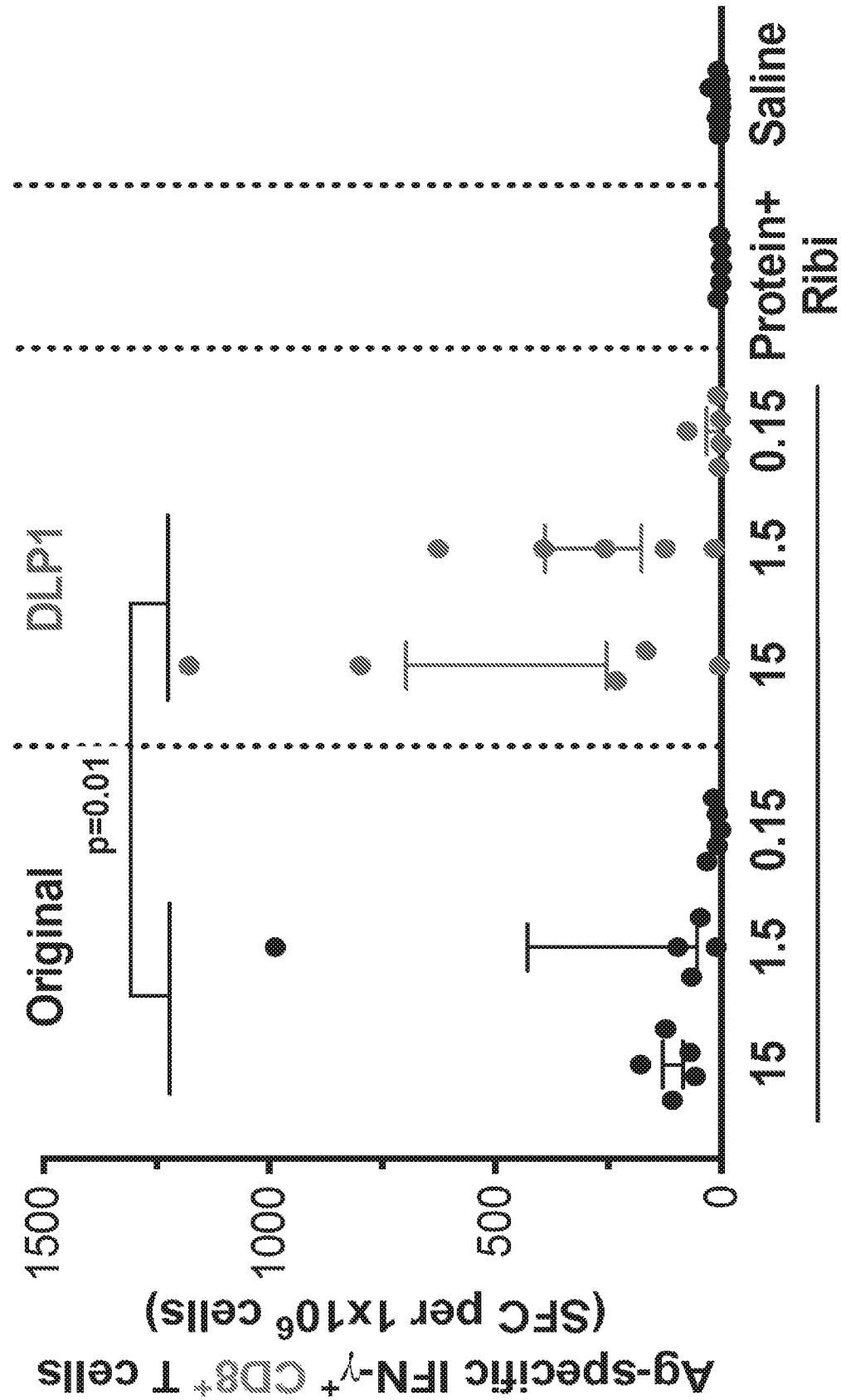

Ag-specific IFN-γ+ CD8+ T cells
(SFC per 1x10^6 cells)
1500
1000
500
0
Original
DLP1
p=0.01
15
1.5
0.15
15
1.5
0.15
Protein+
Ribi
Saline
Alphavirus.Ag (μg)

FIG. 12B

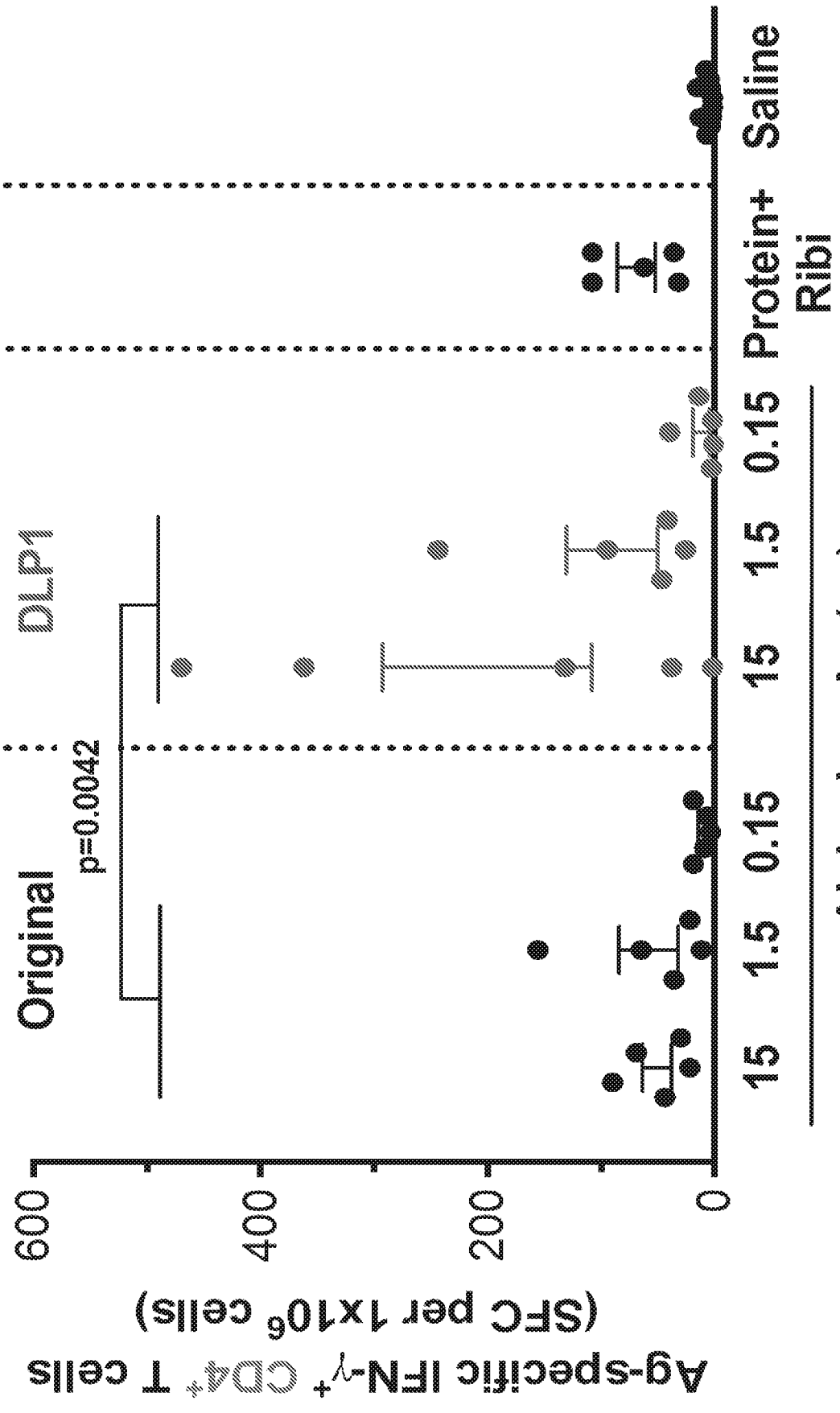

Ag-specific IFN-γ+ CD4+ T cells
(SFC per 1x10^6 cells)
600
400
200
0
Original
DLP1
p=0.0042
15
1.5
0.15
15
1.5
0.15
Alphavirus.Ag (μg)
Protein+
Ribi
Saline

FIG. 12C

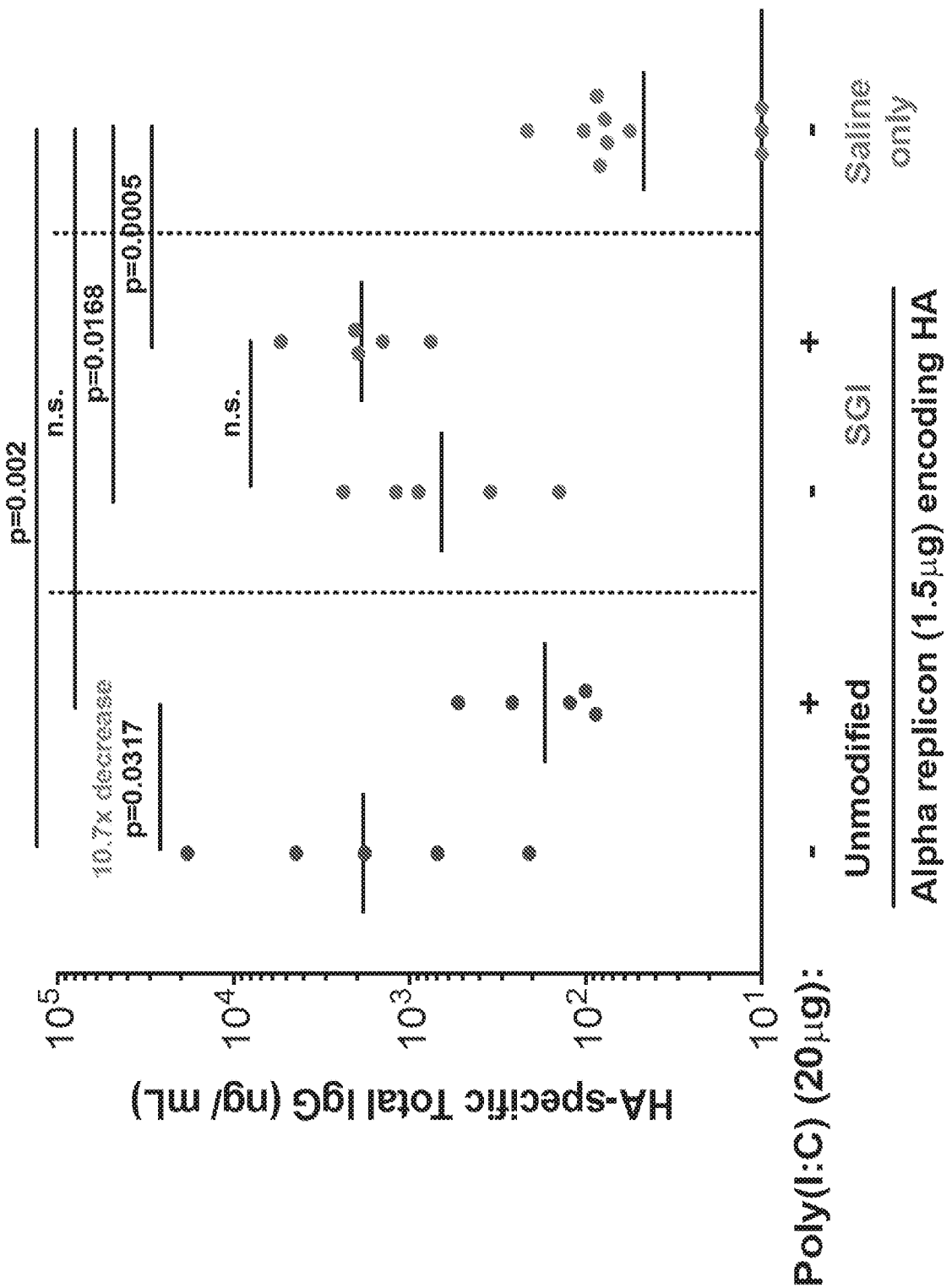

p=0.002
n.s.
p=0.0168
p=0.0005
n.s.
10.7% decrease
p=0.0317
HA-specific Total IgG (ng/ mL)
10^5
10^4
10^3
10^2
10^1
Poly(I:C) (20μg):
-
+
-
+
-
Unmodified
SGI
Saline only
Alpha replicon (1.5μg) encoding HA

FIG. 13

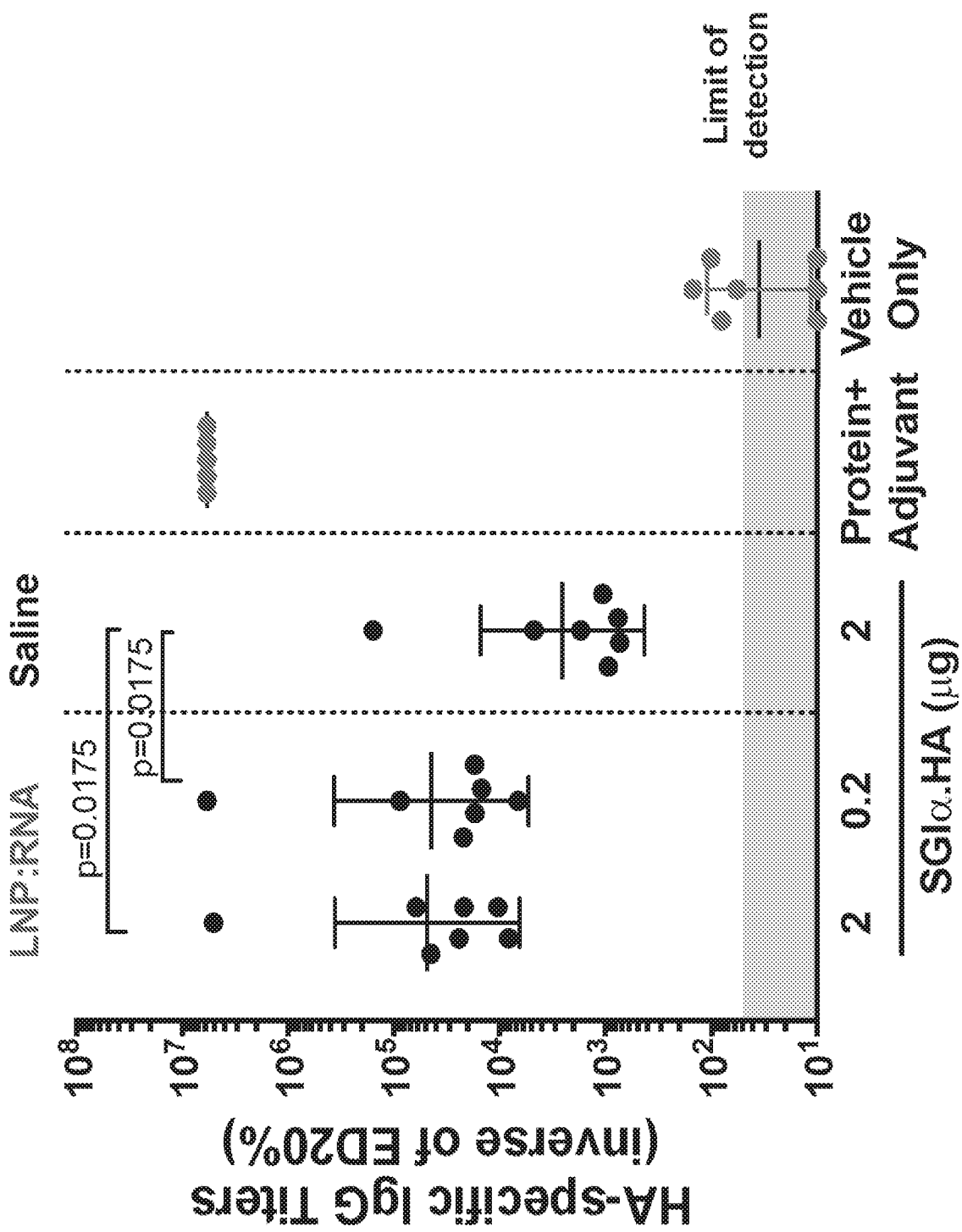

HA-specific IgG Titers
(inverse of ED20%)
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
Limit of detection
p=0.0175
p=0.0175
LNP:RNA
Saline
2
0.2
2
Protein+ Adjuvant
Vehicle Only
SGIα.HA (μg)

FIG. 14A

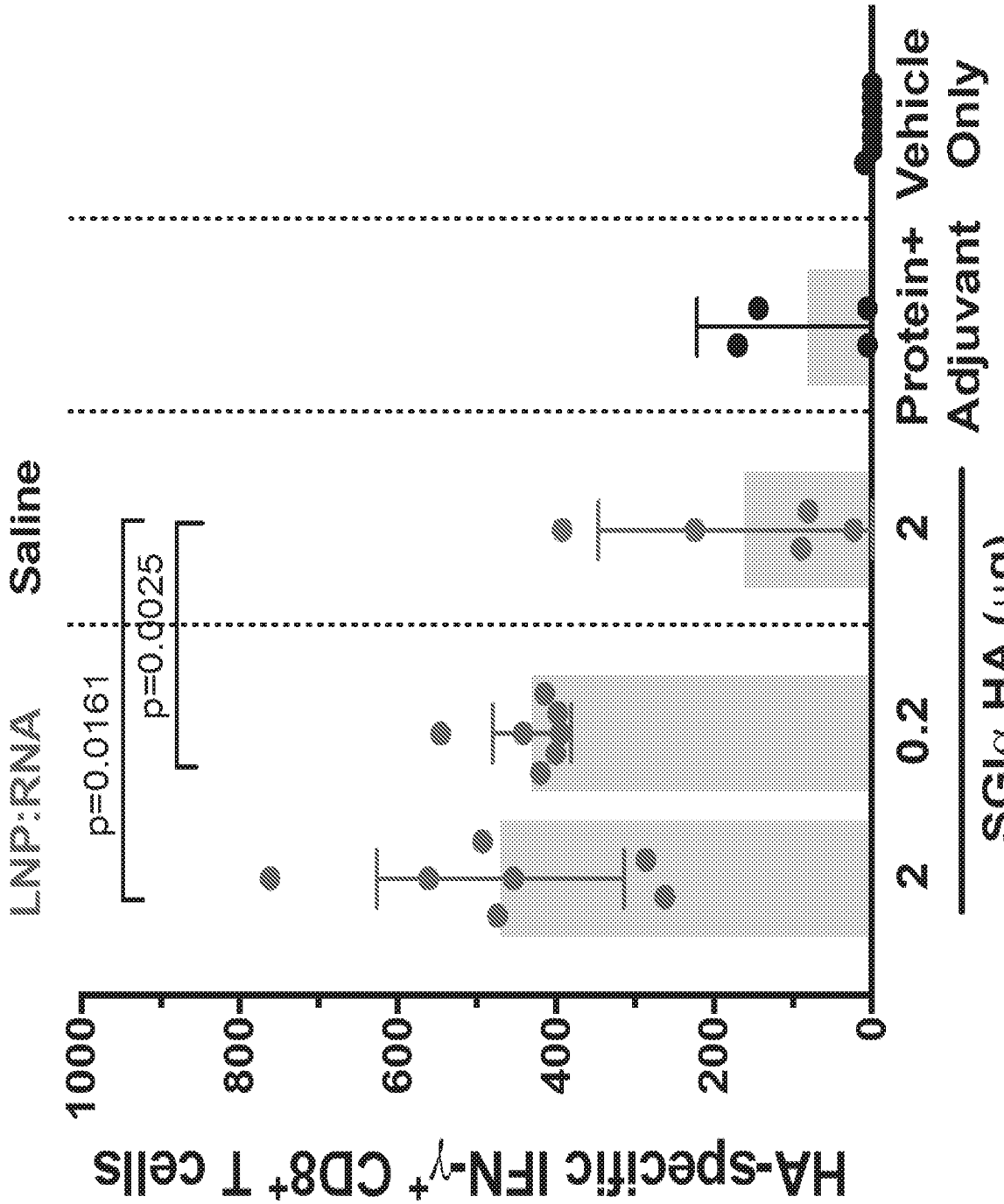

HA-specific IFN-γ+ CD8+ T cells
1000
800
600
400
200
0
p=0.0161
p=0.0025
LNP:RNA
Saline
2
0.2
2
SGIα.HA (μg)
Protein+ Adjuvant
Vehicle Only

FIG. 14B

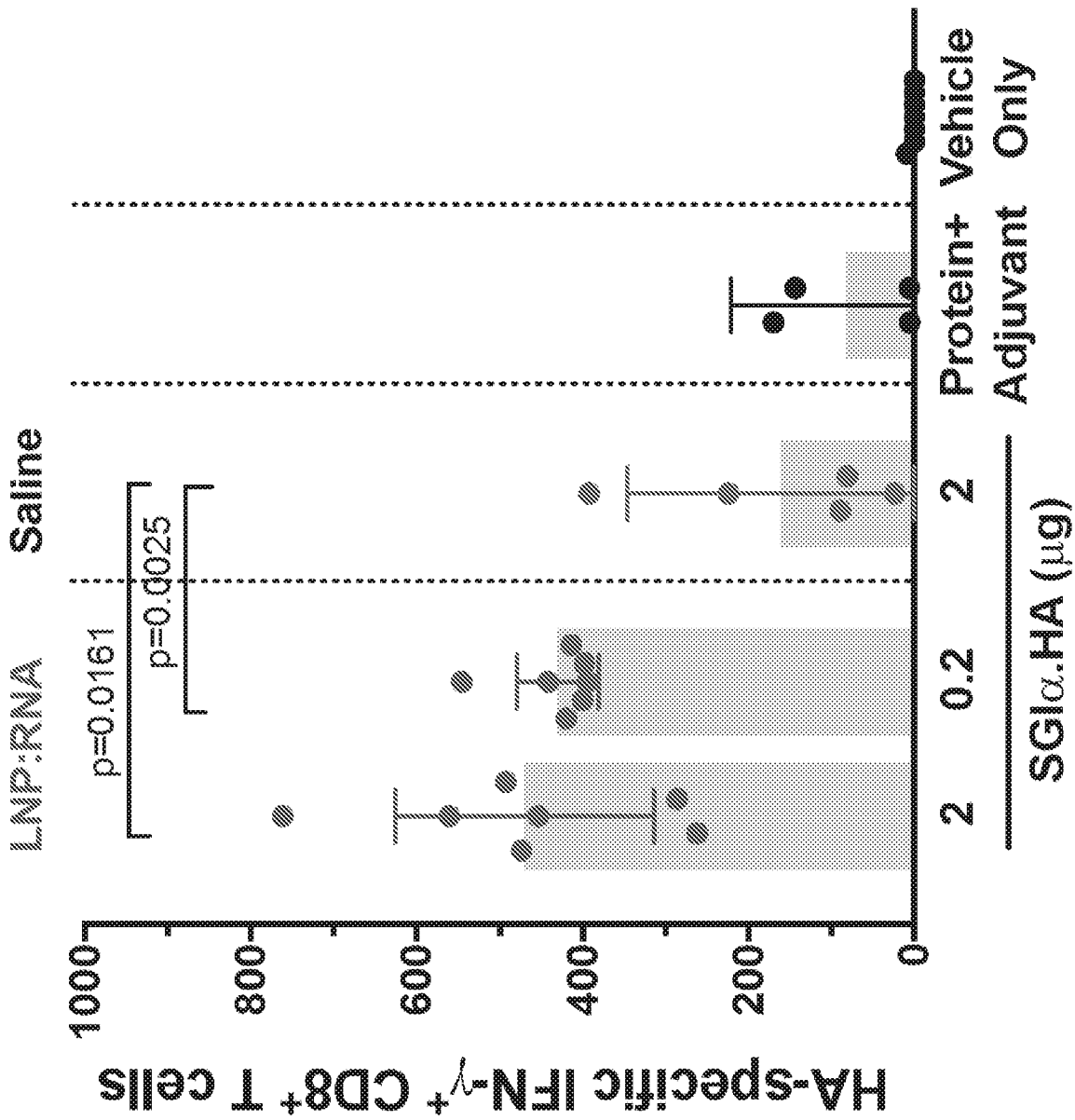

HA-specific IFN-γ+ CD8+ T cells
1000
800
600
400
200
0
LNP:RNA
Saline
p=0.0161
p=0.0025
2
0.2
2
SGIα.HA (μg)
Protein+ Adjuvant
Vehicle Only

FIG. 14C

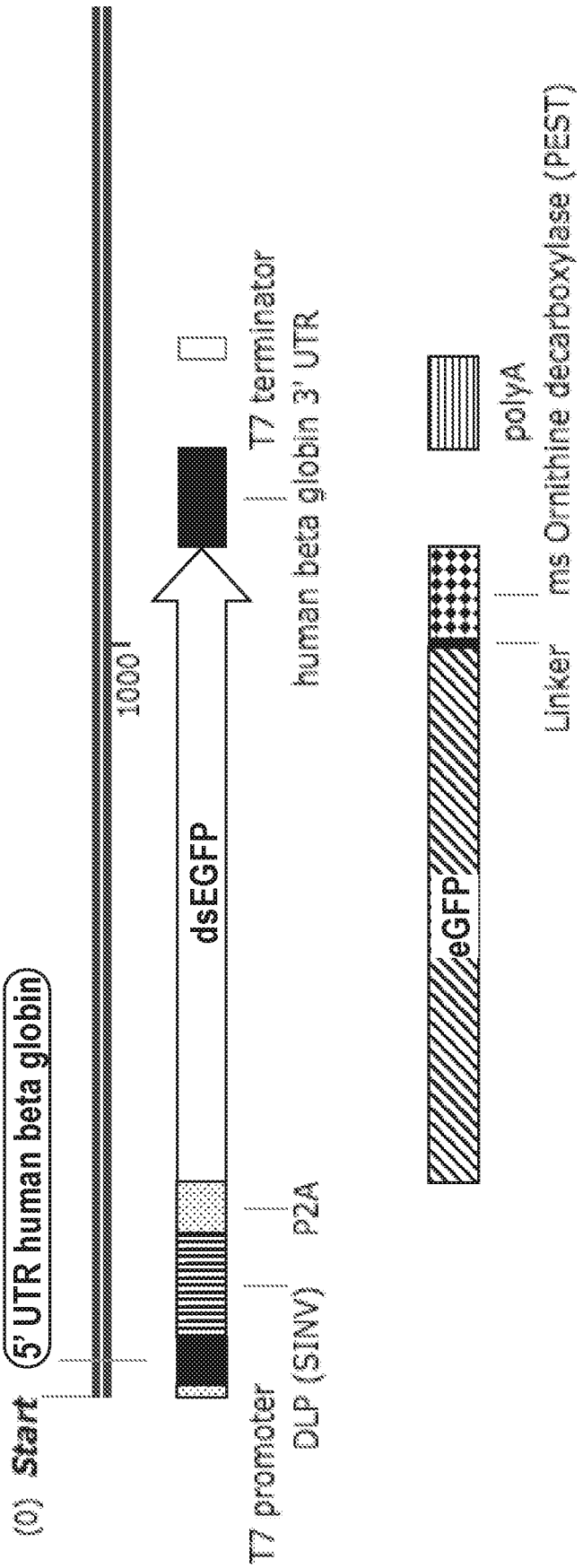

(0) Start
5' UTR human beta globin
1000
T7 promoter
DLP (SINV)
dsEGFP
P2A
T7 terminator
human beta globin 3' UTR
Linker
eGFP
polyA
ms Ornithine decarboxylase (PEST)

FIG. 15

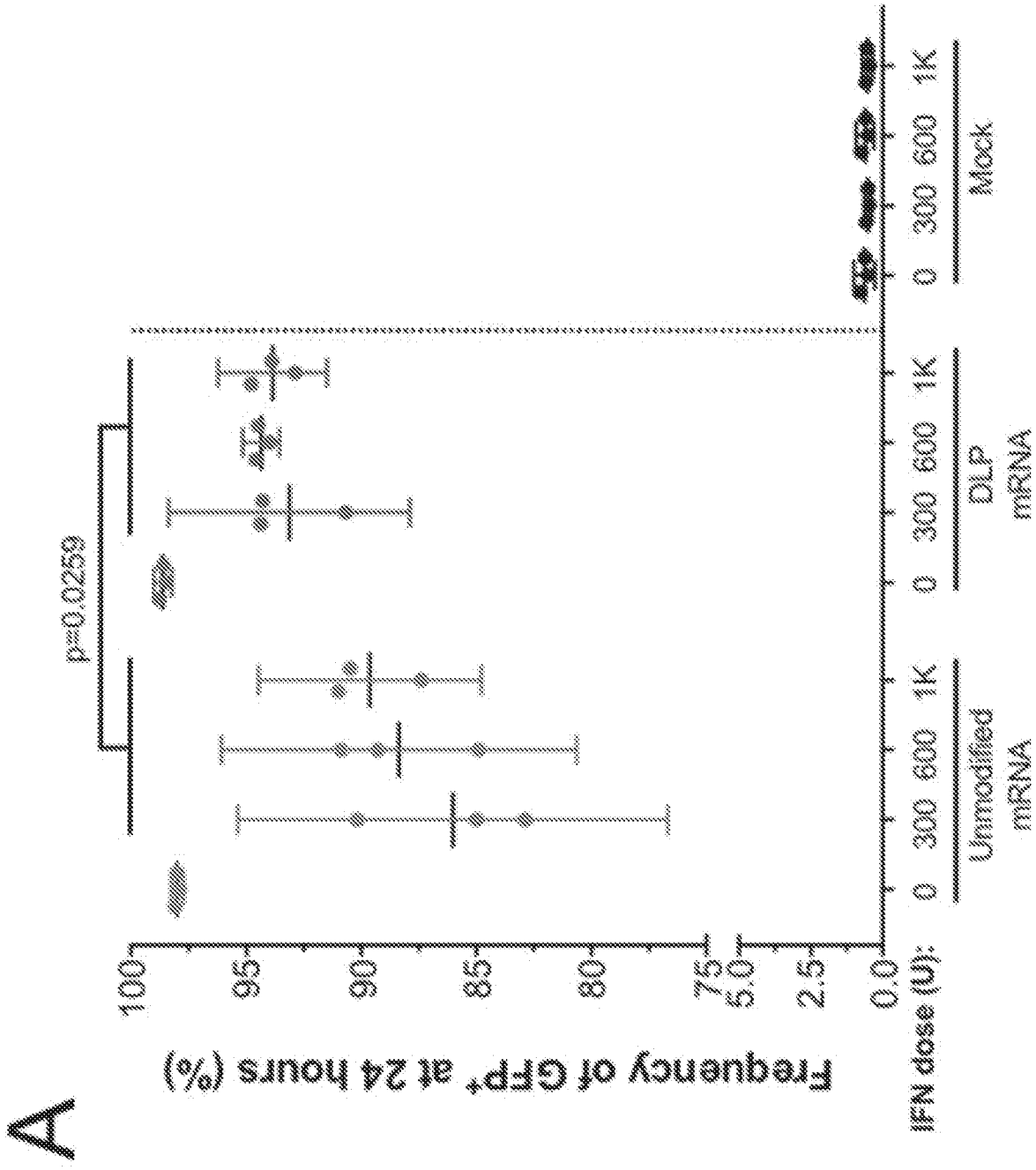

A
Frequency of GFP+ at 24 hours (%)
100
95
90
85
80
75
50
25
0.0
p=0.0259
IFN dose (U):
0
300
600
1K
0
300
600
1K
0
300
600
1K
Unmodified mRNA
DLP mRNA
Mock

FIG. 16A

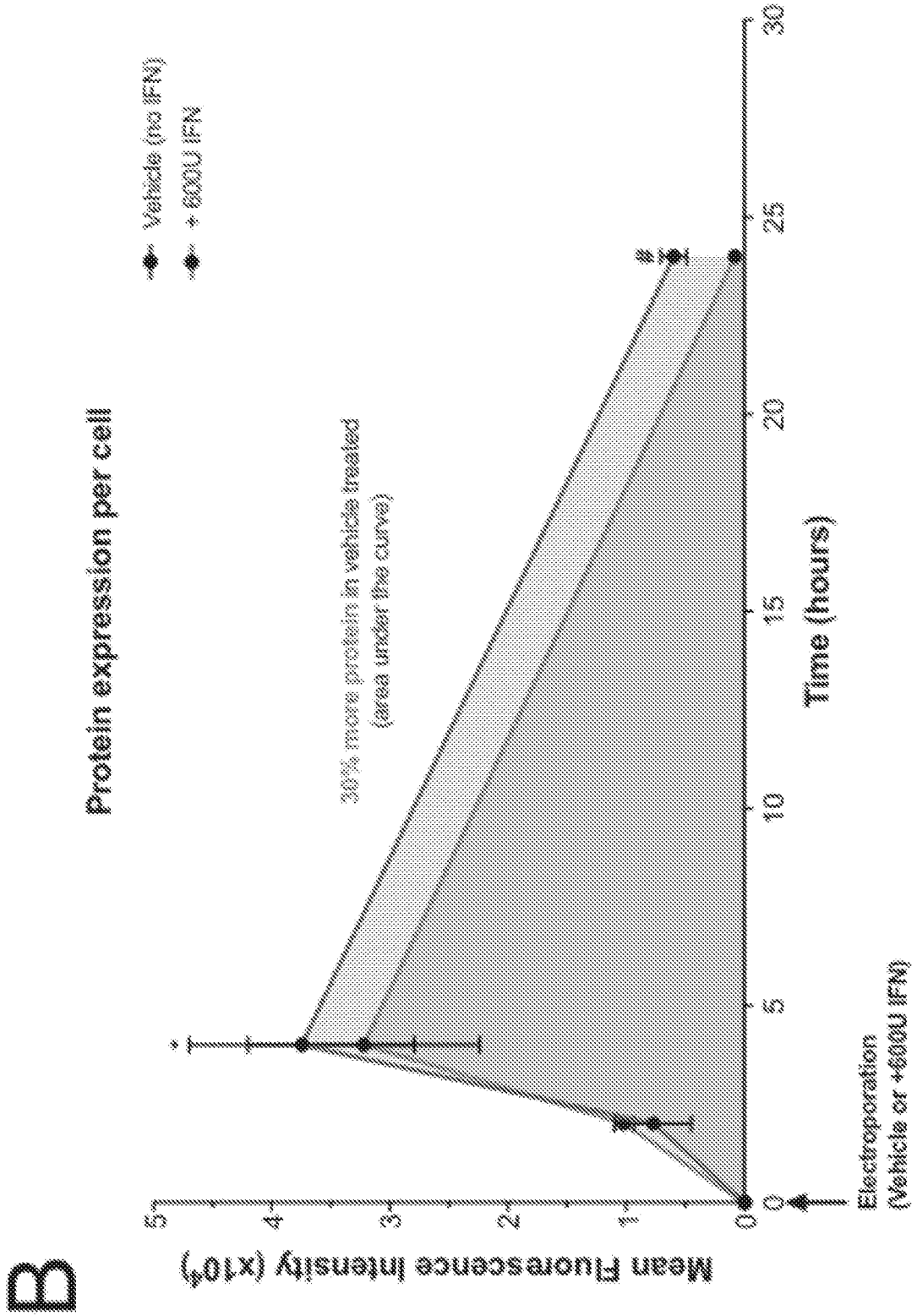

B
Protein expression per cell
Vehicle (no IFN)
+ 600U IFN
30% more protein in vehicle treated
(area under the curve)
Mean Fluorescence Intensity (x10⁴)
Time (hours)
Electroporation
(Vehicle or +600U IFN)
0
1
2
3
4
5
0
5
10
15
20
25
30
*
#

FIG. 16B

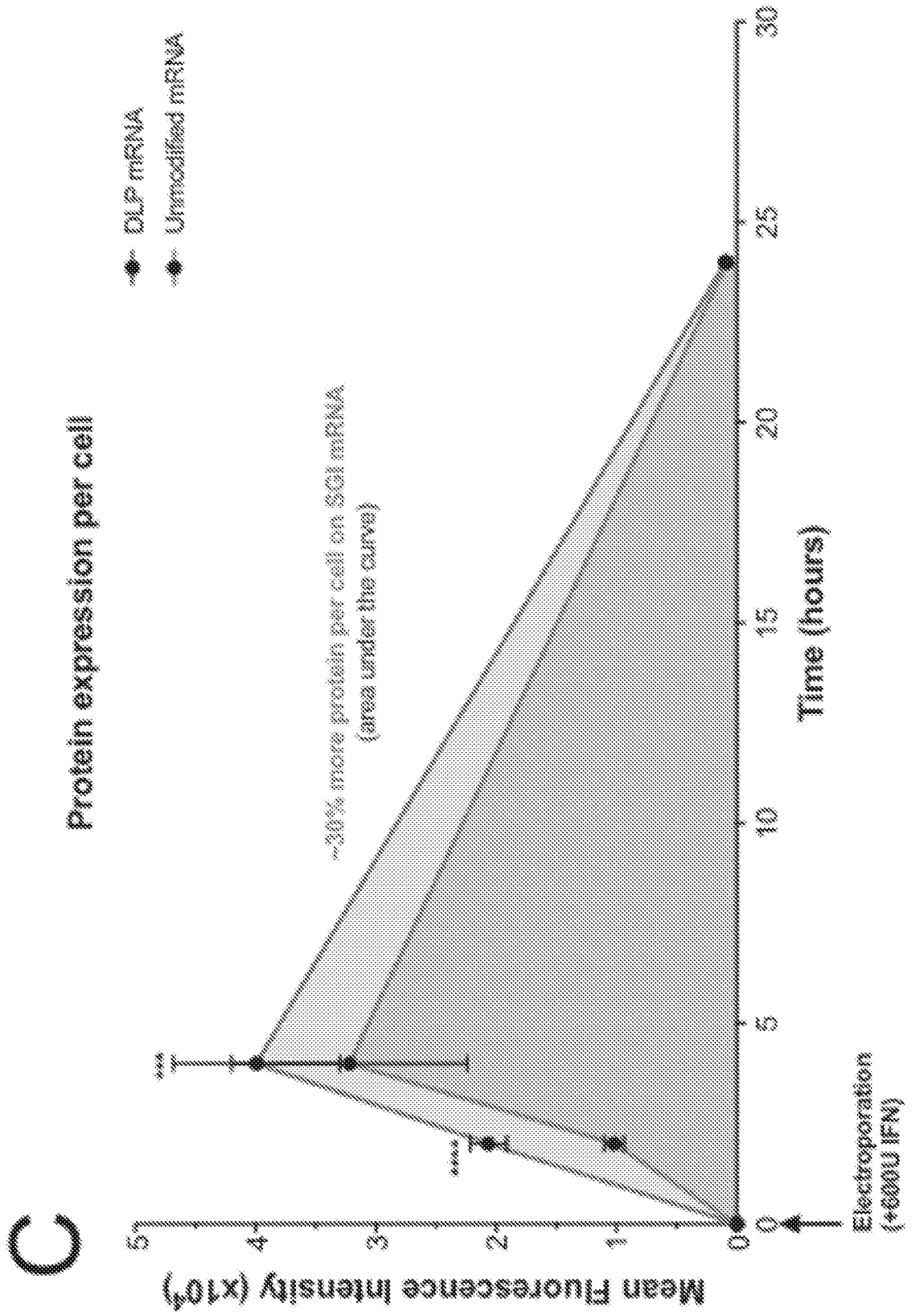

C
Protein expression per cell
DLP mRNA
Unmodified mRNA
~30% more protein per cell on SGI mRNA
(area under the curve)
***
***
Mean Fluorescence Intensity (x10⁶)
Time (hours)
Electroporation
(+4000U IFN)
0
1
2
3
4
5
0
5
10
15
20
25
30

FIG. 16C

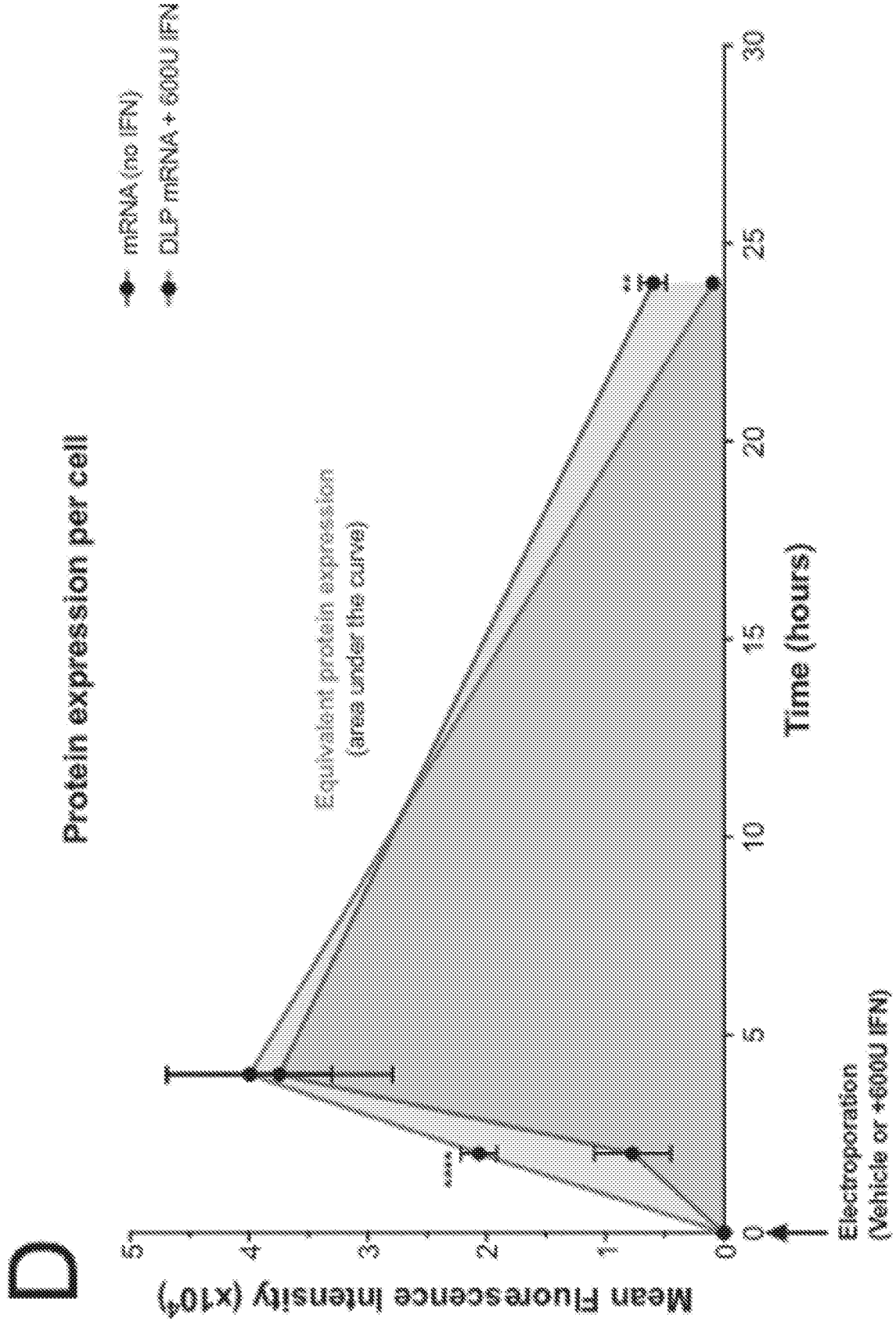

D
Protein expression per cell
mRNA (no IFN)
DLP mRNA + 600U IFN
Equivalent protein expression
(area under the curve)
Mean Fluorescence Intensity (x10⁶)
Time (hours)
Electroporation
(Vehicle or +600U IFN)
0
1
2
3
4
5
0
5
10
15
20
25
30

FIG. 16D

SEQUENCE LISTING

<110> SYNTHETIC GENOMICS INC

<120> COMPOSITIONS AND METHODS FOR ENHANCING GENE EXPRESSION

<130> SGI.012WO

<150> 62/430,250
<151> 2016-12-05

<150> 62/486,361
<151> 2017-04-17

<150> 62/587,954
<151> 2017-11-17

<160> 52

<170> PatentIn version 3.5

<210> 1
<211> 145
<212> DNA
<213> Sindbis virus

<400> 1
atagtcagca tagtacattt catctgacta atactacaac accaccacca tgaatagagg 60

attctttaac atgctcggcc gccgcccctt cccggccccc actgccatgt ggaggccgcg 120

gagaaggagg caggcggccc cgatg 145

<210> 2
<211> 480
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<400> 2
gctcgaagtg tgtatggtgc catatacggc tcaccaccat atacactgca agaattacta 60

ttcttgtggg cccctctcgg taaatcctag agggctttcc tctcgttatt gcgagattcg 120

tcgttagata acggcaagtt ccctttctta ctatcctatt ttcatcttgt ggcttgacgg 180

gtcactgcct acgtcgtcga tctctatcaa ctacccttgc gacttaggca accttctccg 240

ctactggatt tggagggagt tttgttaggg actggtccct ggacttaccc gacgcttgtg 300

agcatagtca gcatagtaca tttcatctga ctaatactac aacaccacca ccatgaatag 360

aggattcttt aacatgctcg gccgccgccc cttcccggcc cccactgcca tgtggaggcc 420

gcggagaagg aggcaggcgg ccccgatgat ggcaaccttc tccgctactg gatttggagg 480

<210> 3
<211> 66

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> 2A proteolytic cleavage site

<400> 3
ggaagcggag ctactaactt cagcctgctg aagcaggctg gagacgtgga ggagaaccct 60

ggacct 66

<210> 4
<211> 1749
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> g-block for construction of rEx-DLP-rFF

<400> 4
cagaggcgca ggacttgatc catggcccac ctacagcatg ccacctgggc caagaaattg 60

acctttggtc caatgagggc ctcgaatatt acaaggaagt caacctgctg tacacacacg 120

tccccatcaa ggatggtgta atacacagtt accctaattg tggccctgcc tgtggctggg 180

aaaagcaatc caacaaaatt tcgtgcctcc cgagagtggc acaaaatttg ggctaccact 240

attccccaga cttaccagga ttttgcccca taccaaaaga actcgctgag cattggcccg 300

tagtgtccaa tgatagatac ccgaattgct tgcaaattac cttacagcaa gtatgtgaac 360

tcagtaaacc gtgctcagcg ggctatatgg ttggacaatc ggttttcgtg cagacgcctg 420

gtgtgacatc ttactggctt actgaatggg tcgacggcaa agcgcgtgct ctaccagatt 480

ccttattctc gtccggtagg ttcgagacta acagccgcgc tttcctcgat gaagccgagg 540

aaaagtttgc cgccgctcac cctcatgcct gtttgggaga aattaataag tccaccgtgg 600

gaggatccca cttcatcttt tcccaatatt taccaccatt gctacccgca gacgctgttg 660

ccctggtagg tgcttcattg gctgggaaag ctgctaaagc tgcttgcagc gttgttgatg 720

tctatgctcc atcatttgaa ccttatctac accctgagac actgagtcgc gtgtacaaga 780

ttatgatcga tttcaagccg tgtaggctta tggtgtggag aaacgcgacc ttttatgtcc 840

aagagggtgt tgatgcagtt acatcagcac tagcagctgt gtccaaactc atcaaagtgc 900

cggccaatga gcctgtttca ttccatgtgg catcagggta cagaaccaac gcgctggtag 960

cgccccaggc taaaatttca attggagcct acgccgccga gtgggcactg tcaactgaac 1020

cgccacctgc tggttatgcg atcgtgcggc gatatattgt aaagaggctc ctcagctcaa 1080

cagaagtgtt cttgtgccgc aggggtgttg tgtcttccac ctcagtgcag accatttgtg 1140

cactagaggg atgtaaacct ctgttcaact tcttacaaat tggttcagtc attgggcccg 1200

tgtgactcta gagtggacct gttcccatcc cccgctcaac tactcaggta gtggttcgcg 1260

gcaacgggta caccgcagtt ggtaacaagc ttgtcgatag tcagcatagt acatttcatc 1320

tgactaatac tacaacacca ccaccatgaa tagaggattc tttaacatgc tcggccgccg 1380

ccccttcccg gcccccactg ccatgtggag gccgcggaga aggaggcagg cggccccgat 1440

gatggaaaat atggaaaacg acgagaacat cgtggtgggc cccaagccct tctaccccat 1500

cgaggaaggc agcgccggca cccagctgcg gaagtacatg gaaagatacg ccaagctggg 1560

cgccattgcc ttcaccaacg ccgtgaccgg cgtggactac agctacgccg agtacctgga 1620

aaagagctgc tgcctgggca aggctctgca gaactacggc ctggtggtgg acggccggat 1680

cgccctgtgc agcgagaact gcgaggaatt cttcatcccc gtgatcgccg gcctgttcat 1740

cggcgtggg 1749

<210> 5
<211> 67
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> primer pp1a-DLP-F

<220>
<221> misc_feature
<223> RP114

<400> 5
gccatgtgga ggccgcggag aaggaggcag gcggccccga tgatggcaac cttctccgct 60

actggat 67

<210> 6
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> primer pBR322-3'SrfI-R

<220>
<221> misc_feature
<223> RP115

<400> 6
acaatgttgc ctcccacatc tgcaa 25

<210> 7
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> primer pBR322-3'SrfI-F

<220>
<221> misc_feature
<223> RP116

<400> 7
gggtcacaag gtagtcgccg tggtt 25

<210> 8
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> primer pBR322-bla-R

<220>
<221> misc_feature
<223> RP117

<400> 8
cgtcaggtgg cacttttcgg ggaa 24

<210> 9
<211> 70
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> primer pp1a-DLP-2A-F

<400> 9
agcctgctga agcaggctgg agacgtggag gagaaccctg gacctatggc aaccttctcc 60

gctactggat 70

<210> 10
<211> 589

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> g-block for rEx-DLP-pp1ab-rFF

<400> 10
aaacaaatag gggttccgcg cacatttccc cgaaaagtgc cacctgacgt ctaagaaacc 60

attattatca tgacattaag catccgcctt tcgttttatt tgaccatgtt ggtatgtaat 120

acgactcact atagctcgaa gtgtgtatgg tgccatatac ggctcaccac catatacact 180

gcaagaatta ctattcttgt gggcccctct cggtaaatcc tagagggctt tcctctcgtt 240

attgcgagat tcgtcgttag ataacggcaa gttccctttc ttactatcct attttcatct 300

tgtggcttga cgggtcactg cctacgtcgt cgatctctat caactaccct tgcgacttag 360

gcaaccttct ccgctactgg atttggaggg agttttgtta gggactggtc cctggactta 420

cccgacgctt gtgagcatag tcagcatagt acatttcatc tgactaatac tacaacacca 480

ccaccatgaa tagaggattc tttaacatgc tcggccgccg ccccttcccg gcccccactg 540

ccatgtggag gccgcggaga aggaggcagg cggccccgat gatggcaac 589

<210> 11
<211> 655
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> g-block for rEx-DLP-2A-pp1ab-rFF

<400> 11
aaacaaatag gggttccgcg cacatttccc cgaaaagtgc cacctgacgt ctaagaaacc 60

attattatca tgacattaag catccgcctt tcgttttatt tgaccatgtt ggtatgtaat 120

acgactcact atagctcgaa gtgtgtatgg tgccatatac ggctcaccac catatacact 180

gcaagaatta ctattcttgt gggcccctct cggtaaatcc tagagggctt tcctctcgtt 240

attgcgagat tcgtcgttag ataacggcaa gttccctttc ttactatcct attttcatct 300

tgtggcttga cgggtcactg cctacgtcgt cgatctctat caactaccct tgcgacttag 360

gcaaccttct ccgctactgg atttggaggg agttttgtta gggactggtc cctggactta 420

cccgacgctt gtgagcatag tcagcatagt acatttcatc tgactaatac tacaacacca 480

ccaccatgaa tagaggattc tttaacatgc tcggccgccg ccccttcccg gcccccactg 540

ccatgtggag gccgcggaga aggaggcagg cggccccgat gggaagcgga gctactaact 600

tcagcctgct gaagcaggct ggagacgtgg aggagaaccc tggacctatg gcaac 655

<210> 12
<211> 655
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> g-block for rEx-DLP-2A-pp1ab-DLP-rFF

<400> 12
aaacaaatag gggttccgcg cacatttccc cgaaaagtgc cacctgacgt ctaagaaacc 60

attattatca tgacattaag catccgcctt tcgttttatt tgaccatgtt ggtatgtaat 120

acgactcact atagctcgaa gtgtgtatgg tgccatatac ggctcaccac catatacact 180

gcaagaatta ctattcttgt gggcccctct cggtaaatcc tagagggctt tcctctcgtt 240

attgcgagat tcgtcgttag ataacggcaa gttccctttc ttactatcct attttcatct 300

tgtggcttga cgggtcactg cctacgtcgt cgatctctat caactaccct tgcgacttag 360

gcaaccttct ccgctactgg atttggaggg agttttgtta gggactggtc cctggactta 420

cccgacgctt gtgagcatag tcagcatagt acatttcatc tgactaatac tacaacacca 480

ccaccatgaa tagaggattc tttaacatgc tcggccgccg ccccttcccg gcccccactg 540

ccatgtggag gccgcggaga aggaggcagg cggccccgat gggaagcgga gctactaact 600

tcagcctgct gaagcaggct ggagacgtgg aggagaaccc tggacctatg gcaac 655

<210> 13
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> primer DLP-pp1ab-screen-F

<220>
<221> misc_feature
<223> RP126

<400> 13
cagcatcttt tactttcacc agcgtttctg 30

<210> 14
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> primer DLP-pp1ab-screen-R

<220>
<221> misc_feature
<223> RP127

<400> 14
ggaactggcg aagccagttt taaca 25

<210> 15
<211> 12529
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> Construct rEx-DLP-rFF

<220>
<221> misc_feature
<222> (1)...(18)
<223> T7 promoter

<400> 15
taatacgact cactatagct cgaagtgtgt atggtgccat atacggctca ccaccatata 60

cactgcaaga attactattc ttgtgggccc ctctcggtaa atcctagagg gctttcctct 120

cgttattgcg agattcgtcg ttagataacg gcaagttccc tttcttacta tcctattttc 180

atcttgtggc ttgacgggtc actgccatcg tcgtcgatct ctatcaacta cccttgcgac 240

tatggcaacc ttctccgcta ctggatttgg agggagtttt gttagggact ggtccctgga 300

cttacccgac gcttgtgagc atggcgcggg attgtgctgc gaagtggacg gctccacctt 360

atgcgccgag tgttttcgcg gttgcgaagg aatggagcaa tgtcctggct tgttcatggg 420

actgttaaaa ctggcttcgc cagttccagt gggacataag ttcctgattg gttggtatcg 480

agctgccaaa gtcaccgggc gttacaattt ccttgagctg ttgcaacacc ctgctttcgc 540

ccagctgcgt gtggttgatg ctaggttagc cattgaagag gcaagtgtgt ttatttccac 600

tgaccacgcg tctgctaagc gtttccctgg cgctagattt gcgctgacac cggtgtatgc 660

taacgcttgg gttgtgagcc cggctgctaa cagtttgata gtgaccactg accaggaaca 720

agatgggttc tgctggttaa aacttttgcc acctgaccgc cgtgaggctg gtttgcggtt 780

gtattacaac cattaccgcg aacaaaggac cgggtggctg tctaaaacag gacttcgctt 840

atggcttgga gacctgggtt tgggcatcaa tgcgagctct ggagggctga aattccacat 900

tatgaggggt tcgcctcagc gagcttggca tatcacaaca cgcagctgca agctgaagag 960

ctactacgtt tgtgacatct ctgaagcaga ctggtcctgt ttgcctgctg gcaactacgg 1020

cggctacaat ccaccagggg acggagcttg cggttacagg tgcttggcct tcatgaatgg 1080

cgccactgtt gtgtcggctg gttgcagttc tgacttgtgg tgtgatgatg agttggctta 1140

tcgagtcttt caattgtcac ccacgttcac ggttaccatc ccaggtgggc gagtttgtcc 1200

gaatgccaag tacgcaatga tttgtgacaa gcagcactgg cgcgtcaaac gtgcaaaggg 1260

cgtcggcctg tgtctcgatg aaagctgttt caggggcatc tgcaattgcc aacgcatgag 1320

tggaccacca cctgcacccg tgtcagccgc cgtgttagat cacatactgg aggcggcgac 1380

gtttggcaac gttcgcgtgg ttacacctga agggcagcca cgccccgtac cagcgccgcg 1440

agttcgtccc agcgccaact cttctggaga tgtcaaagat ccggcgcccg ttccgccagt 1500

accaaaacca aggaccaagc ttgccacacc gaacccaact caggcgccca tcccagcacc 1560

gcgcacgcga cttcaagggg cctcaacaca ggagccactg gcgagtgcag gagttgcttc 1620

tgactcggca cctaaatggc gtgtggccaa aactgtgtac agctccgcgg agcgctttcg 1680

gaccgaactg gtacaacgtg ctcggtccgt tggggacgtt cttgttcaag cgctaccgct 1740

caaaacccca gcagtgcagc ggtataccat gactctgaag atgatgcgtt cacgcttcag 1800

ttggcactgc gacgtgtggt acccttggc tgtaatcgct tgtttgctcc ctatatggcc 1860

atctcttgct ttgctcctta gctttgccat tgggttgata cccagtgtgg gcaataatgt 1920

tgttctgaca gcgcttctgg tttcatcagc taattatgtt gcgtcaatgg accatcaatg 1980

tgaaggtgcg gcttgcttag ccttgctgga agaagaacac tattatagag cggtccgttg 2040

gcgcccgatt acaggcgcgc tgtcgcttgt gctcaattta ctggggcagg taggctatgt 2100

agctcgttcc acctttgatg cagcttatgt tccttgcact gtgttcgatc tttgcagctt 2160

tgctattctg tacctctgcc gcaatcgttg ctggagatgc ttcggacgct gtgtgcgagt 2220

tgggcctgcc acgcatgttt tgggctccac cgggcaacga gtttccaaac tggcgctcat 2280

tgatttgtgt gaccactttt caaagcccac catcgatgtt gtgggcatgg caactggttg 2340

gagcggatgt tacacaggaa ccgccgcaat ggagcgtcag tgtgcctcta cggtggaccc 2400

tcactcgttc gaccagaaga aggcaggagc gactgtttac ctcacccccc ctgtcaacag 2460

cgggtcagcg ctgcagtgcc tcaatgtcat gtggaagcga ccaattgggt ccactgtcct 2520

tggggaacaa acaggagctg ttgtgacggc ggtcaagagt atctctttct cacctccctg 2580

ctgcgtctct accactttgc ccacccgacc cggtgtgacc gttgtcgacc atgctcttta 2640

caaccggttg actgcttcag gggtcgatcc cgctttattg cgtgttgggc aaggtgattt 2700

tctaaaactt aatccggggt tccggctgat aggtggatgg atttatggga tatgctattt 2760

tgtgttggtg gttgtgtcaa cttttacctg cttacctatc aaatgtggca ttggcacccg 2820

cgacccttтc tgccgcagag tgttttctgt acccgtcacc aagacccaag agcactgcca 2880

tgctggaatg tgtgctagcg ctgaaggcat ctctctggac tctctggggt taactcagtt 2940

acaaagttac tggatcgcag ccgtcactag cggattagtg atcttgttgg tctgccaccg 3000

cctggccatc agcgccttgg acttgttgac tctagcttcc cctttagtgt tgcttgtgtt 3060

cccttgggca tctgtggggc ttttacttgc ttgcagtctc gctggtgctg ctgtgaaaat 3120

acagttgttg gcgacgcttt ttgtgaatct attctttccc caagctaccc ttgtcactat 3180

gggatactgg gcgtgcgtgg cggctttggc cgtttacagt ttgatgggct tgcgagtgaa 3240

agtgaatgtg cccatgtgtg tgacacctgc ccattttctg ctgctggcga ggtcagctgg 3300

acagtcaaga gagcagatgc tccgggtcag cgctgctgcc cccaccaatt cactgcttgg 3360

agtggctcgt gattgttatg tcacaggcac aactcggctg tacataccca aggaaggcgg 3420

gatggtgttt gaagggctat tcaggtcacc gaaggcgcgc ggcaacgtcg gcttcgtggc 3480

tggtagcagc tacggcacag ggtcagtgtg gaccaggaac aacgaggtcg tcgtactgac 3540

agcgtcacac gtggttggcc gcgctaacat ggccactctg aagatcggtg acgcaatgct 3600

gactctgact ttcaaaaaga atggcgactt cgccgaggca gtgacgacac agtccgagct 3660

cccaggcaat tggccacagt tgcatttcgc ccaaccaaca accgggcccg cttcatggtg 3720

cactgccaca ggagatgaag aaggcttgct cagtggcgag gtttgtctgg cgtggactac 3780

tagtggcgac tctggatctg cagtggttca gggtgacgct gtggtagggg tccacaccgg 3840

ttcgaacaca agtggtgttg cctacgtgac caccccaagc ggaaaactcc ttggcgccga 3900

caccgtgact ttgtcatcac tgtcaaagca tttcacaggc cctttgacat caatcccgaa 3960

ggacatccct gacaacatta ttgccgatgt tgatgctgtt cctcgttctc tggccatgct 4020

gattgatggc ttatccaata gagagagcag cctttctgga cctcagttgt tgttaattgc 4080

ttgttttatg tggtcttatc ttaaccaacc tgcttacttg ccttatgtgc tgggcttctt 4140

tgccgctaac ttcttcctgc caaaaagtgt tggccgccct gtggtcactg ggcttctatg 4200

gttgtgctgc ctcttcacac cgctttccat gcgcttgtgc ttgttccatc tggtctgtgc 4260

taccgtcacg ggaaacgtga tatctttgtg gttctacatc actgccgctg gcacgtctta 4320

cctttctgag atgtggttcg gaggctatcc caccatgttg tttgtgccac ggttcctagt 4380

gtaccagttc cccggctggg ctattggcac agtactagcg gtatgcagca tcaccatgct 4440

ggctgctgcc ctcggtcaca ccctgttact ggatgtgttc tccgcctcag gtcgctttga 4500

caggactttc atgatgaaat acttcctgga gggaggagtg aaagagagtg tcaccgcctc 4560

agtcacccgc gcttatggca aaccaattac ccaggagagt ctcactgcaa cattagctgc 4620

cctcactgat gatgacttcc aattcctctc tgatgtgctt gactgtcggg ccgtccgatc 4680

ggcaatgaat ctgcgtgccg ctctcacaag ttttcaagtg gcgcagtatc gtaacatcct 4740

taatgcatcc ttgcaagtcg atcgtgacgc tgctcgtagt cgcagactaa tggcaaaact 4800

ggctgatttt gcggttgaac aagaagtaac agctggagac cgtgttgtgg ttatcgacgg 4860

tctggaccgc atggctcact tcaaagacga tttggtgctg gttcctttga ccaccaaagt 4920

agtaggcggt tctaggtgca ccatttgtga cgtcgttaag gaagaagcca atgacacccc 4980

agttaagcca atgcccagca ggagacgccg caagggcctg cctaaaggtg ctcagttgga 5040

gtgggaccgt caccaggaag agaagaggaa cgccggtgat gatgattttg cggtctcgaa 5100

tgattatgtc aagagagtgc caaagtactg ggatcccagc gacacccgag gcacgacagt 5160

gaaaatcgcc ggcactacct atcagaaagt ggttgactat tcaggcaatg tgcattacgt 5220

ggagcatcag gaagatctgc tagactacgt gctgggcaag gggagctatg aaggcctaga 5280

tcaggacaaa gtgttggacc tcacaaacat gcttaaagtg gaccccacgg agctctcctc 5340

caaagacaaa gccaaggcgc gtcagcttgc tcatctgctg ttggatctgg ctaacccagt 5400

tgaggcagtg aatcagttaa actgagagcg ccccacatct ttcccggcga tgtggggcgt 5460

cggacctttg ctgactctaa agacaagggt ttcgtggctc tacacagtcg cacaatgttt 5520

ttagctgccc gggacttttt atttaacatc aaatttgtgt gcgacgaaga gttcacaaag 5580

accccaaaag acacactgct tgggtacgta cgcgcctgcc ctggttactg gtttattttc 5640

cgtcgtacgc accggtcgct gattgatgca tactgggaca gtatggagtg cgtttacgcg 5700

cttcccacca tatctgattt tgatgtgagc ccaggtgacg tcgcagtgac gggcgagcga 5760

tgggattttg aatctcccgg aggaggccgt gcaaaacgtc tcacagctga tctggtgcac 5820

gcttttcaag ggttccacgg agcctcttat tcctatgatg acaaggtggc agctgctgtc 5880

agtggtgacc cgtatcggtc ggacggcgtc ttgtataaca cccgttgggg caacattcca 5940

tattctgtcc caaccaatgc tttggaagcc acagcttgct accgtgctgg atgtgaggcc 6000

gttaccgacg ggaccaacgt catcgcaaca attgggccct tcccggagca acaacccata 6060

ccggacatcc caaagagcgt gcttgacaac tgcgctgaca tcagctgtga cgctttcata 6120

gcgcccgctg cagagacagc cctgtgtgga gatttagaga aatacaacct atccacgcag 6180

ggttttgtgt tgcctagtgt tttctccatg gtgcgggcgt acttaaaaga ggagattgga 6240

gacgctccac cactctactt gccatctact gtaccatcta aaaattcaca agccggaatt 6300

aacggcgctg agtttcctac aaagtcttta cagagctact gtttgattga tgacatggtg 6360

tcacagtcca tgaaaagcaa tctacaaacc gccaccatgg cgacttgtaa acggcaatac 6420

tgttccaaat acaagattag gagcattctg ggcaccaaca attacattgg cctaggtttg 6480

cgtgcctgcc tttcgggggt tacggccgca ttccaaaaag ctggaaagga tgggtcaccg 6540

atttatttgg gcaagtcaaa attcgacccg ataccagctc ctgacaagta ctgccttgaa 6600

acagacctgg agagttgtga tcgctccacc ccggctttgg tgcgttggtt cgctactaat 6660

cttatttttg agctagctgg ccagcccgag ttggtgcaca gctacgtgtt gaattgctgt 6720

cacgatctag ttgtggcggg tagtgtagca ttcaccaaac gcgggggttt gtcatctgga 6780

gaccctatca cttccatttc caataccatc tattcattgg tgctgtacac ccagcacatg 6840

ttgctatgtg gacttgaagg ctatttccca gagattgcag aaaaatatct tgatggcagc 6900

ctggagctgc gggacatgtt caagtacgtt cgagtgtaca tctactcgga cgatgtggtt 6960

ctaaccacac ccaaccagca ttacgcggcc agctttgacc gctgggtccc ccacctgcag 7020

gcgctgctag gtttcaaggt tgacccaaag aaaactgtga acaccagctc cccttccttt 7080

ttgggctgcc ggttcaagca agtggacggc aagtgttatc tagccagtct tcaggaccgc 7140

gttacacgct ctctgttata ccacattggt gcaaagaatc cctcagagta ctatgaagct 7200

gctgtttcca tctttaagga ctccattatc tgctgtgatg aagactggtg gacggacctc 7260

catcgacgta tcagtggcgc tgcgcgtacc gacggagttg agttccccac cattgaaatg 7320

ttaacatcct tccgcaccaa gcagtatgag agtgccgtgt gcacagtttg tggggccgcc 7380

cccgtggcca agtctgcttg tggagggtgg ttctgtggca attgtgtccc gtaccacgcg 7440

ggtcattgtc acacaacctc gctcttcgcc aactgcgggc acgacatcat gtaccgctcc 7500

acttactgca caatgtgtga gggttcccca aaacagatgg taccaaaagt gcctcacccg 7560

atcctggatc atttgctgtg ccacattgat tacggcagta aagaggaact aactctggta 7620

gtggcggatg gtcgaacaac atcaccgccc gggcgctaca aagtgggtca caaggtagtc 7680

gccgtggttg cagatgtggg aggcaacatt gtgtttgggt gcggtcctgg atcacacatc 7740

gcagtaccac ttcaggatac gctcaagggc gtggtggtga ataaagctct gaagaacgcc 7800

gccgcctctg agtacgtgga aggacccccc gggagtggga agacttttca cctggtcaaa 7860

gatgtgctag ccgtggtcgg tagcgcgacc ttggttgtgc ccacccacgc gtccatgctg 7920

gactgcatca acaagctcaa acaagcgggc gccgatccat actttgtggt gcccaagtat 7980

acagttcttg actttccccg gcctggcagt ggaaacatca cagtgcgact gccacaggtc 8040

ggaaccagtg agggagaaac ctttgtggat gaggtggcct acttctcacc agtggatctg 8100

gcgcgcattt taacccaggg tcgagtcaag ggttacggtg atttaaatca gctcgggtgc 8160

gtcggacccg cgagcgtgcc acgtaacctt tggctccgac attttgtcag cctggagccc 8220

ttgcgagtgt gccatcgatt cggcgctgct gtgtgtgatt tgatcaaggg catttatcct 8280

tattatgagc cagctccaca taccactaaa gtggtgtttg tgccaaatcc agactttgag 8340

aaaggtgtag tcatcaccgc ctaccacaaa gatcgcggtc ttggtcaccg cacaattgat 8400

tcaattcaag gctgtacatt ccctgttgtg actcttcgac tgcccacacc ccaatcactg 8460

acgcgcccgc gcgcagttgt ggcggttact agggcgtctc aggaattata catctacgac 8520

ccctttgatc agcttagcgg gttgttgaag ttcaccaagg aagcagaggc gcaggacttg 8580

atccatggcc cacctacagc atgccacctg ggccaagaaa ttgacctttg gtccaatgag 8640

ggcctcgaat attacaagga agtcaacctg ctgtacacac acgtccccat caaggatggt 8700

gtaatacaca gttaccctaa ttgtggccct gcctgtggct gggaaaagca atccaacaaa 8760

atttcgtgcc tcccgagagt ggcacaaaat ttgggctacc actattcccc agacttacca 8820

ggattttgcc ccataccaaa agaactcgct gagcattggc ccgtagtgtc caatgataga 8880

tacccgaatt gcttgcaaat taccttacag caagtatgtg aactcagtaa accgtgctca 8940

gcgggctata tggttggaca atcggttttc gtgcagacgc ctggtgtgac atcttactgg 9000

cttactgaat gggtcgacgg caaagcgcgt gctctaccag attccttatt ctcgtccggt 9060

aggttcgaga ctaacagccg cgctttcctc gatgaagccg aggaaaagtt tgccgccgct 9120

caccctcatg cctgtttggg agaaattaat aagtccaccg tgggaggatc ccacttcatc 9180

ttttcccaat atttaccacc attgctaccc gcagacgctg ttgccctggt aggtgcttca 9240

ttggctggga aagctgctaa agctgcttgc agcgttgttg atgtctatgc tccatcattt 9300

gaaccttatc tacaccctga gacactgagt cgcgtgtaca agattatgat cgatttcaag 9360

ccgtgtaggc ttatggtgtg gagaaacgcg acctttttatg tccaagaggg tgttgatgca 9420

gttacatcag cactagcagc tgtgtccaaa ctcatcaaag tgccggccaa tgagcctgtt 9480

tcattccatg tggcatcagg gtacagaacc aacgcgctgg tagcgcccca ggctaaaatt 9540

tcaattggag cctacgccgc cgagtgggca ctgtcaactg aaccgccacc tgctggttat 9600

gcgatcgtgc ggcgatatat tgtaaagagg ctcctcagct caacagaagt gttcttgtgc 9660

cgcaggggtg ttgtgtcttc cacctcagtg cagaccattt gtgcactaga gggatgtaaa 9720

cctctgttca acttcttaca aattggttca gtcattgggc ccgtgtgact ctagagtgga 9780

cctgttccca tcccccgctc aactactcag gtagtggttc gcggcaacgg gtacaccgca 9840

gttggtaaca agcttgtcga tagtcagcat agtacatttc atctgactaa tactacaaca 9900

ccaccaccat gaatagagga ttctttaaca tgctcggccg ccgccccttc ccggccccca 9960

ctgccatgtg gaggccgcgg agaaggaggc aggcggcccc gatgatggaa aatatggaaa 10020

acgacgagaa catcgtggtg ggccccaagc ccttctaccc catcgaggaa ggcagcgccg 10080

gcacccagct gcggaagtac atggaaagat acgccaagct gggcgccatt gccttcacca 10140

acgccgtgac cggcgtggac tacagctacg ccgagtacct ggaaaagagc tgctgcctgg 10200

gcaaggctct gcagaactac ggcctggtgg tggacggccg gatcgccctg tgcagcgaga 10260

actgcgagga attcttcatc cccgtgatcg ccggcctgtt catcggcgtg ggcgtggctc 10320

ccaccaacga gatctacacc ctgcgggagc tggtgcacag cctgggcatc agcaagccca 10380

ccatcgtgtt cagcagcaag aagggcctgg acaaagtcat caccgtgcag aaaaccgtga 10440

ccaccatcaa gaccatcgtg atcctggaca gcaaggtgga ctaccggggc taccagtgcc 10500

tggacacctt catcaagcgg aacacccccc ctggcttcca ggccagcagc ttcaagaccg 10560

tggaggtgga ccggaaagaa caggtggccc tgatcatgaa cagcagcggc agcaccggcc 10620

tgcccaaggg cgtgcagctg acccacgaga acaccgtgac ccggttcagc cacgccaggg 10680

accccatcta cggcaaccag gtgtcccccg gcaccgccgt gctgaccgtg gtgcccttcc 10740

accacggctt cggcatgttc accaccctgg gctacctgat ctgcggcttc cgggtggtga 10800

tgctgaccaa gttcgacgag gaaaccttcc tgaaaaccct gcaggactac aagtgcacct 10860

acgtgattct ggtgcccacc ctgttcgcca tcctgaacaa gagcgagctg ctgaacaagt 10920

acgacctgag caacctggtg gagatcgcca gcggcggagc ccccctgagc aaagaagtgg 10980

gagaggccgt cgccaggcgg ttcaatctgc ccggcgtgcg gcagggctac ggcctgaccg 11040

agacaaccag cgccatcatc atcacccccg agggcgacga caagcctgga gccagcggca 11100

aggtggtgcc cctgttcaag gccaaagtga tcgacctgga caccaagaag agcctgggcc 11160

ccaacagacg gggcgaagtg tgcgtgaagg gccccatgct gatgaagggc tacgtgaaca 11220

accccgaggc caccaaagag ctgatcgacg aagagggctg gctgcacacc ggcgacatcg 11280

gctactacga cgaagagaag cacttcttca tcgtggaccg gctgaagagc ctgatcaagt 11340

acaagggcta tcaggtgccc cctgccgagc tggaaagcgt cctgctgcag caccccagca 11400

tcttcgacgc cggcgtggcc ggggtgccag atcctgtggc cggcgagctg cctggcgccg 11460

tggtggtgct ggaatccggc aagaacatga ccgagaaaga agtgatggac tacgtcgcca 11520

gccaggtgtc caacgccaag cggctgagag gcggcgtgag attcgtggac gaagtgccaa 11580

agggcctgac cggcaagatc gacggcaggg ccatccggga gatcctgaag aaacccgtgg 11640

ccaagatgtg attataactc gagggagcca tagattcatt ttgtggtgac gggattttag 11700

gtgagtatct agattacttt attctgtccg tcccactctt gctgttgctt actaggtatg 11760

tagcatctgg gttagtgtat gttttgactg ccttgttcta ttcctttgta ttagcagctt 11820

atatttggtt tgttatagtt ggaagagcct tttctactgc ttatgctttt gtgcttttgg 11880

ctgcttttct gttattagta atgaggatga ttgtgggtat gatgcctcgt cttcggtcca 11940

ttttcaacca tcgccaactg gtggtagctg attttgtgga cacacctagt ggacctgttc 12000

ccatcccccg cccaaccact caggtagtgg ttcgcggcaa cgggtacacc gcagttggta 12060

acaagcttgt cgatggcgtc aagacgatca cgtccgcagg ccgcctcttt tcgaaacgga 12120

cggcggcgac agcctacaag ctacaatgac ctactgcgca tgtttggtca gatgcgggtc 12180

cgcaaaccgc ccgcgcaacc cactcaggct attattgcag agcctggaga ccttaggcat 12240

gatttaaatc aacaggagcg cgccaccctt tcgtcgaacg tacaacggtt cttcatgatt 12300

gggcatggtt cactcactgc agatgccgga ggactcacgt acaccgtcag ttgggttcct 12360

accaaacaaa tccagcgcaa agttgcgcct ccagcagggc cgtaagacgt ggatattctc 12420

ctgtgtggcg tcatgttgaa gtagttatta gccacccagg aaccaaaaaa aaaaaaaaaa 12480

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaa 12529

<210> 16
<211> 12608
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> Construct rEx-DLP-pp1ab-rFF

<220>
<221> misc_feature
<222> (1)...(18)
<223> T7 promoter

<400> 16
taatacgact cactatagct cgaagtgtgt atggtgccat atacggctca ccaccatata 60

cactgcaaga attactattc ttgtgggccc ctctcggtaa atcctagagg gctttcctct 120

cgttattgcg agattcgtcg ttagataacg gcaagttccc tttcttacta tcctattttc 180

atcttgtggc ttgacgggtc actgcctacg tcgtcgatct ctatcaacta cccttgcgac 240

ttaggcaacc ttctccgcta ctggatttgg agggagtttt gttagggact ggtccctgga 300

cttacccgac gcttgtgagc atagtcagca tagtacattt catctgacta atactacaac 360

accaccacca tgaatagagg attctttaac atgctcggcc gccgcccctt cccggccccc 420

actgccatgt ggaggccgcg gagaaggagg caggcggccc cgatgatggc aaccttctcc 480

gctactggat ttggagggag ttttgttagg gactggtccc tggacttacc cgacgcttgt 540

gagcatggcg cgggattgtg ctgcgaagtg gacggctcca ccttatgcgc cgagtgtttt 600

cgcggttgcg aaggaatgga gcaatgtcct ggcttgttca tgggactgtt aaaactggct 660

tcgccagttc cagtgggaca taagttcctg attggttggt atcgagctgc caaagtcacc 720

gggcgttaca atttccttga gctgttgcaa caccctgctt tcgcccagct gcgtgtggtt 780

gatgctaggt tagccattga agaggcaagt gtgtttattt ccactgacca cgcgtctgct 840

aagcgtttcc ctggcgctag atttgcgctg acaccggtgt atgctaacgc ttgggttgtg 900

agcccggctg ctaacagttt gatagtgacc actgaccagg aacaagatgg gttctgctgg 960

ttaaaacttt tgccacctga ccgccgtgag gctggtttgc ggttgtatta caaccattac 1020

cgcgaacaaa ggaccgggtg gctgtctaaa acaggacttc gcttatggct tggagacctg 1080

ggtttgggca tcaatgcgag ctctggaggg ctgaaattcc acattatgag gggttcgcct 1140

cagcgagctt ggcatatcac aacacgcagc tgcaagctga agagctacta cgtttgtgac 1200

atctctgaag cagactggtc ctgtttgcct gctggcaact acggcggcta caatccacca 1260

ggggacggag cttgcggtta caggtgcttg gccttcatga atggcgccac tgttgtgtcg 1320

gctggttgca gttctgactt gtggtgtgat gatgagttgg cttatcgagt ctttcaattg 1380

tcacccacgt tcacggttac catcccaggt gggcgagttt gtccgaatgc caagtacgca 1440

atgatttgtg acaagcagca ctggcgcgtc aaacgtgcaa agggcgtcgg cctgtgtctc 1500

gatgaaagct gtttcagggg catctgcaat tgccaacgca tgagtggacc accacctgca 1560

cccgtgtcag ccgccgtgtt agatcacata ctggaggcgg cgacgtttgg caacgttcgc 1620

gtggttacac ctgaagggca gccacgcccc gtaccagcgc cgcgagttcg tcccagcgcc 1680

aactcttctg gagatgtcaa agatccggcg cccgttccgc cagtaccaaa accaaggacc 1740

aagcttgcca caccgaaccc aactcaggcg cccatcccag caccgcgcac gcgacttcaa 1800

ggggcctcaa cacaggagcc actggcgagt gcaggagttg cttctgactc ggcacctaaa 1860

tggcgtgtgg ccaaaactgt gtacagctcc gcggagcgct ttcggaccga actggtacaa 1920

cgtgctcggt ccgttgggga cgttcttgtt caagcgctac cgctcaaaac cccagcagtg 1980

cagcggtata ccatgactct gaagatgatg cgttcacgct tcagttggca ctgcgacgtg 2040

tggtaccctt tggctgtaat cgcttgtttg ctccctatat ggccatctct tgctttgctc 2100

cttagctttg ccattgggtt gatacccagt gtgggcaata atgttgttct gacagcgctt 2160

ctggtttcat cagctaatta tgttgcgtca atggaccatc aatgtgaagg tgcggcttgc 2220

ttagccttgc tggaagaaga acactattat agagcggtcc gttggcgccc gattacaggc 2280

gcgctgtcgc ttgtgctcaa tttactgggg caggtaggct atgtagctcg ttccaccttt 2340

gatgcagctt atgttccttg cactgtgttc gatctttgca gctttgctat tctgtacctc 2400

tgccgcaatc gttgctggag atgcttcgga cgctgtgtgc gagttgggcc tgccacgcat 2460

gttttgggct ccaccgggca acgagtttcc aaactggcgc tcattgattt gtgtgaccac 2520

ttttcaaagc ccaccatcga tgttgtgggc atggcaactg gttggagcgg atgttacaca 2580

ggaaccgccg caatggagcg tcagtgtgcc tctacggtgg accctcactc gttcgaccag 2640

aagaaggcag gagcgactgt ttacctcacc ccccctgtca acagcgggtc agcgctgcag 2700

tgcctcaatg tcatgtggaa gcgaccaatt gggtccactg tccttgggga acaaacagga 2760

gctgttgtga cggcggtcaa gagtatctct ttctcacctc cctgctgcgt ctctaccact 2820

ttgcccaccc gacccggtgt gaccgttgtc gaccatgctc tttacaaccg gttgactgct 2880

tcaggggtcg atcccgcttt attgcgtgtt gggcaaggtg attttctaaa acttaatccg 2940

gggttccggc tgataggtgg atggatttat gggatatgct attttgtgtt ggtggttgtg 3000

tcaactttta cctgcttacc tatcaaatgt ggcattggca cccgcgaccc tttctgccgc 3060

agagtgtttt ctgtacccgt caccaagacc caagagcact gccatgctgg aatgtgtgct 3120

agcgctgaag gcatctctct ggactctctg gggttaactc agttacaaag ttactggatc 3180

gcagccgtca ctagcggatt agtgatcttg ttggtctgcc accgcctggc catcagcgcc 3240

ttggacttgt tgactctagc ttccccttta gtgttgcttg tgttcccttg ggcatctgtg 3300

gggcttttac ttgcttgcag tctcgctggt gctgctgtga aaatacagtt gttggcgacg 3360

ctttttgtga atctattctt tccccaagct acccttgtca ctatgggata ctgggcgtgc 3420

gtggcggctt tggccgttta cagtttgatg ggcttgcgag tgaaagtgaa tgtgcccatg 3480

tgtgtgacac ctgcccattt tctgctgctg gcgaggtcag ctggacagtc aagagagcag 3540

atgctccggg tcagcgctgc tgcccccacc aattcactgc ttggagtggc tcgtgattgt 3600

tatgtcacag gcacaactcg gctgtacata cccaaggaag gcgggatggt gtttgaaggg 3660

ctattcaggt caccgaaggc gcgcggcaac gtcggcttcg tggctggtag cagctacggc 3720

acagggtcag tgtggaccag gaacaacgag gtcgtcgtac tgacagcgtc acacgtggtt 3780

ggccgcgcta acatggccac tctgaagatc ggtgacgcaa tgctgactct gactttcaaa 3840

aagaatggcg acttcgccga ggcagtgacg acacagtccg agctcccagg caattggcca 3900

cagttgcatt tcgcccaacc aacaaccggg cccgcttcat ggtgcactgc cacaggagat 3960

gaagaaggct tgctcagtgg cgaggtttgt ctggcgtgga ctactagtgg cgactctgga 4020

tctgcagtgg ttcagggtga cgctgtggta ggggtccaca ccggttcgaa cacaagtggt 4080

gttgcctacg tgaccacccc aagcggaaaa ctccttggcg ccgacaccgt gactttgtca 4140

tcactgtcaa agcatttcac aggccctttg acatcaatcc cgaaggacat ccctgacaac 4200

attattgccg atgttgatgc tgttcctcgt tctctggcca tgctgattga tggcttatcc 4260

aatagagaga gcagcctttc tggacctcag ttgttgttaa ttgcttgttt tatgtggtct 4320

tatcttaacc aacctgctta cttgccttat gtgctgggct tctttgccgc taacttcttc 4380

ctgccaaaaa gtgttggccg ccctgtggtc actgggcttc tatggttgtg ctgcctcttc 4440

acaccgcttt ccatgcgctt gtgcttgttc catctggtct gtgctaccgt cacgggaaac 4500

gtgatatctt tgtggttcta catcactgcc gctggcacgt cttacctttc tgagatgtgg 4560

ttcggaggct atcccaccat gttgtttgtg ccacggttcc tagtgtacca gttccccggc 4620

tgggctattg gcacagtact agcggtatgc agcatcacca tgctggctgc tgccctcggt 4680

cacaccctgt tactggatgt gttctccgcc tcaggtcgct ttgacaggac tttcatgatg 4740

aaatacttcc tggagggagg agtgaaagag agtgtcaccg cctcagtcac ccgcgcttat 4800

ggcaaaccaa ttacccagga gagtctcact gcaacattag ctgccctcac tgatgatgac 4860

ttccaattcc tctctgatgt gcttgactgt cgggccgtcc gatcggcaat gaatctgcgt 4920

gccgctctca caagttttca agtggcgcag tatcgtaaca tccttaatgc atccttgcaa 4980

gtcgatcgtg acgctgctcg tagtcgcaga ctaatggcaa aactggctga ttttgcggtt 5040

gaacaagaag taacagctgg agaccgtgtt gtggttatcg acggtctgga ccgcatggct 5100

cacttcaaag acgatttggt gctggttcct ttgaccacca aagtagtagg cggttctagg 5160

tgcaccattt gtgacgtcgt taaggaagaa gccaatgaca ccccagttaa gccaatgccc 5220

agcaggagac gccgcaaggg cctgcctaaa ggtgctcagt tggagtggga ccgtcaccag 5280

gaagagaaga ggaacgccgg tgatgatgat tttgcggtct cgaatgatta tgtcaagaga 5340

gtgccaaagt actgggatcc cagcgacacc cgaggcacga cagtgaaaat cgccggcact 5400

acctatcaga aagtggttga ctattcaggc aatgtgcatt acgtggagca tcaggaagat 5460

ctgctagact acgtgctggg caaggggagc tatgaaggcc tagatcagga caaagtgttg 5520

gacctcacaa acatgcttaa agtggacccc acggagctct cctccaaaga caaagccaag 5580

gcgcgtcagc ttgctcatct gctgttggat ctggctaacc cagttgaggc agtgaatcag 5640

ttaaactgag agcgccccac atctttcccg gcgatgtggg gcgtcggacc tttgctgact 5700

ctaaagacaa gggtttcgtg gctctacaca gtcgcacaat gtttttagct gcccgggact 5760

ttttatttaa catcaaattt gtgtgcgacg aagagttcac aaagacccca aaagacacac 5820

tgcttgggta cgtacgcgcc tgccctggtt actggtttat tttccgtcgt acgcaccggt 5880

cgctgattga tgcatactgg gacagtatgg agtgcgttta cgcgcttccc accatatctg 5940

attttgatgt gagcccaggt gacgtcgcag tgacgggcga gcgatgggat tttgaatctc 6000

ccggaggagg ccgtgcaaaa cgtctcacag ctgatctggt gcacgctttt caagggttcc 6060

acggagcctc ttattcctat gatgacaagg tggcagctgc tgtcagtggt gacccgtatc 6120

ggtcggacgg cgtcttgtat aacacccgtt ggggcaacat tccatattct gtcccaacca 6180

atgctttgga agccacagct tgctaccgtg ctggatgtga ggccgttacc gacgggacca 6240

acgtcatcgc aacaattggg cccttcccgg agcaacaacc cataccggac atcccaaaga 6300

gcgtgcttga caactgcgct gacatcagct gtgacgcttt catagcgccc gctgcagaga 6360

cagccctgtg tggagattta gagaaataca acctatccac gcagggtttt gtgttgccta 6420

gtgttttctc catggtgcgg gcgtacttaa aagaggagat tggagacgct ccaccactct 6480

acttgccatc tactgtacca tctaaaaatt cacaagccgg aattaacggc gctgagtttc 6540

ctacaaagtc tttacagagc tactgtttga ttgatgacat ggtgtcacag tccatgaaaa 6600

gcaatctaca aaccgccacc atggcgactt gtaaacggca atactgttcc aaatacaaga 6660

ttaggagcat tctgggcacc aacaattaca ttggcctagg tttgcgtgcc tgcctttcgg 6720

gggttacggc cgcattccaa aaagctggaa aggatgggtc accgatttat ttgggcaagt 6780

caaaattcga cccgatacca gctcctgaca agtactgcct tgaaacagac ctggagagtt 6840

gtgatcgctc caccccggct ttggtgcgtt ggttcgctac taatcttatt tttgagctag 6900

ctggccagcc cgagttggtg cacagctacg tgttgaattg ctgtcacgat ctagttgtgg 6960

cgggtagtgt agcattcacc aaacgcgggg gtttgtcatc tggagaccct atcacttcca 7020

tttccaatac catctattca ttggtgctgt acacccagca catgttgcta tgtggacttg 7080

aaggctattt cccagagatt gcagaaaaat atcttgatgg cagcctggag ctgcgggaca 7140

tgttcaagta cgttcgagtg tacatctact cggacgatgt ggttctaacc acacccaacc 7200

agcattacgc ggccagcttt gaccgctggg tcccccacct gcaggcgctg ctaggtttca 7260

aggttgaccc aaagaaaact gtgaacacca gctccccttc cttttgggc tgccggttca 7320

agcaagtgga cggcaagtgt tatctagcca gtcttcagga ccgcgttaca cgctctctgt 7380

tataccacat tggtgcaaag aatccctcag agtactatga agctgctgtt tccatcttta 7440

aggactccat tatctgctgt gatgaagact ggtggacgga cctccatcga cgtatcagtg 7500

gcgctgcgcg taccgacgga gttgagttcc ccaccattga aatgttaaca tccttccgca 7560

ccaagcagta tgagagtgcc gtgtgcacag tttgtggggc cgcccccgtg gccaagtctg 7620

cttgtggagg gtggttctgt ggcaattgtg tcccgtacca cgcgggtcat tgtcacacaa 7680

cctcgctctt cgccaactgc gggcacgaca tcatgtaccg ctccacttac tgcacaatgt 7740

gtgagggttc cccaaaacag atggtaccaa aagtgcctca cccgatcctg gatcatttgc 7800

tgtgccacat tgattacggc agtaaagagg aactaactct ggtagtggcg gatggtcgaa 7860

caacatcacc gcccgggcgc tacaaagtgg gtcacaaggt agtcgccgtg gttgcagatg 7920

tgggaggcaa cattgtgttt gggtgcggtc ctggatcaca catcgcagta ccacttcagg 7980

atacgctcaa gggcgtggtg gtgaataaag ctctgaagaa cgccgccgcc tctgagtacg 8040

tggaaggacc ccctgggagt gggaagactt ttcacctggt caaagatgtg ctagccgtgg 8100

tcggtagcgc gaccttggtt gtgcccaccc acgcgtccat gctggactgc atcaacaagc 8160

tcaaacaagc gggcgccgat ccatactttg tggtgcccaa gtatacagtt cttgactttc 8220

cccggcctgg cagtggaaac atcacagtgc gactgccaca ggtcggaacc agtgagggag 8280

aaacctttgt ggatgaggtg gcctacttct caccagtgga tctggcgcgc attttaaccc 8340

agggtcgagt caagggttac ggtgatttaa atcagctcgg gtgcgtcgga cccgcgagcg 8400

tgccacgtaa cctttggctc cgacattttg tcagcctgga gcccttgcga gtgtgccatc 8460

gattcggcgc tgctgtgtgt gatttgatca agggcattta tccttattat gagccagctc 8520

cacataccac taaagtggtg tttgtgccaa atccagactt tgagaaaggt gtagtcatca 8580

ccgcctacca caaagatcgc ggtcttggtc accgcacaat tgattcaatt caaggctgta 8640

cattccctgt tgtgactctt cgactgccca caccccaatc actgacgcgc ccgcgcgcag 8700

ttgtggcggt tactagggcg tctcaggaat tatacatcta cgaccccttt gatcagctta 8760

gcgggttgtt gaagttcacc aaggaagcag aggcgcagga cttgatccat ggcccaccta 8820

cagcatgcca cctgggccaa gaaattgacc tttggtccaa tgagggcctc gaatattaca 8880

aggaagtcaa cctgctgtac acacacgtcc ccatcaagga tggtgtaata cacagttacc 8940

ctaattgtgg ccctgcctgt ggctgggaaa agcaatccaa caaaatttcg tgcctcccga 9000

gagtggcaca aaatttgggc taccactatt ccccagactt accaggattt tgccccatac 9060

caaaagaact cgctgagcat tggcccgtag tgtccaatga tagatacccg aattgcttgc 9120

aaattacctt acagcaagta tgtgaactca gtaaaccgtg ctcagcgggc tatatggttg 9180

gacaatcggt tttcgtgcag acgcctggtg tgacatctta ctggcttact gaatgggtcg 9240

acggcaaagc gcgtgctcta ccagattcct tattctcgtc cggtaggttc gagactaaca 9300

gccgcgcttt cctcgatgaa gccgaggaaa agtttgccgc cgctcaccct catgcctgtt 9360

tgggagaaat taataagtcc accgtgggag gatcccactt catcttttcc caatatttac 9420

caccattgct acccgcagac gctgttgccc tggtaggtgc ttcattggct gggaaagctg 9480

ctaaagctgc ttgcagcgtt gttgatgtct atgctccatc atttgaacct tatctacacc 9540

ctgagacact gagtcgcgtg tacaagatta tgatcgattt caagccgtgt aggcttatgg 9600

tgtggagaaa cgcgaccttt tatgtccaag agggtgttga tgcagttaca tcagcactag 9660

cagctgtgtc caaactcatc aaagtgccgg ccaatgagcc tgtttcattc catgtggcat 9720

cagggtacag aaccaacgcg ctggtagcgc cccaggctaa aatttcaatt ggagcctacg 9780

ccgccgagtg ggcactgtca actgaaccgc cacctgctgg ttatgcgatc gtgcggcgat 9840

atattgtaaa gaggctcctc agctcaacag aagtgttctt gtgccgcagg ggtgttgtgt 9900

cttccacctc agtgcagacc atttgtgcac tagagggatg taaacctctg ttcaacttct 9960

tacaaattgg ttcagtcatt gggcccgtgt gactctagag tggacctgtt cccatccccc 10020

gctcaactac tcaggtagtg gttcgcggca acgggtacac cgcagttggt aacaagcttg 10080

tcgatggaaa atatggaaaa cgacgagaac atcgtggtgg gccccaagcc cttctacccc 10140

atcgaggaag gcagcgccgg cacccagctg cggaagtaca tggaaagata cgccaagctg 10200

ggcgccattg ccttcaccaa cgccgtgacc ggcgtggact acagctacgc cgagtacctg 10260

gaaaagagct gctgcctggg caaggctctg cagaactacg gcctggtggt ggacggccgg 10320

atcgccctgt gcagcgagaa ctgcgaggaa ttcttcatcc ccgtgatcgc cggcctgttc 10380

atcggcgtgg gcgtggctcc caccaacgag atctacaccc tgcgggagct ggtgcacagc 10440

ctgggcatca gcaagcccac catcgtgttc agcagcaaga agggcctgga caaagtcatc 10500

accgtgcaga aaaccgtgac caccatcaag accatcgtga tcctggacag caaggtggac 10560

taccggggct accagtgcct ggacaccttc atcaagcgga acacccccc tggcttccag 10620

gccagcagct tcaagaccgt ggaggtggac cggaaagaac aggtggccct gatcatgaac 10680

agcagcggca gcaccggcct gcccaagggc gtgcagctga cccacgagaa caccgtgacc 10740

cggttcagcc acgccaggga ccccatctac ggcaaccagg tgtcccccgg caccgccgtg 10800

ctgaccgtgg tgcccttcca ccacggcttc ggcatgttca ccaccctggg ctacctgatc 10860

tgcggcttcc gggtggtgat gctgaccaag ttcgacgagg aaaccttcct gaaaaccctg 10920

caggactaca agtgcaccta cgtgattctg gtgcccaccc tgttcgccat cctgaacaag 10980

agcgagctgc tgaacaagta cgacctgagc aacctggtgg agatcgccag cggcggagcc 11040

cccctgagca aagaagtggg agaggccgtc gccaggcggt tcaatctgcc cggcgtgcgg 11100

cagggctacg gcctgaccga gacaaccagc gccatcatca tcacccccga gggcgacgac 11160

aagcctggag ccagcggcaa ggtggtgccc ctgttcaagg ccaaagtgat cgacctggac 11220

accaagaaga gcctgggccc caacagacgg ggcgaagtgt gcgtgaaggg ccccatgctg 11280

atgaagggct acgtgaacaa ccccgaggcc accaaagagc tgatcgacga agagggctgg 11340

ctgcacaccg gcgacatcgg ctactacgac gaagagaagc acttcttcat cgtggaccgg 11400

ctgaagagcc tgatcaagta caagggctat caggtgcccc ctgccgagct ggaaagcgtc 11460

ctgctgcagc accccagcat cttcgacgcc ggcgtggccg gggtgccaga tcctgtggcc 11520

ggcgagctgc ctggcgccgt ggtggtgctg gaatccggca agaacatgac cgagaaagaa 11580

gtgatggact acgtcgccag ccaggtgtcc aacgccaagc ggctgagagg cggcgtgaga 11640

ttcgtggacg aagtgccaaa gggcctgacc ggcaagatcg acggcagggc catccgggag 11700

atcctgaaga aacccgtggc caagatgtga ttataactcg agggagccat agattcattt 11760

tgtggtgacg ggattttagg tgagtatcta gattacttta ttctgtccgt cccactcttg 11820

ctgttgctta ctaggtatgt agcatctggg ttagtgtatg ttttgactgc cttgttctat 11880

tcctttgtat tagcagctta tatttggttt gttatagttg gaagagcctt ttctactgct 11940

tatgcttttg tgcttttggc tgcttttctg ttattagtaa tgaggatgat tgtgggtatg 12000

atgcctcgtc ttcggtccat tttcaaccat cgccaactgg tggtagctga ttttgtggac 12060

acacctagtg gacctgttcc catcccccgc ccaaccactc aggtagtggt tcgcggcaac 12120

gggtacaccg cagttggtaa caagcttgtc gatggcgtca agacgatcac gtccgcaggc 12180

cgcctctttt cgaaacggac ggcggcgaca gcctacaagc tacaatgacc tactgcgcat 12240

gtttggtcag atgcgggtcc gcaaaccgcc cgcgcaaccc actcaggcta ttattgcaga 12300

gcctggagac cttaggcatg atttaaatca acaggagcgc gccacccttt cgtcgaacgt 12360

acaacggttc ttcatgattg ggcatggttc actcactgca gatgccggag gactcacgta 12420

caccgtcagt tgggttccta ccaaacaaat ccagcgcaaa gttgcgcctc cagcagggcc 12480

gtaagacgtg gatattctcc tgtgtggcgt catgttgaag tagttattag ccacccagga 12540

accaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 12600

aaaaaaaa 12608

<210> 17
<211> 12674

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> Construct rEx-DLP-2A-pp1ab-rFF

<220>
<221> misc_feature
<222> (1)...(18)
<223> T7 promoter

<400> 17
taatacgact cactatagct cgaagtgtgt atggtgccat atacggctca ccaccatata 60

cactgcaaga attactattc ttgtgggccc ctctcggtaa atcctagagg gctttcctct 120

cgttattgcg agattcgtcg ttagataacg gcaagttccc tttcttacta tcctattttc 180

atcttgtggc ttgacgggtc actgcctacg tcgtcgatct ctatcaacta cccttgcgac 240

ttaggcaacc ttctccgcta ctggatttgg agggagtttt gttagggact ggtccctgga 300

cttacccgac gcttgtgagc atagtcagca tagtacattt catctgacta atactacaac 360

accaccacca tgaatagagg attctttaac atgctcggcc gccgcccctt cccggccccc 420

actgccatgt ggaggccgcg gagaaggagg caggcggccc cgatgggaag cggagctact 480

aacttcagcc tgctgaagca ggctggagac gtggaggaga accctggacc tatggcaacc 540

ttctccgcta ctggatttgg agggagtttt gttagggact ggtccctgga cttacccgac 600

gcttgtgagc atggcgcggg attgtgctgc gaagtggacg gctccacctt atgcgccgag 660

tgttttcgcg gttgcgaagg aatggagcaa tgtcctggct tgttcatggg actgttaaaa 720

ctggcttcgc cagttccagt gggacataag ttcctgattg gttggtatcg agctgccaaa 780

gtcaccgggc gttacaattt ccttgagctg ttgcaacacc ctgctttcgc ccagctgcgt 840

gtggttgatg ctaggttagc cattgaagag gcaagtgtgt ttatttccac tgaccacgcg 900

tctgctaagc gtttccctgg cgctagattt gcgctgacac cggtgtatgc taacgcttgg 960

gttgtgagcc cggctgctaa cagtttgata gtgaccactg accaggaaca agatgggttc 1020

tgctggttaa aacttttgcc acctgaccgc cgtgaggctg gtttgcggtt gtattacaac 1080

cattaccgcg aacaaaggac cgggtggctg tctaaaacag gacttcgctt atggcttgga 1140

gacctgggtt tgggcatcaa tgcgagctct ggagggctga aattccacat tatgaggggt 1200

tcgcctcagc gagcttggca tatcacaaca cgcagctgca agctgaagag ctactacgtt 1260

tgtgacatct ctgaagcaga ctggtcctgt ttgcctgctg gcaactacgg cggctacaat 1320

ccaccagggg acggagcttg cggttacagg tgcttggcct tcatgaatgg cgccactgtt 1380

gtgtcggctg gttgcagttc tgacttgtgg tgtgatgatg agttggctta tcgagtcttt 1440

caattgtcac ccacgttcac ggttaccatc ccaggtgggc gagtttgtcc gaatgccaag 1500

tacgcaatga tttgtgacaa gcagcactgg cgcgtcaaac gtgcaaaggg cgtcggcctg 1560

tgtctcgatg aaagctgttt caggggcatc tgcaattgcc aacgcatgag tggaccacca 1620

cctgcacccg tgtcagccgc cgtgttagat cacatactgg aggcggcgac gtttggcaac 1680

gttcgcgtgg ttacacctga agggcagcca cgccccgtac cagcgccgcg agttcgtccc 1740

agcgccaact cttctggaga tgtcaaagat ccggcgcccg ttccgccagt accaaaacca 1800

aggaccaagc ttgccacacc gaacccaact caggcgccca tcccagcacc gcgcacgcga 1860

cttcaagggg cctcaacaca ggagccactg gcgagtgcag gagttgcttc tgactcggca 1920

cctaaatggc gtgtggccaa aactgtgtac agctccgcgg agcgctttcg gaccgaactg 1980

gtacaacgtg ctcggtccgt tggggacgtt cttgttcaag cgctaccgct caaaacccca 2040

gcagtgcagc ggtataccat gactctgaag atgatgcgtt cacgcttcag ttggcactgc 2100

gacgtgtggt acccttggc tgtaatcgct tgtttgctcc ctatatggcc atctcttgct 2160

ttgctcctta gctttgccat tgggttgata cccagtgtgg gcaataatgt tgttctgaca 2220

gcgcttctgg tttcatcagc taattatgtt gcgtcaatgg accatcaatg tgaaggtgcg 2280

gcttgcttag ccttgctgga agaagaacac tattatagag cggtccgttg gcgcccgatt 2340

acaggcgcgc tgtcgcttgt gctcaattta ctggggcagg taggctatgt agctcgttcc 2400

acctttgatg cagcttatgt tccttgcact gtgttcgatc tttgcagctt tgctattctg 2460

tacctctgcc gcaatcgttg ctggagatgc ttcggacgct gtgtgcgagt tgggcctgcc 2520

acgcatgttt tgggctccac cgggcaacga gtttccaaac tggcgctcat tgatttgtgt 2580

gaccactttt caaagcccac catcgatgtt gtgggcatgg caactggttg gagcggatgt 2640

tacacaggaa ccgccgcaat ggagcgtcag tgtgcctcta cggtggaccc tcactcgttc 2700

gaccagaaga aggcaggagc gactgtttac ctcacccccc ctgtcaacag cgggtcagcg 2760

ctgcagtgcc tcaatgtcat gtggaagcga ccaattgggt ccactgtcct tggggaacaa 2820

acaggagctg ttgtgacggc ggtcaagagt atctctttct cacctccctg ctgcgtctct 2880

accactttgc ccacccgacc cggtgtgacc gttgtcgacc atgctcttta caaccggttg 2940

actgcttcag gggtcgatcc cgctttattg cgtgttgggc aaggtgattt tctaaaactt 3000

aatccggggt tccggctgat aggtggatgg atttatggga tatgctattt tgtgttggtg 3060

gttgtgtcaa cttttacctg cttacctatc aaatgtggca ttggcacccg cgacccttc 3120

tgccgcagag tgttttctgt acccgtcacc aagacccaag agcactgcca tgctggaatg 3180

tgtgctagcg ctgaaggcat ctctctggac tctctggggt taactcagtt acaaagttac 3240

tggatcgcag ccgtcactag cggattagtg atcttgttgg tctgccaccg cctggccatc 3300

agcgccttgg acttgttgac tctagcttcc cctttagtgt tgcttgtgtt cccttgggca 3360

tctgtggggc ttttacttgc ttgcagtctc gctggtgctg ctgtgaaaat acagttgttg 3420

gcgacgcttt ttgtgaatct attctttccc caagctaccc ttgtcactat gggatactgg 3480

gcgtgcgtgg cggctttggc cgtttacagt ttgatgggct tgcgagtgaa agtgaatgtg 3540

cccatgtgtg tgacacctgc ccattttctg ctgctggcga ggtcagctgg acagtcaaga 3600

gagcagatgc tccgggtcag cgctgctgcc cccaccaatt cactgcttgg agtggctcgt 3660

gattgttatg tcacaggcac aactcggctg tacataccca aggaaggcgg gatggtgttt 3720

gaagggctat tcaggtcacc gaaggcgcgc ggcaacgtcg gcttcgtggc tggtagcagc 3780

tacggcacag ggtcagtgtg gaccaggaac aacgaggtcg tcgtactgac agcgtcacac 3840

gtggttggcc gcgctaacat ggccactctg aagatcggtg acgcaatgct gactctgact 3900

ttcaaaaaga atggcgactt cgccgaggca gtgacgacac agtccgagct cccaggcaat 3960

tggccacagt tgcatttcgc ccaaccaaca accgggcccg cttcatggtg cactgccaca 4020

ggagatgaag aaggcttgct cagtggcgag gtttgtctgg cgtggactac tagtggcgac 4080

tctggatctg cagtggttca gggtgacgct gtggtagggg tccacaccgg ttcgaacaca 4140

agtggtgttg cctacgtgac caccccaagc ggaaaactcc ttggcgccga caccgtgact 4200

ttgtcatcac tgtcaaagca tttcacaggc cctttgacat caatcccgaa ggacatccct 4260

gacaacatta ttgccgatgt tgatgctgtt cctcgttctc tggccatgct gattgatggc 4320

ttatccaata gagagagcag cctttctgga cctcagttgt tgttaattgc ttgttttatg 4380

tggtcttatc ttaaccaacc tgcttacttg ccttatgtgc tgggcttctt tgccgctaac 4440

ttcttcctgc caaaaagtgt tggccgccct gtggtcactg ggcttctatg gttgtgctgc 4500

ctcttcacac cgctttccat gcgcttgtgc ttgttccatc tggtctgtgc taccgtcacg 4560

ggaaacgtga tatctttgtg gttctacatc actgccgctg gcacgtctta cctttctgag 4620

atgtggttcg gaggctatcc caccatgttg tttgtgccac ggttcctagt gtaccagttc 4680

cccggctggg ctattggcac agtactagcg gtatgcagca tcaccatgct ggctgctgcc 4740

ctcggtcaca ccctgttact ggatgtgttc tccgcctcag gtcgctttga caggactttc 4800

atgatgaaat acttcctgga gggaggagtg aaagagagtg tcaccgcctc agtcacccgc 4860

gcttatggca aaccaattac ccaggagagt ctcactgcaa cattagctgc cctcactgat 4920

gatgacttcc aattcctctc tgatgtgctt gactgtcggg ccgtccgatc ggcaatgaat 4980

ctgcgtgccg ctctcacaag ttttcaagtg gcgcagtatc gtaacatcct taatgcatcc 5040

ttgcaagtcg atcgtgacgc tgctcgtagt cgcagactaa tggcaaaact ggctgatttt 5100

gcggttgaac aagaagtaac agctggagac cgtgttgtgg ttatcgacgg tctggaccgc 5160

atggctcact tcaaagacga tttggtgctg gttcctttga ccaccaaagt agtaggcggt 5220

tctaggtgca ccatttgtga cgtcgttaag gaagaagcca atgacacccc agttaagcca 5280

atgcccagca ggagacgccg caagggcctg cctaaaggtg ctcagttgga gtgggaccgt 5340

caccaggaag agaagaggaa cgccggtgat gatgattttg cggtctcgaa tgattatgtc 5400

aagagagtgc caaagtactg ggatcccagc gacacccgag gcacgacagt gaaaatcgcc 5460

ggcactacct atcagaaagt ggttgactat tcaggcaatg tgcattacgt ggagcatcag 5520

gaagatctgc tagactacgt gctgggcaag gggagctatg aaggcctaga tcaggacaaa 5580

gtgttggacc tcacaaacat gcttaaagtg gaccccacgg agctctcctc caaagacaaa 5640

gccaaggcgc gtcagcttgc tcatctgctg ttggatctgg ctaacccagt tgaggcagtg 5700

aatcagttaa actgagagcg ccccacatct ttcccggcga tgtggggcgt cggacctttg 5760

ctgactctaa agacaagggt ttcgtggctc tacacagtcg cacaatgttt ttagctgccc 5820

gggacttttt atttaacatc aaatttgtgt gcgacgaaga gttcacaaag accccaaaag 5880

acacactgct tgggtacgta cgcgcctgcc ctggttactg gtttattttc cgtcgtacgc 5940

accggtcgct gattgatgca tactgggaca gtatggagtg cgtttacgcg cttcccacca 6000

tatctgattt tgatgtgagc ccaggtgacg tcgcagtgac gggcgagcga tgggattttg 6060

aatctcccgg aggaggccgt gcaaaacgtc tcacagctga tctggtgcac gcttttcaag 6120

ggttccacgg agcctcttat tcctatgatg acaaggtggc agctgctgtc agtggtgacc 6180

cgtatcggtc ggacggcgtc ttgtataaca cccgttgggg caacattcca tattctgtcc 6240

caaccaatgc tttggaagcc acagcttgct accgtgctgg atgtgaggcc gttaccgacg 6300

ggaccaacgt catcgcaaca attgggccct tcccggagca acaacccata ccggacatcc 6360

caaagagcgt gcttgacaac tgcgctgaca tcagctgtga cgctttcata gcgcccgctg 6420

cagagacagc cctgtgtgga gatttagaga aatacaacct atccacgcag ggttttgtgt 6480

tgcctagtgt tttctccatg gtgcgggcgt acttaaaaga ggagattgga gacgctccac 6540

cactctactt gccatctact gtaccatcta aaaattcaca agccggaatt aacggcgctg 6600

agtttcctac aaagtcttta cagagctact gtttgattga tgacatggtg tcacagtcca 6660

tgaaaagcaa tctacaaacc gccaccatgg cgacttgtaa acggcaatac tgttccaaat 6720

acaagattag gagcattctg ggcaccaaca attacattgg cctaggtttg cgtgcctgcc 6780

tttcgggggt tacggccgca ttccaaaaag ctggaaagga tgggtcaccg atttatttgg 6840

gcaagtcaaa attcgacccg ataccagctc ctgacaagta ctgccttgaa acagacctgg 6900

agagttgtga tcgctccacc ccggctttgg tgcgttggtt cgctactaat cttatttttg 6960

agctagctgg ccagcccgag ttggtgcaca gctacgtgtt gaattgctgt cacgatctag 7020

ttgtggcggg tagtgtagca ttcaccaaac gcgggggttt gtcatctgga gaccctatca 7080

cttccatttc caataccatc tattcattgg tgctgtacac ccagcacatg ttgctatgtg 7140

gacttgaagg ctatttccca gagattgcag aaaaatatct tgatggcagc ctggagctgc 7200

gggacatgtt caagtacgtt cgagtgtaca tctactcgga cgatgtggtt ctaaccacac 7260

ccaaccagca ttacgcggcc agctttgacc gctgggtccc ccacctgcag gcgctgctag 7320

gtttcaaggt tgacccaaag aaaactgtga acaccagctc cccttccttt ttgggctgcc 7380

ggttcaagca agtggacggc aagtgttatc tagccagtct tcaggaccgc gttacacgct 7440

ctctgttata ccacattggt gcaaagaatc cctcagagta ctatgaagct gctgtttcca 7500

tctttaagga ctccattatc tgctgtgatg aagactggtg gacggacctc catcgacgta 7560

tcagtggcgc tgcgcgtacc gacggagttg agttccccac cattgaaatg ttaacatcct 7620

tccgcaccaa gcagtatgag agtgccgtgt gcacagtttg tggggccgcc cccgtggcca 7680

agtctgcttg tggagggtgg ttctgtggca attgtgtccc gtaccacgcg ggtcattgtc 7740

acacaacctc gctcttcgcc aactgcgggc acgacatcat gtaccgctcc acttactgca 7800

caatgtgtga gggttcccca aaacagatgg taccaaaagt gcctcacccg atcctggatc 7860

atttgctgtg ccacattgat tacggcagta aagaggaact aactctggta gtggcggatg 7920

gtcgaacaac atcaccgccc gggcgctaca aagtgggtca caaggtagtc gccgtggttg 7980

cagatgtggg aggcaacatt gtgtttgggt gcggtcctgg atcacacatc gcagtaccac 8040

ttcaggatac gctcaagggc gtggtggtga ataaagctct gaagaacgcc gccgcctctg 8100

agtacgtgga aggacccccc gggagtggga agacttttca cctggtcaaa gatgtgctag 8160

ccgtggtcgg tagcgcgacc ttggttgtgc ccacccacgc gtccatgctg gactgcatca 8220

acaagctcaa acaagcgggc gccgatccat actttgtggt gcccaagtat acagttcttg 8280

actttccccg gcctggcagt ggaaacatca cagtgcgact gccacaggtc ggaaccagtg 8340

agggagaaac ctttgtggat gaggtggcct acttctcacc agtggatctg gcgcgcattt 8400

taacccaggg tcgagtcaag ggttacggtg atttaaatca gctcgggtgc gtcggacccg 8460

cgagcgtgcc acgtaacctt tggctccgac attttgtcag cctggagccc ttgcgagtgt 8520

gccatcgatt cggcgctgct gtgtgtgatt tgatcaaggg catttatcct tattatgagc 8580

cagctccaca taccactaaa gtggtgtttg tgccaaatcc agactttgag aaaggtgtag 8640

tcatcaccgc ctaccacaaa gatcgcggtc ttggtcaccg cacaattgat tcaattcaag 8700

gctgtacatt ccctgttgtg actcttcgac tgcccacacc ccaatcactg acgcgcccgc 8760

gcgcagttgt ggcggttact agggcgtctc aggaattata catctacgac ccctttgatc 8820

agcttagcgg gttgttgaag ttcaccaagg aagcagaggc gcaggacttg atccatggcc 8880

cacctacagc atgccacctg ggccaagaaa ttgacctttg gtccaatgag ggcctcgaat 8940

attacaagga agtcaacctg ctgtacacac acgtccccat caaggatggt gtaatacaca 9000

gttaccctaa ttgtggccct gcctgtggct gggaaaagca atccaacaaa atttcgtgcc 9060

tcccgagagt ggcacaaaat ttgggctacc actattcccc agacttacca ggattttgcc 9120

ccataccaaa agaactcgct gagcattggc ccgtagtgtc caatgataga tacccgaatt 9180

gcttgcaaat taccttacag caagtatgtg aactcagtaa accgtgctca gcgggctata 9240

tggttggaca atcggttttc gtgcagacgc ctggtgtgac atcttactgg cttactgaat 9300

gggtcgacgg caaagcgcgt gctctaccag attccttatt ctcgtccggt aggttcgaga 9360

ctaacagccg cgctttcctc gatgaagccg aggaaaagtt tgccgccgct caccctcatg 9420

cctgtttggg agaaattaat aagtccaccg tgggaggatc ccacttcatc ttttcccaat 9480

atttaccacc attgctaccc gcagacgctg ttgccctggt aggtgcttca ttggctggga 9540

aagctgctaa agctgcttgc agcgttgttg atgtctatgc tccatcattt gaaccttatc 9600

tacaccctga gacactgagt cgcgtgtaca agattatgat cgatttcaag ccgtgtaggc 9660

ttatggtgtg gagaaacgcg acctttatg tccaagaggg tgttgatgca gttacatcag 9720

cactagcagc tgtgtccaaa ctcatcaaag tgccggccaa tgagcctgtt tcattccatg 9780

tggcatcagg gtacagaacc aacgcgctgg tagcgcccca ggctaaaatt tcaattggag 9840

cctacgccgc cgagtgggca ctgtcaactg aaccgccacc tgctggttat gcgatcgtgc 9900

ggcgatatat tgtaaagagg ctcctcagct caacagaagt gttcttgtgc cgcaggggtg 9960

ttgtgtcttc cacctcagtg cagaccattt gtgcactaga gggatgtaaa cctctgttca 10020

acttcttaca aattggttca gtcattgggc ccgtgtgact ctagagtgga cctgttccca 10080

tcccccgctc aactactcag gtagtggttc gcggcaacgg gtacaccgca gttggtaaca 10140

agcttgtcga tggaaaatat ggaaaacgac gagaacatcg tggtgggccc caagcccttc 10200

taccccatcg aggaaggcag cgccggcacc cagctgcgga agtacatgga aagatacgcc 10260

aagctgggcg ccattgcctt caccaacgcc gtgaccggcg tggactacag ctacgccgag 10320

tacctggaaa agagctgctg cctgggcaag gctctgcaga actacggcct ggtggtggac 10380

ggccggatcg ccctgtgcag cgagaactgc gaggaattct tcatccccgt gatcgccggc 10440

ctgttcatcg gcgtgggcgt ggctcccacc aacgagatct acaccctgcg ggagctggtg 10500

cacagcctgg gcatcagcaa gcccaccatc gtgttcagca gcaagaaggg cctggacaaa 10560

gtcatcaccg tgcagaaaac cgtgaccacc atcaagacca tcgtgatcct ggacagcaag 10620

gtggactacc ggggctacca gtgcctggac accttcatca agcggaacac ccccccctggc 10680

ttccaggcca gcagcttcaa gaccgtggag gtggaccgga aagaacaggt ggccctgatc 10740

atgaacagca gcggcagcac cggcctgccc aagggcgtgc agctgaccca cgagaacacc 10800

gtgacccggt tcagccacgc cagggacccc atctacggca accaggtgtc ccccggcacc 10860

gccgtgctga ccgtggtgcc cttccaccac ggcttcggca tgttcaccac cctgggctac 10920

ctgatctgcg gcttccgggt ggtgatgctg accaagttcg acgaggaaac cttcctgaaa 10980

accctgcagg actacaagtg cacctacgtg attctggtgc ccaccctgtt cgccatcctg 11040

aacaagagcg agctgctgaa caagtacgac ctgagcaacc tggtggagat cgccagcggc 11100

ggagcccccc tgagcaaaga agtgggagag gccgtcgcca ggcggttcaa tctgcccggc 11160

gtgcggcagg gctacggcct gaccgagaca accagcgcca tcatcatcac ccccgagggc 11220

gacgacaagc ctggagccag cggcaaggtg gtgcccctgt tcaaggccaa agtgatcgac 11280

ctggacacca agaagagcct gggccccaac agacggggcg aagtgtgcgt gaagggcccc 11340

atgctgatga agggctacgt gaacaacccc gaggccacca aagagctgat cgacgaagag 11400

ggctggctgc acaccggcga catcggctac tacgacgaag agaagcactt cttcatcgtg 11460

gaccggctga agagcctgat caagtacaag ggctatcagg tgcccccctgc cgagctggaa 11520

agcgtcctgc tgcagcaccc cagcatcttc gacgccggcg tggccggggt gccagatcct 11580

gtggccggcg agctgcctgg cgccgtggtg gtgctggaat ccggcaagaa catgaccgag 11640

aaagaagtga tggactacgt cgccagccag gtgtccaacg ccaagcggct gagaggcggc 11700

gtgagattcg tggacgaagt gccaaagggc ctgaccggca agatcgacgg cagggccatc 11760

cgggagatcc tgaagaaacc cgtggccaag atgtgattat aactcgaggg agccatagat 11820

tcattttgtg gtgacgggat tttaggtgag tatctagatt actttattct gtccgtccca 11880

ctcttgctgt tgcttactag gtatgtagca tctgggttag tgtatgtttt gactgccttg 11940

ttctattcct ttgtattagc agcttatatt tggtttgtta tagttggaag agccttttct 12000

actgcttatg cttttgtgct tttggctgct tttctgttat tagtaatgag gatgattgtg 12060

ggtatgatgc ctcgtcttcg gtccattttc aaccatcgcc aactggtggt agctgatttt 12120

gtggacacac ctagtggacc tgttcccatc ccccgcccaa ccactcaggt agtggttcgc 12180

ggcaacgggt acaccgcagt tggtaacaag cttgtcgatg gcgtcaagac gatcacgtcc 12240

gcaggccgcc tcttttcgaa acggacggcg gcgacagcct acaagctaca atgacctact 12300

gcgcatgttt ggtcagatgc gggtccgcaa accgcccgcg caacccactc aggctattat 12360

tgcagagcct ggagacctta ggcatgattt aaatcaacag gagcgcgcca ccctttcgtc 12420

gaacgtacaa cggttcttca tgattgggca tggttcactc actgcagatg ccggaggact 12480

cacgtacacc gtcagttggg ttcctaccaa acaaatccag cgcaaagttg cgcctccagc 12540

agggccgtaa gacgtggata ttctcctgtg tggcgtcatg ttgaagtagt tattagccac 12600

ccaggaacca aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 12660

aaaaaaaaaa aaaa 12674

<210> 18
<211> 12819
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> Construct rEx-DLP-2A-pp1ab-DLP-rFF

<220>
<221> misc_feature
<222> (1)...(18)
<223> T7 promoter

<400> 18
taatacgact cactatagct cgaagtgtgt atggtgccat atacggctca ccaccatata 60

cactgcaaga attactattc ttgtgggccc ctctcggtaa atcctagagg gctttcctct 120

cgttattgcg agattcgtcg ttagataacg gcaagttccc tttcttacta tcctattttc 180

atcttgtggc ttgacgggtc actgcctacg tcgtcgatct ctatcaacta cccttgcgac 240

ttaggcaacc ttctccgcta ctggatttgg agggagtttt gttagggact ggtccctgga 300

cttacccgac gcttgtgagc atagtcagca tagtacattt catctgacta atactacaac 360

accaccacca tgaatagagg attctttaac atgctcggcc gccgcccctt cccggccccc 420

actgccatgt ggaggccgcg gagaaggagg caggcggccc cgatgggaag cggagctact 480

aacttcagcc tgctgaagca ggctggagac gtggaggaga accctggacc tatggcaacc 540

ttctccgcta ctggatttgg agggagtttt gttagggact ggtccctgga cttacccgac 600

gcttgtgagc atggcgcggg attgtgctgc gaagtggacg gctccacctt atgcgccgag 660

tgttttcgcg gttgcgaagg aatggagcaa tgtcctggct tgttcatggg actgttaaaa 720

ctggcttcgc cagttccagt gggacataag ttcctgattg gttggtatcg agctgccaaa 780

gtcaccgggc gttacaattt ccttgagctg ttgcaacacc ctgctttcgc ccagctgcgt 840

gtggttgatg ctaggttagc cattgaagag gcaagtgtgt ttatttccac tgaccacgcg 900

tctgctaagc gtttccctgg cgctagattt gcgctgacac cggtgtatgc taacgcttgg 960

gttgtgagcc cggctgctaa cagtttgata gtgaccactg accaggaaca agatgggttc 1020

tgctggttaa aacttttgcc acctgaccgc cgtgaggctg gtttgcggtt gtattacaac 1080

cattaccgcg aacaaaggac cgggtggctg tctaaaacag gacttcgctt atggcttgga 1140

gacctgggtt tgggcatcaa tgcgagctct ggagggctga aattccacat tatgaggggt 1200

tcgcctcagc gagcttggca tatcacaaca cgcagctgca agctgaagag ctactacgtt 1260

tgtgacatct ctgaagcaga ctggtcctgt ttgcctgctg gcaactacgg cggctacaat 1320

ccaccagggg acggagcttg cggttacagg tgcttggcct tcatgaatgg cgccactgtt 1380

gtgtcggctg gttgcagttc tgacttgtgg tgtgatgatg agttggctta tcgagtcttt 1440

caattgtcac ccacgttcac ggttaccatc ccaggtgggc gagtttgtcc gaatgccaag 1500

tacgcaatga tttgtgacaa gcagcactgg cgcgtcaaac gtgcaaaggg cgtcggcctg 1560

tgtctcgatg aaagctgttt caggggcatc tgcaattgcc aacgcatgag tggaccacca 1620

cctgcacccg tgtcagccgc cgtgttagat cacatactgg aggcggcgac gtttggcaac 1680

gttcgcgtgg ttacacctga agggcagcca cgccccgtac cagcgccgcg agttcgtccc 1740

agcgccaact cttctggaga tgtcaaagat ccggcgcccg ttccgccagt accaaaacca 1800

aggaccaagc ttgccacacc gaacccaact caggcgccca tcccagcacc gcgcacgcga 1860

cttcaagggg cctcaacaca ggagccactg gcgagtgcag gagttgcttc tgactcggca 1920

cctaaatggc gtgtggccaa aactgtgtac agctccgcgg agcgctttcg gaccgaactg 1980

gtacaacgtg ctcggtccgt tggggacgtt cttgttcaag cgctaccgct caaaacccca 2040

gcagtgcagc ggtataccat gactctgaag atgatgcgtt cacgcttcag ttggcactgc 2100

gacgtgtggt acccttggc tgtaatcgct tgtttgctcc ctatatggcc atctcttgct 2160

ttgctcctta gctttgccat tgggttgata cccagtgtgg gcaataatgt tgttctgaca 2220

gcgcttctgg tttcatcagc taattatgtt gcgtcaatgg accatcaatg tgaaggtgcg 2280

gcttgcttag ccttgctgga agaagaacac tattatagag cggtccgttg gcgcccgatt 2340

acaggcgcgc tgtcgcttgt gctcaattta ctggggcagg taggctatgt agctcgttcc 2400

acctttgatg cagcttatgt tccttgcact gtgttcgatc tttgcagctt tgctattctg 2460

tacctctgcc gcaatcgttg ctggagatgc ttcggacgct gtgtgcgagt tgggcctgcc 2520

acgcatgttt tgggctccac cgggcaacga gtttccaaac tggcgctcat tgatttgtgt 2580

gaccactttt caaagcccac catcgatgtt gtgggcatgg caactggttg gagcggatgt 2640

tacacaggaa ccgccgcaat ggagcgtcag tgtgcctcta cggtggaccc tcactcgttc 2700

gaccagaaga aggcaggagc gactgtttac ctcacccccc ctgtcaacag cgggtcagcg 2760

ctgcagtgcc tcaatgtcat gtggaagcga ccaattgggt ccactgtcct tggggaacaa 2820

acaggagctg ttgtgacggc ggtcaagagt atctctttct cacctccctg ctgcgtctct 2880

accactttgc ccacccgacc cggtgtgacc gttgtcgacc atgctcttta caaccggttg 2940

actgcttcag gggtcgatcc cgctttattg cgtgttgggc aaggtgattt tctaaaactt 3000

aatccggggt tccggctgat aggtggatgg atttatggga tatgctattt tgtgttggtg 3060

gttgtgtcaa cttttacctg cttacctatc aaatgtggca ttggcacccg cgacccttc 3120

tgccgcagag tgttttctgt acccgtcacc aagacccaag agcactgcca tgctggaatg 3180

tgtgctagcg ctgaaggcat ctctctggac tctctggggt taactcagtt acaaagttac 3240

tggatcgcag ccgtcactag cggattagtg atcttgttgg tctgccaccg cctggccatc 3300

agcgccttgg acttgttgac tctagcttcc cctttagtgt tgcttgtgtt cccttgggca 3360

tctgtggggc ttttacttgc ttgcagtctc gctggtgctg ctgtgaaaat acagttgttg 3420

gcgacgcttt ttgtgaatct attctttccc caagctaccc ttgtcactat gggatactgg 3480

gcgtgcgtgg cggctttggc cgtttacagt ttgatgggct tgcgagtgaa agtgaatgtg 3540

cccatgtgtg tgacacctgc ccattttctg ctgctggcga ggtcagctgg acagtcaaga 3600

gagcagatgc tccgggtcag cgctgctgcc cccaccaatt cactgcttgg agtggctcgt 3660

gattgttatg tcacaggcac aactcggctg tacataccca aggaaggcgg gatggtgttt 3720

gaagggctat tcaggtcacc gaaggcgcgc ggcaacgtcg gcttcgtggc tggtagcagc 3780

tacggcacag ggtcagtgtg gaccaggaac aacgaggtcg tcgtactgac agcgtcacac 3840

gtggttggcc gcgctaacat ggccactctg aagatcggtg acgcaatgct gactctgact 3900

ttcaaaaaga atggcgactt cgccgaggca gtgacgacac agtccgagct cccaggcaat 3960

tggccacagt tgcatttcgc ccaaccaaca accgggcccg cttcatggtg cactgccaca 4020

ggagatgaag aaggcttgct cagtggcgag gtttgtctgg cgtggactac tagtggcgac 4080

tctggatctg cagtggttca gggtgacgct gtggtagggg tccacaccgg ttcgaacaca 4140

agtggtgttg cctacgtgac caccccaagc ggaaaactcc ttggcgccga caccgtgact 4200

ttgtcatcac tgtcaaagca tttcacaggc cctttgacat caatcccgaa ggacatccct 4260

gacaacatta ttgccgatgt tgatgctgtt cctcgttctc tggccatgct gattgatggc 4320

ttatccaata gagagagcag cctttctgga cctcagttgt tgttaattgc ttgttttatg 4380

tggtcttatc ttaaccaacc tgcttacttg ccttatgtgc tgggcttctt tgccgctaac 4440

ttcttcctgc caaaaagtgt tggccgccct gtggtcactg ggcttctatg gttgtgctgc 4500

ctcttcacac cgctttccat gcgcttgtgc ttgttccatc tggtctgtgc taccgtcacg 4560

ggaaacgtga tatctttgtg gttctacatc actgccgctg gcacgtctta cctttctgag 4620

atgtggttcg gaggctatcc caccatgttg tttgtgccac ggttcctagt gtaccagttc 4680

cccggctggg ctattggcac agtactagcg gtatgcagca tcaccatgct ggctgctgcc 4740

ctcggtcaca ccctgttact ggatgtgttc tccgcctcag gtcgctttga caggactttc 4800

atgatgaaat acttcctgga gggaggagtg aaagagagtg tcaccgcctc agtcacccgc 4860

gcttatggca aaccaattac ccaggagagt ctcactgcaa cattagctgc cctcactgat 4920

gatgacttcc aattcctctc tgatgtgctt gactgtcggg ccgtccgatc ggcaatgaat 4980

ctgcgtgccg ctctcacaag ttttcaagtg gcgcagtatc gtaacatcct taatgcatcc 5040

ttgcaagtcg atcgtgacgc tgctcgtagt cgcagactaa tggcaaaact ggctgatttt 5100

gcggttgaac aagaagtaac agctggagac cgtgttgtgg ttatcgacgg tctggaccgc 5160

atggctcact tcaaagacga tttggtgctg gttcctttga ccaccaaagt agtaggcggt 5220

tctaggtgca ccatttgtga cgtcgttaag gaagaagcca atgacacccc agttaagcca 5280

atgcccagca ggagacgccg caagggcctg cctaaaggtg ctcagttgga gtgggaccgt 5340

caccaggaag agaagaggaa cgccggtgat gatgattttg cggtctcgaa tgattatgtc 5400

aagagagtgc caaagtactg ggatcccagc gacacccgag gcacgacagt gaaaatcgcc 5460

ggcactacct atcagaaagt ggttgactat tcaggcaatg tgcattacgt ggagcatcag 5520

gaagatctgc tagactacgt gctgggcaag gggagctatg aaggcctaga tcaggacaaa 5580

gtgttggacc tcacaaacat gcttaaagtg gaccccacgg agctctcctc caaagacaaa 5640

gccaaggcgc gtcagcttgc tcatctgctg ttggatctgg ctaacccagt tgaggcagtg 5700

aatcagttaa actgagagcg ccccacatct ttcccggcga tgtggggcgt cggacctttg 5760

ctgactctaa agacaagggt ttcgtggctc tacacagtcg cacaatgttt ttagctgccc 5820

gggacttttt atttaacatc aaatttgtgt gcgacgaaga gttcacaaag accccaaaag 5880

acacactgct tgggtacgta cgcgcctgcc ctggttactg gtttattttc cgtcgtacgc 5940

accggtcgct gattgatgca tactgggaca gtatggagtg cgtttacgcg cttcccacca 6000

tatctgattt tgatgtgagc ccaggtgacg tcgcagtgac gggcgagcga tgggattttg 6060

aatctcccgg aggaggccgt gcaaaacgtc tcacagctga tctggtgcac gcttttcaag 6120

ggttccacgg agcctcttat tcctatgatg acaaggtggc agctgctgtc agtggtgacc 6180

cgtatcggtc ggacggcgtc ttgtataaca cccgttgggg caacattcca tattctgtcc 6240

caaccaatgc tttggaagcc acagcttgct accgtgctgg atgtgaggcc gttaccgacg 6300

ggaccaacgt catcgcaaca attgggccct tcccggagca acaacccata ccggacatcc 6360

caaagagcgt gcttgacaac tgcgctgaca tcagctgtga cgctttcata gcgcccgctg 6420

cagagacagc cctgtgtgga gatttagaga aatacaacct atccacgcag ggttttgtgt 6480

tgcctagtgt tttctccatg gtgcgggcgt acttaaaaga ggagattgga gacgctccac 6540

cactctactt gccatctact gtaccatcta aaaattcaca agccggaatt aacggcgctg 6600

agtttcctac aaagtcttta cagagctact gtttgattga tgacatggtg tcacagtcca 6660

tgaaaagcaa tctacaaacc gccaccatgg cgacttgtaa acggcaatac tgttccaaat 6720

acaagattag gagcattctg ggcaccaaca attacattgg cctaggtttg cgtgcctgcc 6780

tttcgggggt tacggccgca ttccaaaaag ctggaaagga tgggtcaccg atttatttgg 6840

gcaagtcaaa attcgacccg ataccagctc ctgacaagta ctgccttgaa acagacctgg 6900

agagttgtga tcgctccacc ccggctttgg tgcgttggtt cgctactaat cttatttttg 6960

agctagctgg ccagcccgag ttggtgcaca gctacgtgtt gaattgctgt cacgatctag 7020

ttgtggcggg tagtgtagca ttcaccaaac gcgggggttt gtcatctgga gaccctatca 7080

cttccatttc caataccatc tattcattgg tgctgtacac ccagcacatg ttgctatgtg 7140

gacttgaagg ctatttccca gagattgcag aaaaatatct tgatggcagc ctggagctgc 7200

gggacatgtt caagtacgtt cgagtgtaca tctactcgga cgatgtggtt ctaaccacac 7260

ccaaccagca ttacgcggcc agctttgacc gctgggtccc ccacctgcag gcgctgctag 7320

gtttcaaggt tgacccaaag aaaactgtga acaccagctc cccttccttt ttgggctgcc 7380

ggttcaagca agtggacggc aagtgttatc tagccagtct tcaggaccgc gttacacgct 7440

ctctgttata ccacattggt gcaaagaatc cctcagagta ctatgaagct gctgtttcca 7500

tctttaagga ctccattatc tgctgtgatg aagactggtg gacggacctc catcgacgta 7560

tcagtggcgc tgcgcgtacc gacggagttg agttccccac cattgaaatg ttaacatcct 7620

tccgcaccaa gcagtatgag agtgccgtgt gcacagtttg tggggccgcc cccgtggcca 7680

agtctgcttg tggagggtgg ttctgtggca attgtgtccc gtaccacgcg ggtcattgtc 7740

acacaacctc gctcttcgcc aactgcgggc acgacatcat gtaccgctcc acttactgca 7800

caatgtgtga gggttcccca aaacagatgg taccaaaagt gcctcacccg atcctggatc 7860

atttgctgtg ccacattgat tacggcagta aagaggaact aactctggta gtggcggatg 7920

gtcgaacaac atcaccgccc gggcgctaca aagtgggtca caaggtagtc gccgtggttg 7980

cagatgtggg aggcaacatt gtgtttgggt gcggtcctgg atcacacatc gcagtaccac 8040

ttcaggatac gctcaagggc gtggtggtga ataaagctct gaagaacgcc gccgcctctg 8100

agtacgtgga aggaccccct gggagtggga agacttttca cctggtcaaa gatgtgctag 8160

ccgtggtcgg tagcgcgacc ttggttgtgc ccacccacgc gtccatgctg gactgcatca 8220

acaagctcaa acaagcgggc gccgatccat actttgtggt gcccaagtat acagttcttg 8280

actttccccg gcctggcagt ggaaacatca cagtgcgact gccacaggtc ggaaccagtg 8340

agggagaaac ctttgtggat gaggtggcct acttctcacc agtggatctg gcgcgcattt 8400

taacccaggg tcgagtcaag ggttacggtg atttaaatca gctcgggtgc gtcggacccg 8460

cgagcgtgcc acgtaacctt tggctccgac attttgtcag cctggagccc ttgcgagtgt 8520

gccatcgatt cggcgctgct gtgtgtgatt tgatcaaggg catttatcct tattatgagc 8580

cagctccaca taccactaaa gtggtgtttg tgccaaatcc agactttgag aaaggtgtag 8640

tcatcaccgc ctaccacaaa gatcgcggtc ttggtcaccg cacaattgat tcaattcaag 8700

gctgtacatt ccctgttgtg actcttcgac tgcccacacc ccaatcactg acgcgcccgc 8760

gcgcagttgt ggcggttact agggcgtctc aggaattata catctacgac ccctttgatc 8820

agcttagcgg gttgttgaag ttcaccaagg aagcagaggc gcaggacttg atccatggcc 8880

cacctacagc atgccacctg ggccaagaaa ttgacctttg gtccaatgag ggcctcgaat 8940

attacaagga agtcaacctg ctgtacacac acgtccccat caaggatggt gtaatacaca 9000

gttaccctaa ttgtggccct gcctgtggct gggaaaagca atccaacaaa atttcgtgcc 9060

tcccgagagt ggcacaaaat ttgggctacc actattcccc agacttacca ggattttgcc 9120

ccataccaaa agaactcgct gagcattggc ccgtagtgtc caatgataga tacccgaatt 9180

gcttgcaaat taccttacag caagtatgtg aactcagtaa accgtgctca gcgggctata 9240

tggttggaca atcggttttc gtgcagacgc ctggtgtgac atcttactgg cttactgaat 9300

gggtcgacgg caaagcgcgt gctctaccag attccttatt ctcgtccggt aggttcgaga 9360

ctaacagccg cgctttcctc gatgaagccg aggaaaagtt tgccgccgct caccctcatg 9420

cctgtttggg agaaattaat aagtccaccg tgggaggatc ccacttcatc ttttcccaat 9480

atttaccacc attgctaccc gcagacgctg ttgccctggt aggtgcttca ttggctggga 9540

aagctgctaa agctgcttgc agcgttgttg atgtctatgc tccatcattt gaaccttatc 9600

tacaccctga gacactgagt cgcgtgtaca agattatgat cgatttcaag ccgtgtaggc 9660

ttatggtgtg gagaaacgcg acctttttatg tccaagaggg tgttgatgca gttacatcag 9720

cactagcagc tgtgtccaaa ctcatcaaag tgccggccaa tgagcctgtt tcattccatg 9780

tggcatcagg gtacagaacc aacgcgctgg tagcgcccca ggctaaaatt tcaattggag 9840

cctacgccgc cgagtgggca ctgtcaactg aaccgccacc tgctggttat gcgatcgtgc 9900

ggcgatatat tgtaaagagg ctcctcagct caacagaagt gttcttgtgc cgcaggggtg 9960

ttgtgtcttc cacctcagtg cagaccattt gtgcactaga gggatgtaaa cctctgttca 10020

acttcttaca aattggttca gtcattgggc ccgtgtgact ctagagtgga cctgttccca 10080

tcccccgctc aactactcag gtagtggttc gcggcaacgg gtacaccgca gttggtaaca 10140

agcttgtcga tagtcagcat agtacatttc atctgactaa tactacaaca ccaccaccat 10200

gaatagagga ttctttaaca tgctcggccg ccgccccttc ccggccccca ctgccatgtg 10260

gaggccgcgg agaaggaggc aggcggcccc gatgatggaa aatatggaaa acgacgagaa 10320

catcgtggtg ggccccaagc ccttctaccc catcgaggaa ggcagcgccg gcacccagct 10380

gcggaagtac atggaaagat acgccaagct gggcgccatt gccttcacca acgccgtgac 10440

cggcgtggac tacagctacg ccgagtacct ggaaaagagc tgctgcctgg gcaaggctct 10500

gcagaactac ggcctggtgg tggacggccg gatcgccctg tgcagcgaga actgcgagga 10560

attcttcatc cccgtgatcg ccggcctgtt catcggcgtg ggcgtggctc ccaccaacga 10620

gatctacacc ctgcgggagc tggtgcacag cctgggcatc agcaagccca ccatcgtgtt 10680

cagcagcaag aagggcctgg acaaagtcat caccgtgcag aaaaccgtga ccaccatcaa 10740

gaccatcgtg atcctggaca gcaaggtgga ctaccggggc taccagtgcc tggacacctt 10800

catcaagcgg aacacccccc ctggcttcca ggccagcagc ttcaagaccg tggaggtgga 10860

ccggaaagaa caggtggccc tgatcatgaa cagcagcggc agcaccggcc tgcccaaggg 10920

cgtgcagctg acccacgaga acaccgtgac ccggttcagc cacgccaggg accccatcta 10980

cggcaaccag gtgtcccccg gcaccgccgt gctgaccgtg gtgcccttcc accacggctt 11040

cggcatgttc accaccctgg gctacctgat ctgcggcttc cgggtggtga tgctgaccaa 11100

gttcgacgag gaaaccttcc tgaaaaccct gcaggactac aagtgcacct acgtgattct 11160

ggtgcccacc ctgttcgcca tcctgaacaa gagcgagctg ctgaacaagt acgacctgag 11220

caacctggtg gagatcgcca gcggcggagc ccccctgagc aaagaagtgg gagaggccgt 11280

cgccaggcgg ttcaatctgc ccggcgtgcg gcagggctac ggcctgaccg agacaaccag 11340

cgccatcatc atcacccccg agggcgacga caagcctgga gccagcggca aggtggtgcc 11400

cctgttcaag gccaaagtga tcgacctgga caccaagaag agcctgggcc ccaacagacg 11460

gggcgaagtg tgcgtgaagg gccccatgct gatgaagggc tacgtgaaca accccgaggc 11520

caccaaagag ctgatcgacg aagagggctg gctgcacacc ggcgacatcg gctactacga 11580

cgaagagaag cacttcttca tcgtggaccg gctgaagagc ctgatcaagt acaagggcta 11640

tcaggtgccc cctgccgagc tggaaagcgt cctgctgcag caccccagca tcttcgacgc 11700

cggcgtggcc ggggtgccag atcctgtggc cggcgagctg cctggcgccg tggtggtgct 11760

ggaatccggc aagaacatga ccgagaaaga agtgatggac tacgtcgcca gccaggtgtc 11820

caacgccaag cggctgagag gcggcgtgag attcgtggac gaagtgccaa agggcctgac 11880

cggcaagatc gacggcaggg ccatccggga gatcctgaag aaacccgtgg ccaagatgtg 11940

attataactc gagggagcca tagattcatt ttgtggtgac gggattttag gtgagtatct 12000

agattacttt attctgtccg tcccactctt gctgttgctt actaggtatg tagcatctgg 12060

gttagtgtat gttttgactg ccttgttcta ttcctttgta ttagcagctt atatttggtt 12120

tgttatagtt ggaagagcct tttctactgc ttatgctttt gtgcttttgg ctgcttttct 12180

gttattagta atgaggatga ttgtgggtat gatgcctcgt cttcggtcca ttttcaacca 12240

tcgccaactg gtggtagctg attttgtgga cacacctagt ggacctgttc ccatcccccg 12300

cccaaccact caggtagtgg ttcgcggcaa cgggtacacc gcagttggta acaagcttgt 12360

cgatggcgtc aagacgatca cgtccgcagg ccgcctcttt tcgaaacgga cggcggcgac 12420

agcctacaag ctacaatgac ctactgcgca tgtttggtca gatgcgggtc cgcaaaccgc 12480

ccgcgcaacc cactcaggct attattgcag agcctggaga ccttaggcat gatttaaatc 12540

aacaggagcg cgccaccctt tcgtcgaacg tacaacggtt cttcatgatt gggcatggtt 12600

cactcactgc agatgccgga ggactcacgt acaccgtcag ttgggttcct accaaacaaa 12660

tccagcgcaa agttgcgcct ccagcagggc cgtaagacgt ggatattctc ctgtgtggcg 12720

tcatgttgaa gtagttatta gccacccagg aaccaaaaaa aaaaaaaaaa aaaaaaaaaa 12780

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaa 12819

<210> 19
<211> 464
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> Construct Alpha-R-DLP-2A-nsp-rFF

<400> 19
gataggcggc gcatgagaga agcccagacc aattacctac ccaaatagga gaaagttcac 60

gttgacatcg aggaagacag cccattcctc agagctttgc agcggagctt cccgcagttt 120

gaggtagaag ccaagcaggt cactgataat gaccatgcta atgccagagc gttttcgcat 180

ctggcttcaa aactgatcga aacggaggtg gacccatccg acacgatcct tgacattgga 240

atagtcagca tagtacattt catctgacta atactacaac accaccacca tgaatagagg 300

attctttaac atgctcggcc gccgcccctt cccggccccc actgccatgt ggaggccgcg 360

gagaaggagg caggcggccc cgggaagcgg agctactaac ttcagcctgc tgaagcaggc 420

tggagacgtg gaggagaacc ctggacctat ggagaaagtt cacg 464

<210> 20
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> primer DLP-rFF-F

<220>
<221> misc_feature
<223> RP112

<400> 20
cctgaatgga ctacgacata gtctagtccg ccaagatatc gcaccatagt cagcatagta 60

catttcatct gactaatact 80

<210> 21
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> primer DLP-rFF-R

<220>
<221> misc_feature
<223> RP113

<400> 21
gcagcttgcc aattgctgct gtatcgatca attaatcaca tcttggccac gggtttcttc 60

<210> 22
<211> 67
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> primer 5'Alpha-P2A-F

<220>
<221> misc_feature
<223> RP124

<400> 22
gaagcaggct ggagacgtgg aggagaaccc tggacctgag aaagttcacg ttgacatcga 60

ggaagac 67

<210> 23
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> primer 5'ScaI-R

<220>
<221> misc_feature
<223> RP125

<400> 23
caccagtcac agaaaagcat cttacggatg 30

<210> 24
<211> 1014
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> g-block for Alpha-R-DLP-2A-nsp-rFF

<400> 24
tgtcatgcca tccgtaagat gcttttctgt gactggtgag tactcaacca agtcattctg 60

agaatagtgt atgcggcgac cgagttgctc ttgcccggcg tcaacacggg ataataccgc 120

gccacatagc agaactttaa aagtgctcat cattggaaaa cgttcttcgg ggcgaaaact 180

ctcaaggatc ttaccgctgt tgagatccag ttcgatgtaa cccactcgtg cacccaactg 240

atcttcagca tcttttactt tcaccagcgt ttctgggtga gcaaaaacag gaaggcaaaa 300

tgccgcaaaa aagggaataa gggcgacacg gaaatgttga atactcatac tcttcctttt 360

tcaatattat tgaagcattt atcagggtta ttgtctcatg agcggataca tatttgaatg 420

tatttagaaa aataaacaaa taggggttcc gcgcacattt ccccgaaaag tgccacctga 480

cgtctaagaa accattatta tcatgacatt aagcatccgc ctttcgtttt atttgaccat 540

gttggtatgt aatacgactc actatagata ggcggcgcat gagagaagcc cagaccaatt 600

acctacccaa ataggagaaa gttcacgttg acatcgagga agacagccca ttcctcagag 660

ctttgcagcg gagcttcccg cagtttgagg tagaagccaa gcaggtcact gataatgacc 720

atgctaatgc cagagcgttt tcgcatctgg cttcaaaact gatcgaaacg gaggtggacc 780

catccgacac gatccttgac attggaatag tcagcatagt acatttcatc tgactaatac 840

tacaacacca ccaccatgaa tagaggattc tttaacatgc tcggccgccg ccccttcccg 900

gcccccactg ccatgtggag gccgcggaga aggaggcagg cggccccggg aagcggagct 960

actaacttca gcctgctgaa gcaggctgga gacgtggagg agaaccctgg acct 1014

<210> 25
<211> 1014
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> g-block for Alpha-R-DLP-2A-nsp-DLP-rFF

<400> 25
tgtcatgcca tccgtaagat gcttttctgt gactggtgag tactcaacca agtcattctg 60

agaatagtgt atgcggcgac cgagttgctc ttgcccggcg tcaacacggg ataataccgc 120

gccacatagc agaactttaa aagtgctcat cattggaaaa cgttcttcgg ggcgaaaact 180

ctcaaggatc ttaccgctgt tgagatccag ttcgatgtaa cccactcgtg cacccaactg 240

atcttcagca tcttttactt tcaccagcgt ttctgggtga gcaaaaacag gaaggcaaaa 300

tgccgcaaaa aagggaataa gggcgacacg gaaatgttga atactcatac tcttcctttt 360

tcaatattat tgaagcattt atcagggtta ttgtctcatg agcggataca tatttgaatg 420

tatttagaaa aataaacaaa taggggttcc gcgcacattt ccccgaaaag tgccacctga 480

cgtctaagaa accattatta tcatgacatt aagcatccgc ctttcgtttt atttgaccat 540

gttggtatgt aatacgactc actatagata ggcggcgcat gagagaagcc cagaccaatt 600

acctacccaa ataggagaaa gttcacgttg acatcgagga agacagccca ttcctcagag 660

ctttgcagcg gagcttcccg cagtttgagg tagaagccaa gcaggtcact gataatgacc 720

atgctaatgc cagagcgttt tcgcatctgg cttcaaaact gatcgaaacg gaggtggacc 780

catccgacac gatccttgac attggaatag tcagcatagt acatttcatc tgactaatac 840

tacaacacca ccaccatgaa tagaggattc tttaacatgc tcggccgccg ccccttcccg 900

gcccccactg ccatgtggag gccgcggaga aggaggcagg cggccccggg aagcggagct 960

actaacttca gcctgctgaa gcaggctgga gacgtggagg agaaccctgg acct 1014

<210> 26
<211> 9476
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> construct Alpha-R-rFF

<220>
<221> misc_feature
<222> (1)...(18)
<223> T7 promoter

<400> 26
taatacgact cactatagat aggcggcgca tgagagaagc ccagaccaat tacctaccca 60

aaatggagaa agttcacgtt gacatcgagg aagacagccc attcctcaga gctttgcagc 120

ggagcttccc gcagtttgag gtagaagcca agcaggtcac tgataatgac catgctaatg 180

ccagagcgtt ttcgcatctg gcttcaaaac tgatcgaaac ggaggtggac ccatccgaca 240

cgatccttga cattggaagt gcgcccgccc gcagaatgta ttctaagcac aagtatcatt 300

gtatctgtcc gatgagatgt gcggaagatc cggacagatt gtataagtat gcaactaagc 360

tgaagaaaaa ctgtaaggaa ataactgata aggaattgga caagaaaatg aaggagctcg 420

ccgccgtcat gagcgaccct gacctggaaa ctgagactat gtgcctccac gacgacgagt 480

cgtgtcgcta cgaagggcaa gtcgctgttt accaggatgt atacgcggtt gacggaccga 540

caagtctcta tcaccaagcc aataagggag ttagagtcgc ctactggata ggctttgaca 600

ccacccttt tatgtttaag aacttggctg gagcatatcc atcatactct accaactggg 660

ccgacgaaac cgtgttaacg gctcgtaaca taggcctatg cagctctgac gttatggagc 720

ggtcacgtag agggatgtcc attcttagaa agaagtattt gaaaccatcc aacaatgttc 780

tattctctgt tggctcgacc atctaccacg agaagaggga cttactgagg agctggcacc 840

tgccgtctgt atttcactta cgtggcaagc aaaattacac atgtcggtgt gagactatag 900

ttagttgcga cgggtacgtc gttaaaagaa tagctatcag tccaggcctg tatgggaagc 960

cttcaggcta tgctgctacg atgcaccgcg agggattctt gtgctgcaaa gtgacagaca 1020

cattgaacgg ggagagggtc tcttttcccg tgtgcacgta tgtgccagct acattgtgtg 1080

accaaatgac tggcatactg gcaacagatg tcagtgcgga cgacgcgcaa aaactgctgg 1140

ttgggctcaa ccagcgtata gtcgtcaacg gtcgcaccca gagaaacacc aataccatga 1200

aaaattacct tttgcccgta gtggcccagg catttgctag gtgggcaaag gaatataagg 1260

aagatcaaga agatgaaagg ccactaggac tacgagatag acagttagtc atggggtgtt 1320

gttgggcttt tagaaggcac aagataacat ctatttataa gcgcccggat acccaaacca 1380

tcatcaaagt gaacagcgat ttccactcat tcgtgctgcc caggataggc agtaacacat 1440

tggagatcgg gctgagaaca agaatcagga aaatgttaga ggagcacaag gagccgtcac 1500

ctctcattac cgccgaggac gtacaagaag ctaagtgcgc agccgatgag gctaaggagg 1560

tgcgtgaagc cgaggagttg cgcgcagctc taccaccttt ggcagctgat gttgaggagc 1620

ccactctgga agccgatgtc gacttgatgt tacaagaggc tggggccggc tcagtggaga 1680

cacctcgtgg cttgataaag gttaccagct acgatggcga ggacaagatc ggctcttacg 1740

ctgtgctttc tccgcaggct gtactcaaga gtgaaaaatt atcttgcatc caccctctcg 1800

ctgaacaagt catagtgata acacactctg gccgaaaagg gcgttatgcc gtggaaccat 1860

accatggtaa agtagtggtg ccagagggac atgcaatacc cgtccaggac tttcaagctc 1920

tgagtgaaag tgccaccatt gtgtacaacg aacgtgagtt cgtaaacagg tacctgcacc 1980

atattgccac acatggagga gcgctgaaca ctgatgaaga atattacaaa actgtcaagc 2040

ccagcgagca cgacggcgaa tacctgtacg acatcgacag gaaacagtgc gtcaagaaag 2100

aactagtcac tgggctaggg ctcacaggcg agctggtgga tcctcccttc catgaattcg 2160

cctacgagag tctgagaaca cgaccagccg ctccttacca agtaccaacc ataggggtgt 2220

atggcgtgcc aggatcaggc aagtctggca tcattaaaag cgcagtcacc aaaaaagatc 2280

tagtggtgag cgccaagaaa gaaaactgtg cagaaattat aagggacgtc aagaaaatga 2340

aagggctgga cgtcaatgcc agaactgtgg actcagtgct cttgaatgga tgcaaacacc 2400

ccgtagagac cctgtatatt gacgaagctt ttgcttgtca tgcaggtact ctcagagcgc 2460

tcatagccat tataagacct aaaaaggcag tgctctgcgg ggatcccaaa cagtgcggtt 2520

tttttaacat gatgtgcctg aaagtgcatt ttaaccacga gatttgcaca caagtcttcc 2580

acaaaagcat ctctcgccgt tgcactaaat ctgtgacttc ggtcgtctca accttgtttt 2640

acgacaaaaa aatgagaacg acgaatccga aagagactaa gattgtgatt gacactaccg 2700

gcagtaccaa acctaagcag gacgatctca ttctcacttg tttcagaggg tgggtgaagc 2760

agttgcaaat agattacaaa ggcaacgaaa taatgacggc agctgcctct caagggctga 2820

cccgtaaagg tgtgtatgcc gttcggtaca aggtgaatga aaatcctctg tacgcaccca 2880

cctctgaaca tgtgaacgtc ctactgaccc gcacggagga ccgcatcgtg tggaaaacac 2940

tagccggcga cccatggata aaaacactga ctgccaagta ccctgggaat ttcactgcca 3000

cgatagagga gtggcaagca gagcatgatg ccatcatgag gcacatcttg gagagaccgg 3060

accctaccga cgtcttccag aataaggcaa acgtgtgttg ggccaaggct ttagtgccgg 3120

tgctgaagac cgctggcata gacatgacca ctgaacaatg gaacactgtg gattattttg 3180

aaacggacaa agctcactca gcagagatag tattgaacca actatgcgtg aggttctttg 3240

gactcgatct ggactccggt ctattttctg cacccactgt tccgttatcc attaggaata 3300

atcactggga taactccccg tcgcctaaca tgtacgggct gaataaagaa gtggtccgtc 3360

agctctctcg caggtaccca caactgcctc gggcagttgc cactggaaga gtctatgaca 3420

tgaacactgg tacactgcgc aattatgatc cgcgcataaa cctagtacct gtaaacagaa 3480

gactgcctca tgctttagtc ctccaccata atgaacaccc acagagtgac ttttcttcat 3540

tcgtcagcaa attgaagggc agaactgtcc tggtggtcgg ggaaaagttg tccgtcccag 3600

gcaaaatggt tgactggttg tcagaccggc ctgaggctac cttcagagct cggctggatt 3660

taggcatccc aggtgatgtg cccaaatatg acataatatt tgttaatgtg aggaccccat 3720

ataaatacca tcactatcag cagtgtgaag accatgccat taagcttagc atgttgacca 3780

agaaagcttg tctgcatctg aatcccggcg gaacctgtgt cagcataggt tatggttacg 3840

ctgacagggc cagcgaaagc atcattggtg ctatagcgcg gcagttcaag ttttcccggg 3900

tatgcaaacc gaaatcctca cttgaagaga cggaagttct gtttgtattc attgggtacg 3960

atcgcaaggc ccgtacgcac aatccttaca agctttcatc aaccttgacc aacatttata 4020

caggttccag actccacgaa gccggatgtg caccctcata tcatgtggtg cgaggggata 4080

ttgccacggc caccgaagga gtgattataa atgctgctaa cagcaaagga caacctggcg 4140

gaggggtgtg cggagcgctg tataagaaat tcccggaaag cttcgattta cagccgatcg 4200

aagtaggaaa agcgcgactg gtcaaaggtg cagctaaaca tatcattcat gccgtaggac 4260

caaacttcaa caaagtttcg gaggttgaag gtgacaaaca gttggcagag gcttatgagt 4320

ccatcgctaa gattgtcaac gataacaatt acaagtcagt agcgattcca ctgttgtcca 4380

ccggcatctt ttccgggaac aaagatcgac taacccaatc attgaaccat ttgctgacag 4440

ctttagacac cactgatgca gatgtagcca tatactgcag ggacaagaaa tgggaaatga 4500

ctctcaagga agcagtggct aggagagaag cagtggagga gatatgcata tccgacgact 4560

cttcagtgac agaacctgat gcagagctgg tgagggtgca tccgaagagt tctttggctg 4620

gaaggaaggg ctacagcaca agcgatggca aaactttctc atatttggaa gggaccaagt 4680

ttcaccaggc ggccaaggat atagcagaaa ttaatgccat gtggcccgtt gcaacggagg 4740

ccaatgagca ggtatgcatg tatatcctcg gagaaagcat gagcagtatt aggtcgaaat 4800

gccccgtcga agagtcggaa gcctccacac cacctagcac gctgccttgc ttgtgcatcc 4860

atgccatgac tccagaaaga gtacagcgcc taaaagcctc acgtccagaa caaattactg 4920

tgtgctcatc ctttccattg ccgaagtata gaatcactgg tgtgcagaag atccaatgct 4980

cccagcctat attgttctca ccgaaagtgc ctgcgtatat tcatccaagg aagtatctcg 5040

tggaaacacc accggtagac gagactccgg agccatcggc agagaaccaa tccacagagg 5100

ggacacctga acaaccacca cttataaccg aggatgagac caggactaga acgcctgagc 5160

cgatcatcat cgaagaggaa gaagaggata gcataagttt gctgtcagat ggcccgaccc 5220

accaggtgct gcaagtcgag gcagacattc acgggccgcc ctctgtatct agctcatcct 5280

ggtccattcc tcatgcatcc gactttgatg tggacagttt atccatactt gacaccctgg 5340

agggagctag cgtgaccagc ggggcaacgt cagccgagac taactcttac ttcgcaaaga 5400

gtatggagtt tctggcgcga ccggtgcctg cgcctcgaac agtattcagg aaccctccac 5460

atcccgctcc gcgcacaaga acaccgtcac ttgcacccag cagggcctgc tcgagaacca 5520

gcctagtttc caccccgcca ggcgtgaata gggtgatcac tagagaggag ctcgaggcgc 5580

ttaccccgtc acgcactcct agcaggtcgg tctcgagaac cagcctggtc tccaacccgc 5640

caggcgtaaa tagggtgatt acaagagagg agtttgaggc gttcgtagca caacaacaat 5700

gacggtttga tgcgggtgca tacatctttt cctccgacac cggtcaaggg catttacaac 5760

aaaaatcagt aaggcaaacg gtgctatccg aagtggtgtt ggagaggacc gaattggaga 5820

tttcgtatgc cccgcgcctc gaccaagaaa aagaagaatt actacgcaag aaattacagt 5880

taaatcccac acctgctaac agaagcagat accagtccag gaaggtggag aacatgaaag 5940

ccataacagc tagacgtatt ctgcaaggcc tagggcatta tttgaaggca gaaggaaaag 6000

tggagtgcta ccgaaccctg catcctgttc ctttgtattc atctagtgtg aaccgtgcct 6060

tttcaagccc caaggtcgca gtggaagcct gtaacgccat gttgaaagag aactttccga 6120

ctgtggcttc ttactgtatt attccagagt acgatgccta tttggacatg gttgacggag 6180

cttcatgctg cttagacact gccagttttt gccctgcaaa gctgcgcagc tttccaaaga 6240

aacactccta tttggaaccc acaatacgat cggcagtgcc ttcagcgatc cagaacacgc 6300

tccagaacgt cctggcagct gccacaaaaa gaaattgcaa tgtcacgcaa atgagagaat 6360

tgcccgtatt ggattcggcg gcctttaatg tggaatgctt caagaaatat gcgtgtaata 6420

atgaatattg ggaaacgttt aaagaaaacc ccatcaggct tactgaagaa aacgtggtaa 6480

attacattac caaattaaaa ggaccaaaag ctgctgctct ttttgcgaag acacataatt 6540

tgaatatgtt gcaggacata ccaatggaca ggtttgtaat ggacttaaag agagacgtga 6600

aagtgactcc aggaacaaaa catactgaag aacggcccaa ggtacaggtg atccaggctg 6660

ccgatccgct agcaacagcg tatctgtgcg gaatccaccg agagctggtt aggagattaa 6720

atgcggtcct gcttccgaac attcatacac tgtttgatat gtcggctgaa gactttgacg 6780

ctattatagc cgagcacttc cagcctgggg attgtgttct ggaaactgac atcgcgtcgt 6840

ttgataaaag tgaggacgac gccatggctc tgaccgcgtt aatgattctg gaagacttag 6900

gtgtggacgc agagctgttg acgctgattg aggcggcttt cggcgaaatt tcatcaatac 6960

atttgcccac taaaactaaa tttaaattcg gagccatgat gaaatctgga atgttcctca 7020

cactgtttgt gaacacagtc attaacattg taatcgcaag cagagtgttg agagaacggc 7080

taaccggatc accatgtgca gcattcattg gagatgacaa tatcgtgaaa ggagtcaaat 7140

cggacaaatt aatggcagac aggtgcgcca cctggttgaa tatggaagtc aagattatag 7200

atgctgtggt gggcgagaaa gcgccttatt tctgtggagg gtttattttg tgtgactccg 7260

tgaccggcac agcgtgccgt gtggcagacc ccctaaaaag gctgtttaag cttggcaaac 7320

ctctggcagc agacgatgaa catgatgatg acaggagaag ggcattgcat gaagagtcaa 7380

cacgctggaa ccgagtgggt attctttcag agctgtgcaa ggcagtagaa tcaaggtatg 7440

aaaccgtagg aacttccatc atagttatgg ccatgactac tctagctagc agtgttaaat 7500

cattcagcta cctgagaggg gcccctataa ctctctacgg ctaacctgaa tggactacga 7560

catagtctag tccgccaaga tatcgcacca tggaaaatat ggaaaacgac gagaacatcg 7620

tggtgggccc caagcccttc taccccatcg aggaaggcag cgccggcacc cagctgcgga 7680

agtacatgga aagatacgcc aagctgggcg ccattgcctt caccaacgcc gtgaccggcg 7740

tggactacag ctacgccgag tacctggaaa agagctgctg cctgggcaag gctctgcaga 7800

actacggcct ggtggtggac ggccggatcg ccctgtgcag cgagaactgc gaggaattct 7860

tcatccccgt gatcgccggc ctgttcatcg gcgtgggcgt ggctcccacc aacgagatct 7920

acaccctgcg ggagctggtg cacagcctgg gcatcagcaa gcccaccatc gtgttcagca 7980

gcaagaaggg cctggacaaa gtcatcaccg tgcagaaaac cgtgaccacc atcaagacca 8040

tcgtgatcct ggacagcaag gtggactacc ggggctacca gtgcctggac accttcatca 8100

agcggaacac ccccccctggc ttccaggcca gcagcttcaa gaccgtggag gtggaccgga 8160

aagaacaggt ggccctgatc atgaacagca gcggcagcac cggcctgccc aagggcgtgc 8220

agctgaccca cgagaacacc gtgacccggt tcagccacgc cagggacccc atctacggca 8280

accaggtgtc ccccggcacc gccgtgctga ccgtggtgcc cttccaccac ggcttcggca 8340

tgttcaccac cctgggctac ctgatctgcg gcttccgggt ggtgatgctg accaagttcg 8400

acgaggaaac cttcctgaaa accctgcagg actacaagtg cacctacgtg attctggtgc 8460

ccaccctgtt cgccatcctg aacaagagcg agctgctgaa caagtacgac ctgagcaacc 8520

tggtggagat cgccagcggc ggagcccccc tgagcaaaga agtgggagag gccgtcgcca 8580

ggcggttcaa tctgcccggc gtgcggcagg gctacggcct gaccgagaca accagcgcca 8640

tcatcatcac ccccgagggc gacgacaagc ctggagccag cggcaaggtg gtgcccctgt 8700

tcaaggccaa agtgatcgac ctggacacca agaagagcct gggccccaac agacggggcg 8760

aagtgtgcgt gaagggcccc atgctgatga agggctacgt gaacaacccc gaggccacca 8820

aagagctgat cgacgaagag ggctggctgc acaccggcga catcggctac tacgacgaag 8880

agaagcactt cttcatcgtg gaccggctga agagcctgat caagtacaag ggctatcagg 8940

tgccccctgc cgagctggaa agcgtcctgc tgcagcaccc cagcatcttc gacgccggcg 9000

tggccggggt gccagatcct gtggccggcg agctgcctgg cgccgtggtg gtgctggaat 9060

ccggcaagaa catgaccgag aaagaagtga tggactacgt cgccagccag gtgtccaacg 9120

ccaagcggct gagaggcggc gtgagattcg tggacgaagt gccaaagggc ctgaccggca 9180

agatcgacgg cagggccatc cgggagatcc tgaagaaacc cgtggccaag atgtgattaa 9240

ttgatcgata cagcagcaat tggcaagctg cttacataga aggcgcgccg tttaaacggc 9300

cggccttaat taagtaacga tacagcagca attggcaagc tgcttacata gaactcgcgg 9360

cgattggcat gccgctttaa aatttttatt ttatttttct tttcttttcc gaatcggatt 9420

ttgtttttaa tatttcaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaa 9476

<210> 27
<211> 9621
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> construct Alpha-R-DLP-rFF

<220>
<221> misc_feature
<222> (1)...(18)
<223> T7 promoter

<400> 27
taatacgact cactatagat aggcggcgca tgagagaagc ccagaccaat tacctaccca 60

aaatggagaa agttcacgtt gacatcgagg aagacagccc attcctcaga gctttgcagc 120

ggagcttccc gcagtttgag gtagaagcca agcaggtcac tgataatgac catgctaatg 180

ccagagcgtt ttcgcatctg gcttcaaaac tgatcgaaac ggaggtggac ccatccgaca 240

cgatccttga cattggaagt gcgcccgccc gcagaatgta ttctaagcac aagtatcatt 300

gtatctgtcc gatgagatgt gcggaagatc cggacagatt gtataagtat gcaactaagc 360

tgaagaaaaa ctgtaaggaa ataactgata aggaattgga caagaaaatg aaggagctcg 420

ccgccgtcat gagcgaccct gacctggaaa ctgagactat gtgcctccac gacgacgagt 480

cgtgtcgcta cgaagggcaa gtcgctgttt accaggatgt atacgcggtt gacggaccga 540

caagtctcta tcaccaagcc aataagggag ttagagtcgc ctactggata ggctttgaca 600

ccaccccttt tatgtttaag aacttggctg gagcatatcc atcatactct accaactggg 660

ccgacgaaac cgtgttaacg gctcgtaaca taggcctatg cagctctgac gttatggagc 720

ggtcacgtag agggatgtcc attcttagaa agaagtattt gaaaccatcc aacaatgttc 780

tattctctgt tggctcgacc atctaccacg agaagaggga cttactgagg agctggcacc 840

tgccgtctgt atttcactta cgtggcaagc aaaattacac atgtcggtgt gagactatag 900

ttagttgcga cgggtacgtc gttaaaagaa tagctatcag tccaggcctg tatgggaagc 960

cttcaggcta tgctgctacg atgcaccgcg agggattctt gtgctgcaaa gtgacagaca 1020

cattgaacgg ggagagggtc tcttttcccg tgtgcacgta tgtgccagct acattgtgtg 1080

accaaatgac tggcatactg gcaacagatg tcagtgcgga cgacgcgcaa aaactgctgg 1140

ttgggctcaa ccagcgtata gtcgtcaacg gtcgcaccca gagaaacacc aataccatga 1200

aaaattacct tttgcccgta gtggcccagg catttgctag gtgggcaaag gaatataagg 1260

aagatcaaga agatgaaagg ccactaggac tacgagatag acagttagtc atggggtgtt 1320

gttgggcttt tagaaggcac aagataacat ctatttataa gcgcccggat acccaaacca 1380

tcatcaaagt gaacagcgat ttccactcat tcgtgctgcc caggataggc agtaacacat 1440

tggagatcgg gctgagaaca agaatcagga aaatgttaga ggagcacaag gagccgtcac 1500

ctctcattac cgccgaggac gtacaagaag ctaagtgcgc agccgatgag gctaaggagg 1560

tgcgtgaagc cgaggagttg cgcgcagctc taccaccttt ggcagctgat gttgaggagc 1620

ccactctgga agccgatgtc gacttgatgt tacaagaggc tggggccggc tcagtggaga 1680

cacctcgtgg cttgataaag gttaccagct acgatggcga ggacaagatc ggctcttacg 1740

ctgtgctttc tccgcaggct gtactcaaga gtgaaaaatt atcttgcatc caccctctcg 1800

ctgaacaagt catagtgata acacactctg gccgaaaagg gcgttatgcc gtggaaccat 1860

accatggtaa agtagtggtg ccagagggac atgcaatacc cgtccaggac tttcaagctc 1920

tgagtgaaag tgccaccatt gtgtacaacg aacgtgagtt cgtaaacagg tacctgcacc 1980

atattgccac acatggagga gcgctgaaca ctgatgaaga atattacaaa actgtcaagc 2040

ccagcgagca cgacggcgaa tacctgtacg acatcgacag gaaacagtgc gtcaagaaag 2100

aactagtcac tgggctaggg ctcacaggcg agctggtgga tcctcccttc catgaattcg 2160

cctacgagag tctgagaaca cgaccagccg ctccttacca agtaccaacc ataggggtgt 2220

atggcgtgcc aggatcaggc aagtctggca tcattaaaag cgcagtcacc aaaaaagatc 2280

tagtggtgag cgccaagaaa gaaaactgtg cagaaattat aagggacgtc aagaaaatga 2340

aagggctgga cgtcaatgcc agaactgtgg actcagtgct cttgaatgga tgcaaacacc 2400

ccgtagagac cctgtatatt gacgaagctt ttgcttgtca tgcaggtact ctcagagcgc 2460

tcatagccat tataagacct aaaaaggcag tgctctgcgg ggatcccaaa cagtgcggtt 2520

tttttaacat gatgtgcctg aaagtgcatt ttaaccacga gatttgcaca caagtcttcc 2580

acaaaagcat ctctcgccgt tgcactaaat ctgtgacttc ggtcgtctca accttgtttt 2640

acgacaaaaa aatgagaacg acgaatccga aagagactaa gattgtgatt gacactaccg 2700

gcagtaccaa acctaagcag gacgatctca ttctcacttg tttcagaggg tgggtgaagc 2760

agttgcaaat agattacaaa ggcaacgaaa taatgacggc agctgcctct caagggctga 2820

cccgtaaagg tgtgtatgcc gttcggtaca aggtgaatga aaatcctctg tacgcaccca 2880

cctctgaaca tgtgaacgtc ctactgaccc gcacggagga ccgcatcgtg tggaaaacac 2940

tagccggcga cccatggata aaaacactga ctgccaagta ccctgggaat ttcactgcca 3000

cgatagagga gtggcaagca gagcatgatg ccatcatgag gcacatcttg gagagaccgg 3060

accctaccga cgtcttccag aataaggcaa acgtgtgttg ggccaaggct ttagtgccgg 3120

tgctgaagac cgctggcata gacatgacca ctgaacaatg gaacactgtg gattattttg 3180

aaacggacaa agctcactca gcagagatag tattgaacca actatgcgtg aggttctttg 3240

gactcgatct ggactccggt ctattttctg cacccactgt tccgttatcc attaggaata 3300

atcactggga taactccccg tcgcctaaca tgtacgggct gaataaagaa gtggtccgtc 3360

agctctctcg caggtaccca caactgcctc gggcagttgc cactggaaga gtctatgaca 3420

tgaacactgg tacactgcgc aattatgatc cgcgcataaa cctagtacct gtaaacagaa 3480

gactgcctca tgctttagtc ctccaccata atgaacaccc acagagtgac ttttcttcat 3540

tcgtcagcaa attgaagggc agaactgtcc tggtggtcgg ggaaaagttg tccgtcccag 3600

gcaaaatggt tgactggttg tcagaccggc ctgaggctac cttcagagct cggctggatt 3660

taggcatccc aggtgatgtg cccaaatatg acataatatt tgttaatgtg aggaccccat 3720

ataaatacca tcactatcag cagtgtgaag accatgccat taagcttagc atgttgacca 3780

agaaagcttg tctgcatctg aatcccggcg gaacctgtgt cagcataggt tatggttacg 3840

ctgacagggc cagcgaaagc atcattggtg ctatagcgcg gcagttcaag ttttcccggg 3900

tatgcaaacc gaaatcctca cttgaagaga cggaagttct gtttgtattc attgggtacg 3960

atcgcaaggc ccgtacgcac aatccttaca agctttcatc aaccttgacc aacatttata 4020

caggttccag actccacgaa gccggatgtg caccctcata tcatgtggtg cgaggggata 4080

ttgccacggc caccgaagga gtgattataa atgctgctaa cagcaaagga caacctggcg 4140

gaggggtgtg cggagcgctg tataagaaat tcccggaaag cttcgattta cagccgatcg 4200

aagtaggaaa agcgcgactg gtcaaaggtg cagctaaaca tatcattcat gccgtaggac 4260

caaacttcaa caaagtttcg gaggttgaag gtgacaaaca gttggcagag gcttatgagt 4320

ccatcgctaa gattgtcaac gataacaatt acaagtcagt agcgattcca ctgttgtcca 4380

ccggcatctt ttccgggaac aaagatcgac taacccaatc attgaaccat ttgctgacag 4440

ctttagacac cactgatgca gatgtagcca tatactgcag ggacaagaaa tgggaaatga 4500

ctctcaagga agcagtggct aggagagaag cagtggagga gatatgcata tccgacgact 4560

cttcagtgac agaacctgat gcagagctgg tgagggtgca tccgaagagt tctttggctg 4620

gaaggaaggg ctacagcaca agcgatggca aaactttctc atatttggaa gggaccaagt 4680

ttcaccaggc ggccaaggat atagcagaaa ttaatgccat gtggcccgtt gcaacggagg 4740

ccaatgagca ggtatgcatg tatatcctcg gagaaagcat gagcagtatt aggtcgaaat 4800

gccccgtcga agagtcggaa gcctccacac cacctagcac gctgccttgc ttgtgcatcc 4860

atgccatgac tccagaaaga gtacagcgcc taaaagcctc acgtccagaa caaattactg 4920

tgtgctcatc ctttccattg ccgaagtata gaatcactgg tgtgcagaag atccaatgct 4980

cccagcctat attgttctca ccgaaagtgc ctgcgtatat tcatccaagg aagtatctcg 5040

tggaaacacc accggtagac gagactccgg agccatcggc agagaaccaa tccacagagg 5100

ggacacctga acaaccacca cttataaccg aggatgagac caggactaga acgcctgagc 5160

cgatcatcat cgaagaggaa gaagaggata gcataagttt gctgtcagat ggcccgaccc 5220

accaggtgct gcaagtcgag gcagacattc acgggccgcc ctctgtatct agctcatcct 5280

ggtccattcc tcatgcatcc gactttgatg tggacagttt atccatactt gacaccctgg 5340

agggagctag cgtgaccagc ggggcaacgt cagccgagac taactcttac ttcgcaaaga 5400

gtatggagtt tctggcgcga ccggtgcctg cgcctcgaac agtattcagg aaccctccac 5460

atcccgctcc gcgcacaaga acaccgtcac ttgcacccag cagggcctgc tcgagaacca 5520

gcctagtttc caccccgcca ggcgtgaata gggtgatcac tagagaggag ctcgaggcgc 5580

ttaccccgtc acgcactcct agcaggtcgg tctcgagaac cagcctggtc tccaacccgc 5640

caggcgtaaa tagggtgatt acaagagagg agtttgaggc gttcgtagca caacaacaat 5700

gacggtttga tgcgggtgca tacatctttt cctccgacac cggtcaaggg catttacaac 5760

aaaaatcagt aaggcaaacg gtgctatccg aagtggtgtt ggagaggacc gaattggaga 5820

tttcgtatgc cccgcgcctc gaccaagaaa aagaagaatt actacgcaag aaattacagt 5880

taaatcccac acctgctaac agaagcagat accagtccag gaaggtggag aacatgaaag 5940

ccataacagc tagacgtatt ctgcaaggcc tagggcatta tttgaaggca gaaggaaaag 6000

tggagtgcta ccgaaccctg catcctgttc ctttgtattc atctagtgtg aaccgtgcct 6060

tttcaagccc caaggtcgca gtggaagcct gtaacgccat gttgaaagag aactttccga 6120

ctgtggcttc ttactgtatt attccagagt acgatgccta tttggacatg gttgacggag 6180

cttcatgctg cttagacact gccagttttt gccctgcaaa gctgcgcagc tttccaaaga 6240

aacactccta tttggaaccc acaatacgat cggcagtgcc ttcagcgatc cagaacacgc 6300

tccagaacgt cctggcagct gccacaaaaa gaaattgcaa tgtcacgcaa atgagagaat 6360

tgcccgtatt ggattcggcg gcctttaatg tggaatgctt caagaaatat gcgtgtaata 6420

atgaatattg ggaaacgttt aaagaaaacc ccatcaggct tactgaagaa aacgtggtaa 6480

attacattac caaattaaaa ggaccaaaag ctgctgctct ttttgcgaag acacataatt 6540

tgaatatgtt gcaggacata ccaatggaca ggtttgtaat ggacttaaag agagacgtga 6600

aagtgactcc aggaacaaaa catactgaag aacggcccaa ggtacaggtg atccaggctg 6660

ccgatccgct agcaacagcg tatctgtgcg gaatccaccg agagctggtt aggagattaa 6720

atgcggtcct gcttccgaac attcatacac tgtttgatat gtcggctgaa gactttgacg 6780

ctattatagc cgagcacttc cagcctgggg attgtgttct ggaaactgac atcgcgtcgt 6840

ttgataaaag tgaggacgac gccatggctc tgaccgcgtt aatgattctg gaagacttag 6900

gtgtggacgc agagctgttg acgctgattg aggcggcttt cggcgaaatt tcatcaatac 6960

atttgcccac taaaactaaa tttaaattcg gagccatgat gaaatctgga atgttcctca 7020

cactgtttgt gaacacagtc attaacattg taatcgcaag cagagtgttg agagaacggc 7080

taaccggatc accatgtgca gcattcattg gagatgacaa tatcgtgaaa ggagtcaaat 7140

cggacaaatt aatggcagac aggtgcgcca cctggttgaa tatggaagtc aagattatag 7200

atgctgtggt gggcgagaaa gcgccttatt tctgtggagg gtttattttg tgtgactccg 7260

tgaccggcac agcgtgccgt gtggcagacc ccctaaaaag gctgtttaag cttggcaaac 7320

ctctggcagc agacgatgaa catgatgatg acaggagaag ggcattgcat gaagagtcaa 7380

cacgctggaa ccgagtgggt attctttcag agctgtgcaa ggcagtagaa tcaaggtatg 7440

aaaccgtagg aacttccatc atagttatgg ccatgactac tctagctagc agtgttaaat 7500

cattcagcta cctgagaggg gcccctataa ctctctacgg ctaacctgaa tggactacga 7560

catagtctag tccgccaaga tatcgcacca tagtcagcat agtacatttc atctgactaa 7620

tactacaaca ccaccaccat gaatagagga ttctttaaca tgctcggccg ccgccccttc 7680

ccggccccca ctgccatgtg gaggccgcgg agaaggaggc aggcggcccc gatgatggaa 7740

aatatggaaa acgacgagaa catcgtggtg ggccccaagc ccttctaccc catcgaggaa 7800

ggcagcgccg gcacccagct gcggaagtac atggaaagat acgccaagct gggcgccatt 7860

gccttcacca acgccgtgac cggcgtggac tacagctacg ccgagtacct ggaaaagagc 7920

tgctgcctgg gcaaggctct gcagaactac ggcctggtgg tggacggccg gatcgccctg 7980

tgcagcgaga actgcgagga attcttcatc cccgtgatcg ccggcctgtt catcggcgtg 8040

ggcgtggctc ccaccaacga gatctacacc ctgcgggagc tggtgcacag cctgggcatc 8100

agcaagccca ccatcgtgtt cagcagcaag aagggcctgg acaaagtcat caccgtgcag 8160

aaaaccgtga ccaccatcaa gaccatcgtg atcctggaca gcaaggtgga ctaccggggc 8220

taccagtgcc tggacacctt catcaagcgg aacacccccc ctggcttcca ggccagcagc 8280

ttcaagaccg tggaggtgga ccggaaagaa caggtggccc tgatcatgaa cagcagcggc 8340

agcaccggcc tgcccaaggg cgtgcagctg acccacgaga acaccgtgac ccggttcagc 8400

cacgccaggg accccatcta cggcaaccag gtgtcccccg gcaccgccgt gctgaccgtg 8460

gtgcccttcc accacggctt cggcatgttc accaccctgg gctacctgat ctgcggcttc 8520

cgggtggtga tgctgaccaa gttcgacgag gaaaccttcc tgaaaaccct gcaggactac 8580

aagtgcacct acgtgattct ggtgcccacc ctgttcgcca tcctgaacaa gagcgagctg 8640

ctgaacaagt acgacctgag caacctggtg gagatcgcca gcggcggagc ccccctgagc 8700

aaagaagtgg gagaggccgt cgccaggcgg ttcaatctgc ccggcgtgcg gcagggctac 8760

ggcctgaccg agacaaccag cgccatcatc atcacccccg agggcgacga caagcctgga 8820

gccagcggca aggtggtgcc cctgttcaag gccaaagtga tcgacctgga caccaagaag 8880

agcctgggcc ccaacagacg gggcgaagtg tgcgtgaagg gccccatgct gatgaagggc 8940

tacgtgaaca accccgaggc caccaaagag ctgatcgacg aagagggctg gctgcacacc 9000

ggcgacatcg gctactacga cgaagagaag cacttcttca tcgtggaccg gctgaagagc 9060

ctgatcaagt acaagggcta tcaggtgccc cctgccgagc tggaaagcgt cctgctgcag 9120

caccccagca tcttcgacgc cggcgtggcc ggggtgccag atcctgtggc cggcgagctg 9180

cctggcgccg tggtggtgct ggaatccggc aagaacatga ccgagaaaga agtgatggac 9240

tacgtcgcca gccaggtgtc caacgccaag cggctgagag gcggcgtgag attcgtggac 9300

gaagtgccaa agggcctgac cggcaagatc gacggcaggg ccatccggga gatcctgaag 9360

aaacccgtgg ccaagatgtg attaattgat cgatacagca gcaattggca agctgcttac 9420

atagaaggcg cgccgtttaa acggccggcc ttaattaagt aacgatacag cagcaattgg 9480

caagctgctt acatagaact cgcggcgatt ggcatgccgc tttaaaattt ttattttatt 9540

tttcttttct tttccgaatc ggattttgtt tttaatattt caaaaaaaaa aaaaaaaaaa 9600

aaaaaaaaaa aaaaaaaaaa a 9621

<210> 28
<211> 9876
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> construct Alpha-R-DLP-2A-nsp-rFF

<220>
<221> misc_feature
<222> (1)...(18)
<223> T7 promoter

<400> 28

taatacgact cactatagat aggcggcgca tgagagaagc ccagaccaat tacctaccca 60

aataggagaa agttcacgtt gacatcgagg aagacagccc attcctcaga gctttgcagc 120

ggagcttccc gcagtttgag gtagaagcca agcaggtcac tgataatgac catgctaatg 180

ccagagcgtt ttcgcatctg gcttcaaaac tgatcgaaac ggaggtggac ccatccgaca 240

cgatccttga cattggaata gtcagcatag tacatttcat ctgactaata ctacaacacc 300

accaccatga atagaggatt ctttaacatg ctcggccgcc gccccttccc ggcccccact 360

gccatgtgga ggccgcggag aaggaggcag gcggccccgg gaagcggagc tactaacttc 420

agcctgctga agcaggctgg agacgtggag gagaaccctg gacctgagaa agttcacgtt 480

gacatcgagg aagacagccc attcctcaga gctttgcagc ggagcttccc gcagtttgag 540

gtagaagcca agcaggtcac tgataatgac catgctaatg ccagagcgtt ttcgcatctg 600

gcttcaaaac tgatcgaaac ggaggtggac ccatccgaca cgatccttga cattggaagt 660

gcgcccgccc gcagaatgta ttctaagcac aagtatcatt gtatctgtcc gatgagatgt 720

gcggaagatc cggacagatt gtataagtat gcaactaagc tgaagaaaaa ctgtaaggaa 780

ataactgata aggaattgga caagaaaatg aaggagctcg ccgccgtcat gagcgaccct 840

gacctggaaa ctgagactat gtgcctccac gacgacgagt cgtgtcgcta cgaagggcaa 900

gtcgctgttt accaggatgt atacgcggtt gacggaccga caagtctcta tcaccaagcc 960

aataagggag ttagagtcgc ctactggata ggctttgaca ccaccccttt tatgtttaag 1020

aacttggctg gagcatatcc atcatactct accaactggg ccgacgaaac cgtgttaacg 1080

gctcgtaaca taggcctatg cagctctgac gttatggagc ggtcacgtag agggatgtcc 1140

attcttagaa agaagtattt gaaaccatcc aacaatgttc tattctctgt tggctcgacc 1200

atctaccacg agaagaggga cttactgagg agctggcacc tgccgtctgt atttcactta 1260

cgtggcaagc aaaattacac atgtcggtgt gagactatag ttagttgcga cgggtacgtc 1320

gttaaaagaa tagctatcag tccaggcctg tatgggaagc cttcaggcta tgctgctacg 1380

atgcaccgcg agggattctt gtgctgcaaa gtgacagaca cattgaacgg ggagagggtc 1440

tcttttcccg tgtgcacgta tgtgccagct acattgtgtg accaaatgac tggcatactg 1500

gcaacagatg tcagtgcgga cgacgcgcaa aaactgctgg ttgggctcaa ccagcgtata 1560

gtcgtcaacg gtcgcaccca gagaaacacc aataccatga aaaattacct tttgcccgta 1620

gtggcccagg catttgctag gtgggcaaag gaatataagg aagatcaaga agatgaaagg 1680

ccactaggac tacgagatag acagttagtc atggggtgtt gttgggcttt tagaaggcac 1740

aagataacat ctatttataa gcgcccggat acccaaacca tcatcaaagt gaacagcgat 1800

ttccactcat tcgtgctgcc caggataggc agtaacacat tggagatcgg gctgagaaca 1860

agaatcagga aaatgttaga ggagcacaag gagccgtcac ctctcattac cgccgaggac 1920

gtacaagaag ctaagtgcgc agccgatgag gctaaggagg tgcgtgaagc cgaggagttg 1980

cgcgcagctc taccaccttt ggcagctgat gttgaggagc ccactctgga agccgatgtc 2040

gacttgatgt tacaagaggc tggggccggc tcagtggaga cacctcgtgg cttgataaag 2100

gttaccagct acgatggcga ggacaagatc ggctcttacg ctgtgctttc tccgcaggct 2160

gtactcaaga gtgaaaaatt atcttgcatc caccctctcg ctgaacaagt catagtgata 2220

acacactctg gccgaaaagg gcgttatgcc gtggaaccat accatggtaa agtagtggtg 2280

ccagagggac atgcaatacc cgtccaggac tttcaagctc tgagtgaaag tgccaccatt 2340

gtgtacaacg aacgtgagtt cgtaaacagg tacctgcacc atattgccac acatggagga 2400

gcgctgaaca ctgatgaaga atattacaaa actgtcaagc ccagcgagca cgacggcgaa 2460

tacctgtacg acatcgacag gaaacagtgc gtcaagaaag aactagtcac tgggctaggg 2520

ctcacaggcg agctggtgga tcctcccttc catgaattcg cctacgagag tctgagaaca 2580

cgaccagccg ctccttacca agtaccaacc ataggggtgt atggcgtgcc aggatcaggc 2640

aagtctggca tcattaaaag cgcagtcacc aaaaaagatc tagtggtgag cgccaagaaa 2700

gaaaactgtg cagaaattat aagggacgtc aagaaaatga aagggctgga cgtcaatgcc 2760

agaactgtgg actcagtgct cttgaatgga tgcaaacacc ccgtagagac cctgtatatt 2820

gacgaagctt ttgcttgtca tgcaggtact ctcagagcgc tcatagccat tataagacct 2880

aaaaaggcag tgctctgcgg ggatcccaaa cagtgcggtt tttttaacat gatgtgcctg 2940

aaagtgcatt ttaaccacga gatttgcaca caagtcttcc acaaaagcat ctctcgccgt 3000

tgcactaaat ctgtgacttc ggtcgtctca accttgtttt acgacaaaaa aatgagaacg 3060

acgaatccga aagagactaa gattgtgatt gacactaccg gcagtaccaa acctaagcag 3120

gacgatctca ttctcacttg tttcagaggg tgggtgaagc agttgcaaat agattacaaa 3180

ggcaacgaaa taatgacggc agctgcctct caagggctga cccgtaaagg tgtgtatgcc 3240

gttcggtaca aggtgaatga aaatcctctg tacgcaccca cctctgaaca tgtgaacgtc 3300

ctactgaccc gcacggagga ccgcatcgtg tggaaaacac tagccggcga cccatggata 3360

aaaacactga ctgccaagta ccctgggaat ttcactgcca cgatagagga gtggcaagca 3420

gagcatgatg ccatcatgag gcacatcttg gagagaccgg accctaccga cgtcttccag 3480

aataaggcaa acgtgtgttg ggccaaggct ttagtgccgg tgctgaagac cgctggcata 3540

gacatgacca ctgaacaatg gaacactgtg gattattttg aaacggacaa agctcactca 3600

gcagagatag tattgaacca actatgcgtg aggttctttg gactcgatct ggactccggt 3660

ctattttctg cacccactgt tccgttatcc attaggaata atcactggga taactccccg 3720

tcgcctaaca tgtacgggct gaataaagaa gtggtccgtc agctctctcg caggtaccca 3780

caactgcctc gggcagttgc cactggaaga gtctatgaca tgaacactgg tacactgcgc 3840

aattatgatc cgcgcataaa cctagtacct gtaaacagaa gactgcctca tgctttagtc 3900

ctccaccata atgaacaccc acagagtgac ttttcttcat tcgtcagcaa attgaagggc 3960

agaactgtcc tggtggtcgg ggaaaagttg tccgtcccag gcaaaatggt tgactggttg 4020

tcagaccggc ctgaggctac cttcagagct cggctggatt taggcatccc aggtgatgtg 4080

cccaaatatg acataatatt tgttaatgtg aggaccccat ataaatacca tcactatcag 4140

cagtgtgaag accatgccat taagcttagc atgttgacca agaaagcttg tctgcatctg 4200

aatcccggcg gaacctgtgt cagcataggt tatggttacg ctgacagggc cagcgaaagc 4260

atcattggtg ctatagcgcg gcagttcaag ttttcccggg tatgcaaacc gaaatcctca 4320

cttgaagaga cggaagttct gtttgtattc attgggtacg atcgcaaggc ccgtacgcac 4380

aatccttaca agctttcatc aaccttgacc aacatttata caggttccag actccacgaa 4440

gccggatgtg caccctcata tcatgtggtg cgaggggata ttgccacggc caccgaagga 4500

gtgattataa atgctgctaa cagcaaagga caacctggcg gaggggtgtg cggagcgctg 4560

tataagaaat tcccggaaag cttcgattta cagccgatcg aagtaggaaa agcgcgactg 4620

gtcaaaggtg cagctaaaca tatcattcat gccgtaggac caaacttcaa caaagtttcg 4680

gaggttgaag gtgacaaaca gttggcagag gcttatgagt ccatcgctaa gattgtcaac 4740

gataacaatt acaagtcagt agcgattcca ctgttgtcca ccggcatctt ttccgggaac 4800

aaagatcgac taacccaatc attgaaccat ttgctgacag ctttagacac cactgatgca 4860

gatgtagcca tatactgcag ggacaagaaa tgggaaatga ctctcaagga agcagtggct 4920

aggagagaag cagtggagga gatatgcata tccgacgact cttcagtgac agaacctgat 4980

gcagagctgg tgagggtgca tccgaagagt tctttggctg gaaggaaggg ctacagcaca 5040

agcgatggca aaactttctc atatttggaa gggaccaagt ttcaccaggc ggccaaggat 5100

atagcagaaa ttaatgccat gtggcccgtt gcaacggagg ccaatgagca ggtatgcatg 5160

tatatcctcg gagaaagcat gagcagtatt aggtcgaaat gccccgtcga agagtcggaa 5220

gcctccacac cacctagcac gctgccttgc ttgtgcatcc atgccatgac tccagaaaga 5280

gtacagcgcc taaaagcctc acgtccagaa caaattactg tgtgctcatc ctttccattg 5340

ccgaagtata gaatcactgg tgtgcagaag atccaatgct cccagcctat attgttctca 5400

ccgaaagtgc ctgcgtatat tcatccaagg aagtatctcg tggaaacacc accggtagac 5460

gagactccgg agccatcggc agagaaccaa tccacagagg ggacacctga acaaccacca 5520

cttataaccg aggatgagac caggactaga acgcctgagc cgatcatcat cgaagaggaa 5580

gaagaggata gcataagttt gctgtcagat ggcccgaccc accaggtgct gcaagtcgag 5640

gcagacattc acgggccgcc ctctgtatct agctcatcct ggtccattcc tcatgcatcc 5700

gactttgatg tggacagttt atccatactt gacaccctgg agggagctag cgtgaccagc 5760

ggggcaacgt cagccgagac taactcttac ttcgcaaaga gtatggagtt tctggcgcga 5820

ccggtgcctg cgcctcgaac agtattcagg aaccctccac atcccgctcc gcgcacaaga 5880

acaccgtcac ttgcacccag cagggcctgc tcgagaacca gcctagtttc caccccgcca 5940

ggcgtgaata gggtgatcac tagagaggag ctcgaggcgc ttaccccgtc acgcactcct 6000

agcaggtcgg tctcgagaac cagcctggtc tccaacccgc caggcgtaaa tagggtgatt 6060

acaagagagg agtttgaggc gttcgtagca caacaacaat gacggtttga tgcgggtgca 6120

tacatctttt cctccgacac cggtcaaggg catttacaac aaaaatcagt aaggcaaacg 6180

gtgctatccg aagtggtgtt ggagaggacc gaattggaga tttcgtatgc cccgcgcctc 6240

gaccaagaaa aagaagaatt actacgcaag aaattacagt taaatcccac acctgctaac 6300

agaagcagat accagtccag gaaggtggag aacatgaaag ccataacagc tagacgtatt 6360

ctgcaaggcc tagggcatta tttgaaggca gaaggaaaag tggagtgcta ccgaaccctg 6420

catcctgttc ctttgtattc atctagtgtg aaccgtgcct tttcaagccc caaggtcgca 6480

gtggaagcct gtaacgccat gttgaaagag aactttccga ctgtggcttc ttactgtatt 6540

attccagagt acgatgccta tttggacatg gttgacggag cttcatgctg cttagacact 6600

gccagttttt gccctgcaaa gctgcgcagc tttccaaaga aacactccta tttggaaccc 6660

acaatacgat cggcagtgcc ttcagcgatc cagaacacgc tccagaacgt cctggcagct 6720

gccacaaaaa gaaattgcaa tgtcacgcaa atgagagaat tgcccgtatt ggattcggcg 6780

gcctttaatg tggaatgctt caagaaatat gcgtgtaata atgaatattg ggaaacgttt 6840

aaagaaaacc ccatcaggct tactgaagaa aacgtggtaa attacattac caaattaaaa 6900

ggaccaaaag ctgctgctct ttttgcgaag acacataatt tgaatatgtt gcaggacata 6960

ccaatggaca ggtttgtaat ggacttaaag agagacgtga aagtgactcc aggaacaaaa 7020

catactgaag aacggcccaa ggtacaggtg atccaggctg ccgatccgct agcaacagcg 7080

tatctgtgcg gaatccaccg agagctggtt aggagattaa atgcggtcct gcttccgaac 7140

attcatacac tgtttgatat gtcggctgaa gactttgacg ctattatagc cgagcacttc 7200

cagcctgggg attgtgttct ggaaactgac atcgcgtcgt ttgataaaag tgaggacgac 7260

gccatggctc tgaccgcgtt aatgattctg gaagacttag gtgtggacgc agagctgttg 7320

acgctgattg aggcggcttt cggcgaaatt tcatcaatac atttgcccac taaaactaaa 7380

tttaaattcg gagccatgat gaaatctgga atgttcctca cactgtttgt gaacacagtc 7440

attaacattg taatcgcaag cagagtgttg agagaacggc taaccggatc accatgtgca 7500

gcattcattg gagatgacaa tatcgtgaaa ggagtcaaat cggacaaatt aatggcagac 7560

aggtgcgcca cctggttgaa tatggaagtc aagattatag atgctgtggt gggcgagaaa 7620

gcgccttatt tctgtggagg gtttattttg tgtgactccg tgaccggcac agcgtgccgt 7680

gtggcagacc ccctaaaaag gctgtttaag cttggcaaac ctctggcagc agacgatgaa 7740

catgatgatg acaggagaag ggcattgcat gaagagtcaa cacgctggaa ccgagtgggt 7800

attctttcag agctgtgcaa ggcagtagaa tcaaggtatg aaaccgtagg aacttccatc 7860

atagttatgg ccatgactac tctagctagc agtgttaaat cattcagcta cctgagaggg 7920

gcccctataa ctctctacgg ctaacctgaa tggactacga catagtctag tccgccaaga 7980

tatcgcacca tggaaaatat ggaaaacgac gagaacatcg tggtgggccc caagcccttc 8040

taccccatcg aggaaggcag cgccggcacc cagctgcgga agtacatgga aagatacgcc 8100

aagctgggcg ccattgcctt caccaacgcc gtgaccggcg tggactacag ctacgccgag 8160

tacctggaaa agagctgctg cctgggcaag gctctgcaga actacggcct ggtggtggac 8220

ggccggatcg ccctgtgcag cgagaactgc gaggaattct tcatccccgt gatcgccggc 8280

ctgttcatcg gcgtgggcgt ggctcccacc aacgagatct acaccctgcg ggagctggtg 8340

cacagcctgg gcatcagcaa gcccaccatc gtgttcagca gcaagaaggg cctggacaaa 8400

gtcatcaccg tgcagaaaac cgtgaccacc atcaagacca tcgtgatcct ggacagcaag 8460

gtggactacc ggggctacca gtgcctggac accttcatca agcggaacac ccccctggc 8520

ttccaggcca gcagcttcaa gaccgtggag gtggaccgga aagaacaggt ggccctgatc 8580

atgaacagca gcggcagcac cggcctgccc aagggcgtgc agctgaccca cgagaacacc 8640

gtgacccggt tcagccacgc cagggacccc atctacggca accaggtgtc ccccggcacc 8700

gccgtgctga ccgtggtgcc cttccaccac ggcttcggca tgttcaccac cctgggctac 8760

ctgatctgcg gcttccgggt ggtgatgctg accaagttcg acgaggaaac cttcctgaaa 8820

accctgcagg actacaagtg cacctacgtg attctggtgc ccaccctgtt cgccatcctg 8880

aacaagagcg agctgctgaa caagtacgac ctgagcaacc tggtggagat cgccagcggc 8940

ggagcccccc tgagcaaaga agtgggagag gccgtcgcca ggcggttcaa tctgcccggc 9000

gtgcggcagg gctacggcct gaccgagaca accagcgcca tcatcatcac ccccgagggc 9060

gacgacaagc ctggagccag cggcaaggtg gtgcccctgt tcaaggccaa agtgatcgac 9120

ctggacacca agaagagcct gggccccaac agacggggcg aagtgtgcgt gaagggcccc 9180

atgctgatga agggctacgt gaacaacccc gaggccacca aagagctgat cgacgaagag 9240

ggctggctgc acaccggcga catcggctac tacgacgaag agaagcactt cttcatcgtg 9300

gaccggctga agagcctgat caagtacaag ggctatcagg tgcccctgc cgagctggaa 9360

agcgtcctgc tgcagcaccc cagcatcttc gacgccggcg tggccggggt gccagatcct 9420

gtggccggcg agctgcctgg cgccgtggtg gtgctggaat ccggcaagaa catgaccgag 9480

aaagaagtga tggactacgt cgccagccag gtgtccaacg ccaagcggct gagaggcggc 9540

gtgagattcg tggacgaagt gccaaagggc ctgaccggca agatcgacgg cagggccatc 9600

cgggagatcc tgaagaaacc cgtggccaag atgtgattaa ttgatcgata cagcagcaat 9660

tggcaagctg cttacataga aggcgcgccg tttaaacggc cggccttaat taagtaacga 9720

tacagcagca attggcaagc tgcttacata gaactcgcgg cgattggcat gccgctttaa 9780

aatttttatt ttatttttct tttcttttcc gaatcggatt ttgtttttaa tatttcaaaa 9840

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaa 9876

<210> 29
<211> 10021
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> construct Alpha-R-DLP-2A-nsp-DLP-rFF

<220>
<221> misc_feature
<222> (1)...(18)
<223> T7 promoter

<400> 29
taatacgact cactatagat aggcggcgca tgagagaagc ccagaccaat tacctaccca 60

aataggagaa agttcacgtt gacatcgagg aagacagccc attcctcaga gctttgcagc 120

ggagcttccc gcagtttgag gtagaagcca agcaggtcac tgataatgac catgctaatg 180

ccagagcgtt ttcgcatctg gcttcaaaac tgatcgaaac ggaggtggac ccatccgaca 240

cgatccttga cattggaata gtcagcatag tacatttcat ctgactaata ctacaacacc 300

accaccatga atagaggatt ctttaacatg ctcggccgcc gccccttccc ggcccccact 360

gccatgtgga ggccgcggag aaggaggcag gcggccccgg gaagcggagc tactaacttc 420

agcctgctga agcaggctgg agacgtggag gagaaccctg gacctgagaa agttcacgtt 480

gacatcgagg aagacagccc attcctcaga gctttgcagc ggagcttccc gcagtttgag 540

gtagaagcca agcaggtcac tgataatgac catgctaatg ccagagcgtt ttcgcatctg 600

gcttcaaaac tgatcgaaac ggaggtggac ccatccgaca cgatccttga cattggaagt 660

gcgcccgccc gcagaatgta ttctaagcac aagtatcatt gtatctgtcc gatgagatgt 720

gcggaagatc cggacagatt gtataagtat gcaactaagc tgaagaaaaa ctgtaaggaa 780

ataactgata aggaattgga caagaaaatg aaggagctcg ccgccgtcat gagcgaccct 840

gacctggaaa ctgagactat gtgcctccac gacgacgagt cgtgtcgcta cgaagggcaa 900

gtcgctgttt accaggatgt atacgcggtt gacggaccga caagtctcta tcaccaagcc 960

aataagggag ttagagtcgc ctactggata ggctttgaca ccaccccttt tatgtttaag 1020

aacttggctg gagcatatcc atcatactct accaactggg ccgacgaaac cgtgttaacg 1080

gctcgtaaca taggcctatg cagctctgac gttatggagc ggtcacgtag agggatgtcc 1140

attcttagaa agaagtattt gaaaccatcc aacaatgttc tattctctgt tggctcgacc 1200

atctaccacg agaagaggga cttactgagg agctggcacc tgccgtctgt atttcactta 1260

cgtggcaagc aaaattacac atgtcggtgt gagactatag ttagttgcga cgggtacgtc 1320

gttaaaagaa tagctatcag tccaggcctg tatgggaagc cttcaggcta tgctgctacg 1380

atgcaccgcg agggattctt gtgctgcaaa gtgacagaca cattgaacgg ggagagggtc 1440

tcttttcccg tgtgcacgta tgtgccagct acattgtgtg accaaatgac tggcatactg 1500

gcaacagatg tcagtgcgga cgacgcgcaa aaactgctgg ttgggctcaa ccagcgtata 1560

gtcgtcaacg gtcgcaccca gagaaacacc aataccatga aaaattacct tttgcccgta 1620

gtggcccagg catttgctag gtgggcaaag gaatataagg aagatcaaga agatgaaagg 1680

ccactaggac tacgagatag acagttagtc atggggtgtt gttgggcttt tagaaggcac 1740

aagataacat ctatttataa gcgcccggat acccaaacca tcatcaaagt gaacagcgat 1800

ttccactcat tcgtgctgcc caggataggc agtaacacat tggagatcgg gctgagaaca 1860

agaatcagga aaatgttaga ggagcacaag gagccgtcac ctctcattac cgccgaggac 1920

gtacaagaag ctaagtgcgc agccgatgag gctaaggagg tgcgtgaagc cgaggagttg 1980

cgcgcagctc taccaccttt ggcagctgat gttgaggagc ccactctgga agccgatgtc 2040

gacttgatgt tacaagaggc tggggccggc tcagtggaga cacctcgtgg cttgataaag 2100

gttaccagct acgatggcga ggacaagatc ggctcttacg ctgtgctttc tccgcaggct 2160

gtactcaaga gtgaaaaatt atcttgcatc caccctctcg ctgaacaagt catagtgata 2220

acacactctg gccgaaaagg gcgttatgcc gtggaaccat accatggtaa agtagtggtg 2280

ccagagggac atgcaatacc cgtccaggac tttcaagctc tgagtgaaag tgccaccatt 2340

gtgtacaacg aacgtgagtt cgtaaacagg tacctgcacc atattgccac acatggagga 2400

gcgctgaaca ctgatgaaga atattacaaa actgtcaagc ccagcgagca cgacggcgaa 2460

tacctgtacg acatcgacag gaaacagtgc gtcaagaaag aactagtcac tgggctaggg 2520

ctcacaggcg agctggtgga tcctcccttc catgaattcg cctacgagag tctgagaaca 2580

cgaccagccg ctccttacca agtaccaacc ataggggtgt atggcgtgcc aggatcaggc 2640

aagtctggca tcattaaaag cgcagtcacc aaaaaagatc tagtggtgag cgccaagaaa 2700

gaaaactgtg cagaaattat aagggacgtc aagaaaatga aagggctgga cgtcaatgcc 2760

agaactgtgg actcagtgct cttgaatgga tgcaaacacc ccgtagagac cctgtatatt 2820

gacgaagctt ttgcttgtca tgcaggtact ctcagagcgc tcatagccat tataagacct 2880

aaaaaggcag tgctctgcgg ggatcccaaa cagtgcggtt tttttaacat gatgtgcctg 2940

aaagtgcatt ttaaccacga gatttgcaca caagtcttcc acaaaagcat ctctcgccgt 3000

tgcactaaat ctgtgacttc ggtcgtctca accttgtttt acgacaaaaa aatgagaacg 3060

acgaatccga aagagactaa gattgtgatt gacactaccg gcagtaccaa acctaagcag 3120

gacgatctca ttctcacttg tttcagaggg tgggtgaagc agttgcaaat agattacaaa 3180

ggcaacgaaa taatgacggc agctgcctct caagggctga cccgtaaagg tgtgtatgcc 3240

gttcggtaca aggtgaatga aaatcctctg tacgcaccca cctctgaaca tgtgaacgtc 3300

ctactgaccc gcacggagga ccgcatcgtg tggaaaacac tagccggcga cccatggata 3360

aaaacactga ctgccaagta ccctgggaat ttcactgcca cgatagagga gtggcaagca 3420

gagcatgatg ccatcatgag gcacatcttg gagagaccgg accctaccga cgtcttccag 3480

aataaggcaa acgtgtgttg ggccaaggct ttagtgccgg tgctgaagac cgctggcata 3540

gacatgacca ctgaacaatg gaacactgtg gattattttg aaacggacaa agctcactca 3600

gcagagatag tattgaacca actatgcgtg aggttctttg gactcgatct ggactccggt 3660

ctattttctg cacccactgt tccgttatcc attaggaata atcactggga taactccccg 3720

tcgcctaaca tgtacgggct gaataaagaa gtggtccgtc agctctctcg caggtaccca 3780

caactgcctc gggcagttgc cactggaaga gtctatgaca tgaacactgg tacactgcgc 3840

aattatgatc cgcgcataaa cctagtacct gtaaacagaa gactgcctca tgctttagtc 3900

ctccaccata atgaacaccc acagagtgac ttttcttcat tcgtcagcaa attgaagggc 3960

agaactgtcc tggtggtcgg ggaaaagttg tccgtcccag gcaaaatggt tgactggttg 4020

tcagaccggc ctgaggctac cttcagagct cggctggatt taggcatccc aggtgatgtg 4080

cccaaatatg acataatatt tgttaatgtg aggaccccat ataaatacca tcactatcag 4140

cagtgtgaag accatgccat taagcttagc atgttgacca agaaagcttg tctgcatctg 4200

aatcccggcg gaacctgtgt cagcataggt tatggttacg ctgacagggc cagcgaaagc 4260

atcattggtg ctatagcgcg gcagttcaag ttttcccggg tatgcaaacc gaaatcctca 4320

cttgaagaga cggaagttct gtttgtattc attgggtacg atcgcaaggc ccgtacgcac 4380

aatccttaca agctttcatc aaccttgacc aacatttata caggttccag actccacgaa 4440

gccggatgtg caccctcata tcatgtggtg cgaggggata ttgccacggc caccgaagga 4500

gtgattataa atgctgctaa cagcaaagga caacctggcg gaggggtgtg cggagcgctg 4560

tataagaaat tcccggaaag cttcgattta cagccgatcg aagtaggaaa agcgcgactg 4620

gtcaaaggtg cagctaaaca tatcattcat gccgtaggac caaacttcaa caaagtttcg 4680

gaggttgaag gtgacaaaca gttggcagag gcttatgagt ccatcgctaa gattgtcaac 4740

gataacaatt acaagtcagt agcgattcca ctgttgtcca ccggcatctt ttccgggaac 4800

aaagatcgac taacccaatc attgaaccat ttgctgacag ctttagacac cactgatgca 4860

gatgtagcca tatactgcag ggacaagaaa tgggaaatga ctctcaagga agcagtggct 4920

aggagagaag cagtggagga gatatgcata tccgacgact cttcagtgac agaacctgat 4980

gcagagctgg tgagggtgca tccgaagagt tctttggctg gaaggaaggg ctacagcaca 5040

agcgatggca aaactttctc atatttggaa gggaccaagt ttcaccaggc ggccaaggat 5100

atagcagaaa ttaatgccat gtggcccgtt gcaacggagg ccaatgagca ggtatgcatg 5160

tatatcctcg gagaaagcat gagcagtatt aggtcgaaat gccccgtcga agagtcggaa 5220

gcctccacac cacctagcac gctgccttgc ttgtgcatcc atgccatgac tccagaaaga 5280

gtacagcgcc taaaagcctc acgtccagaa caaattactg tgtgctcatc ctttccattg 5340

ccgaagtata gaatcactgg tgtgcagaag atccaatgct cccagcctat attgttctca 5400

ccgaaagtgc ctgcgtatat tcatccaagg aagtatctcg tggaaacacc accggtagac 5460

gagactccgg agccatcggc agagaaccaa tccacagagg ggacacctga acaaccacca 5520

cttataaccg aggatgagac caggactaga acgcctgagc cgatcatcat cgaagaggaa 5580

gaagaggata gcataagttt gctgtcagat ggcccgaccc accaggtgct gcaagtcgag 5640

gcagacattc acgggccgcc ctctgtatct agctcatcct ggtccattcc tcatgcatcc 5700

gactttgatg tggacagttt atccatactt gacaccctgg agggagctag cgtgaccagc 5760

ggggcaacgt cagccgagac taactcttac ttcgcaaaga gtatggagtt tctggcgcga 5820

ccggtgcctg cgcctcgaac agtattcagg aaccctccac atcccgctcc gcgcacaaga 5880

acaccgtcac ttgcacccag cagggcctgc tcgagaacca gcctagtttc caccccgcca 5940

ggcgtgaata gggtgatcac tagagaggag ctcgaggcgc ttaccccgtc acgcactcct 6000

agcaggtcgg tctcgagaac cagcctggtc tccaacccgc caggcgtaaa tagggtgatt 6060

acaagagagg agtttgaggc gttcgtagca caacaacaat gacggtttga tgcgggtgca 6120

tacatctttt cctccgacac cggtcaaggg catttacaac aaaaatcagt aaggcaaacg 6180

gtgctatccg aagtggtgtt ggagaggacc gaattggaga tttcgtatgc cccgcgcctc 6240

gaccaagaaa aagaagaatt actacgcaag aaattacagt taaatcccac acctgctaac 6300

agaagcagat accagtccag gaaggtggag aacatgaaag ccataacagc tagacgtatt 6360

ctgcaaggcc tagggcatta tttgaaggca gaaggaaaag tggagtgcta ccgaaccctg 6420

catcctgttc ctttgtattc atctagtgtg aaccgtgcct tttcaagccc caaggtcgca 6480

gtggaagcct gtaacgccat gttgaaagag aactttccga ctgtggcttc ttactgtatt 6540

attccagagt acgatgccta tttggacatg gttgacggag cttcatgctg cttagacact 6600

gccagttttt gccctgcaaa gctgcgcagc tttccaaaga aacactccta tttggaaccc 6660

acaatacgat cggcagtgcc ttcagcgatc cagaacacgc tccagaacgt cctggcagct 6720

gccacaaaaa gaaattgcaa tgtcacgcaa atgagagaat tgcccgtatt ggattcggcg 6780

gcctttaatg tggaatgctt caagaaatat gcgtgtaata atgaatattg ggaaacgttt 6840

aaagaaaacc ccatcaggct tactgaagaa aacgtggtaa attacattac caaattaaaa 6900

ggaccaaaag ctgctgctct ttttgcgaag acacataatt tgaatatgtt gcaggacata 6960

ccaatggaca ggtttgtaat ggacttaaag agagacgtga aagtgactcc aggaacaaaa 7020

catactgaag aacggcccaa ggtacaggtg atccaggctg ccgatccgct agcaacagcg 7080

tatctgtgcg gaatccaccg agagctggtt aggagattaa atgcggtcct gcttccgaac 7140

attcatacac tgtttgatat gtcggctgaa gactttgacg ctattatagc cgagcacttc 7200

cagcctgggg attgtgttct ggaaactgac atcgcgtcgt ttgataaaag tgaggacgac 7260

gccatggctc tgaccgcgtt aatgattctg gaagacttag gtgtggacgc agagctgttg 7320

acgctgattg aggcggcttt cggcgaaatt tcatcaatac atttgcccac taaaactaaa 7380

tttaaattcg gagccatgat gaaatctgga atgttcctca cactgtttgt gaacacagtc 7440

attaacattg taatcgcaag cagagtgttg agagaacggc taaccggatc accatgtgca 7500

gcattcattg gagatgacaa tatcgtgaaa ggagtcaaat cggacaaatt aatggcagac 7560

aggtgcgcca cctggttgaa tatggaagtc aagattatag atgctgtggt gggcgagaaa 7620

gcgccttatt tctgtggagg gtttattttg tgtgactccg tgaccggcac agcgtgccgt 7680

gtggcagacc ccctaaaaag gctgtttaag cttggcaaac ctctggcagc agacgatgaa 7740

catgatgatg acaggagaag ggcattgcat gaagagtcaa cacgctggaa ccgagtgggt 7800

attctttcag agctgtgcaa ggcagtagaa tcaaggtatg aaaccgtagg aacttccatc 7860

atagttatgg ccatgactac tctagctagc agtgttaaat cattcagcta cctgagaggg 7920

gcccctataa ctctctacgg ctaacctgaa tggactacga catagtctag tccgccaaga 7980

tatcgcacca tagtcagcat agtacatttc atctgactaa tactacaaca ccaccaccat 8040

gaatagagga ttctttaaca tgctcggccg ccgccccttc ccggccccca ctgccatgtg 8100

gaggccgcgg agaaggaggc aggcggcccc gatgatggaa aatatggaaa acgacgagaa 8160

catcgtggtg ggccccaagc ccttctaccc catcgaggaa ggcagcgccg gcacccagct 8220

gcggaagtac atggaaagat acgccaagct gggcgccatt gccttcacca acgccgtgac 8280

cggcgtggac tacagctacg ccgagtacct ggaaaagagc tgctgcctgg gcaaggctct 8340

gcagaactac ggcctggtgg tggacggccg gatcgccctg tgcagcgaga actgcgagga 8400

attcttcatc cccgtgatcg ccggcctgtt catcggcgtg ggcgtggctc ccaccaacga 8460

gatctacacc ctgcgggagc tggtgcacag cctgggcatc agcaagccca ccatcgtgtt 8520

cagcagcaag aagggcctgg acaaagtcat caccgtgcag aaaaccgtga ccaccatcaa 8580

gaccatcgtg atcctggaca gcaaggtgga ctaccggggc taccagtgcc tggacacctt 8640

catcaagcgg aacacccccc ctggcttcca ggccagcagc ttcaagaccg tggaggtgga 8700

ccggaaagaa caggtggccc tgatcatgaa cagcagcggc agcaccggcc tgcccaaggg 8760

cgtgcagctg acccacgaga acaccgtgac ccggttcagc cacgccaggg accccatcta 8820

cggcaaccag gtgtcccccg gcaccgccgt gctgaccgtg gtgcccttcc accacggctt 8880

cggcatgttc accaccctgg gctacctgat ctgcggcttc cgggtggtga tgctgaccaa 8940

gttcgacgag gaaaccttcc tgaaaaccct gcaggactac aagtgcacct acgtgattct 9000

ggtgcccacc ctgttcgcca tcctgaacaa gagcgagctg ctgaacaagt acgacctgag 9060

caacctggtg gagatcgcca gcggcggagc ccccctgagc aaagaagtgg gagaggccgt 9120

cgccaggcgg ttcaatctgc ccggcgtgcg gcagggctac ggcctgaccg agacaaccag 9180

cgccatcatc atcacccccg agggcgacga caagcctgga gccagcggca aggtggtgcc 9240

cctgttcaag gccaaagtga tcgacctgga caccaagaag agcctgggcc ccaacagacg 9300

gggcgaagtg tgcgtgaagg gccccatgct gatgaagggc tacgtgaaca accccgaggc 9360

caccaaagag ctgatcgacg aagagggctg gctgcacacc ggcgacatcg gctactacga 9420

cgaagagaag cacttcttca tcgtggaccg gctgaagagc ctgatcaagt acaagggcta 9480

tcaggtgccc cctgccgagc tggaaagcgt cctgctgcag caccccagca tcttcgacgc 9540

cggcgtggcc ggggtgccag atcctgtggc cggcgagctg cctggcgccg tggtggtgct 9600

ggaatccggc aagaacatga ccgagaaaga agtgatggac tacgtcgcca gccaggtgtc 9660

caacgccaag cggctgagag gcggcgtgag attcgtggac gaagtgccaa agggcctgac 9720

cggcaagatc gacggcaggg ccatccggga gatcctgaag aaacccgtgg ccaagatgtg 9780

attaattgat cgatacagca gcaattggca agctgcttac atagaaggcg cgccgtttaa 9840

acggccggcc ttaattaagt aacgatacag cagcaattgg caagctgctt acatagaact 9900

cgcggcgatt ggcatgccgc tttaaaattt ttattttatt tttcttttct tttccgaatc 9960

ggattttgtt tttaatattt caaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 10020

a 10021

<210> 30
<211> 1150
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> g-block for Alpha-R-DLP-2A-rFF

<400> 30
tctacggcta acctgaatgg actacgacat agtctagtcc gccaagatat cgcaccatag 60

tcagcatagt acatttcatc tgactaatac tacaacacca ccaccatgaa tagaggattc 120

tttaacatgc tcggccgccg ccccttcccg gcccccactg ccatgtggag gccgcggaga 180

aggaggcagg cggccccggg aagcggagct actaacttca gcctgctgaa gcaggctgga 240

gacgtggagg agaaccctgg acctatggaa aatatggaaa acgacgagaa catcgtggtg 300

ggccccaagc ccttctaccc catcgaggaa ggcagcgccg gcacccagct gcggaagtac 360

atggaaagat acgccaagct gggcgccatt gccttcacca acgccgtgac cggcgtggac 420

tacagctacg ccgagtacct ggaaaagagc tgctgcctgg gcaaggctct gcagaactac 480

ggcctggtgg tggacggccg gatcgccctg tgcagcgaga actgcgagga attcttcatc 540

cccgtgatcg ccggcctgtt catcggcgtg ggcgtggctc ccaccaacga gatctacacc 600

ctgcgggagc tggtgcacag cctgggcatc agcaagccca ccatcgtgtt cagcagcaag 660

aagggcctgg acaaagtcat caccgtgcag aaaaccgtga ccaccatcaa gaccatcgtg 720

atcctggaca gcaaggtgga ctaccggggc taccagtgcc tggacacctt catcaagcgg 780

aacacccccc ctggcttcca ggccagcagc ttcaagaccg tggaggtgga ccggaaagaa 840

caggtggccc tgatcatgaa cagcagcggc agcaccggcc tgcccaaggg cgtgcagctg 900

acccacgaga acaccgtgac ccggttcagc cacgccaggg accccatcta cggcaaccag 960

gtgtcccccg gcaccgccgt gctgaccgtg gtgcccttcc accacggctt cggcatgttc 1020

accaccctgg gctacctgat ctgcggcttc cgggtggtga tgctgaccaa gttcgacgag 1080

gaaaccttcc tgaaaaccct gcaggactac aagtgcacct acgtgattct ggtgcccacc 1140

ctgttcgcca 1150

<210> 31
<211> 1150
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> g-block for Alpha-R-DLP-2A-nsp-DLP-2A-rFF

<400> 31
tctacggcta acctgaatgg actacgacat agtctagtcc gccaagatat cgcaccatag 60

tcagcatagt acatttcatc tgactaatac tacaacacca ccaccatgaa tagaggattc 120

tttaacatgc tcggccgccg ccccttcccg gcccccactg ccatgtggag gccgcggaga 180

aggaggcagg cggccccggg aagcggagct actaacttca gcctgctgaa gcaggctgga 240

gacgtggagg agaaccctgg acctatggaa aatatggaaa acgacgagaa catcgtggtg 300

ggccccaagc ccttctaccc catcgaggaa ggcagcgccg gcacccagct gcggaagtac 360

atggaaagat acgccaagct gggcgccatt gccttcacca acgccgtgac cggcgtggac 420

tacagctacg ccgagtacct ggaaaagagc tgctgcctgg gcaaggctct gcagaactac 480

ggcctggtgg tggacggccg gatcgccctg tgcagcgaga actgcgagga attcttcatc 540

cccgtgatcg ccggcctgtt catcggcgtg ggcgtggctc ccaccaacga gatctacacc 600

ctgcgggagc tggtgcacag cctgggcatc agcaagccca ccatcgtgtt cagcagcaag 660

aagggcctgg acaaagtcat caccgtgcag aaaaccgtga ccaccatcaa gaccatcgtg 720

atcctggaca gcaaggtgga ctaccggggc taccagtgcc tggacacctt catcaagcgg 780

aacacccccc ctggcttcca ggccagcagc ttcaagaccg tggaggtgga ccggaaagaa 840

caggtggccc tgatcatgaa cagcagcggc agcaccggcc tgcccaaggg cgtgcagctg 900

acccacgaga acaccgtgac ccggttcagc cacgccaggg accccatcta cggcaaccag 960

gtgtcccccg gcaccgccgt gctgaccgtg gtgcccttcc accacggctt cggcatgttc 1020

accaccctgg gctacctgat ctgcggcttc cgggtggtga tgctgaccaa gttcgacgag 1080

gaaaccttcc tgaaaaccct gcaggactac aagtgcacct acgtgattct ggtgcccacc 1140

ctgttcgcca 1150

<210> 32
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> primer Alpha-3'nsp4-F

<220>
<221> misc_feature
<223> RP123

<400> 32
ggctgtttaa gcttggcaaa cctct 25

<210> 33
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> primer Alpha-3'nsp4-F

<220>
<221> misc_feature
<223> rFF-seq1

<400> 33
agcgagaact gcgaggaatt ctt 23

<210> 34
<211> 12342
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polypeptide

<220>
<221> misc_feature
<223> Construct rEx-rFF

<400> 34
gctcgaagtg tgtatggtgc catatacggc tcaccaccat atacactgca agaattacta 60

ttcttgtggg cccctctcgg taaatcctag agggctttcc tctcgttatt gcgagattcg 120

tcgttagata acggcaagtt ccctttctta ctatcctatt ttcatcttgt ggcttgacgg 180

gtcactgcca tcgtcgtcga tctctatcaa ctacccttgc gactatggca accttctccg 240

ctactggatt tggagggagt tttgttaggg actggtccct ggacttaccc gacgcttgtg 300

agcatggcgc gggattgtgc tgcgaagtgg acggctccac cttatgcgcc gagtgttttc 360

gcggttgcga aggaatggag caatgtcctg gcttgttcat gggactgtta aaactggctt 420

cgccagttcc agtgggacat aagttcctga ttggttggta tcgagctgcc aaagtcaccg 480

ggcgttacaa tttccttgag ctgttgcaac accctgcttt cgcccagctg cgtgtggttg 540

atgctaggtt agccattgaa gaggcaagtg tgtttatttc cactgaccac gcgtctgcta 600

agcgtttccc tggcgctaga tttgcgctga caccggtgta tgctaacgct tgggttgtga 660

gcccggctgc taacagtttg atagtgacca ctgaccagga acaagatggg ttctgctggt 720

taaaactttt gccacctgac cgccgtgagg ctggtttgcg gttgtattac aaccattacc 780

gcgaacaaag gaccgggtgg ctgtctaaaa caggacttcg cttatggctt ggagacctgg 840

gtttgggcat caatgcgagc tctggagggc tgaaattcca cattatgagg ggttcgcctc 900

agcgagcttg gcatatcaca acacgcagct gcaagctgaa gagctactac gtttgtgaca 960

tctctgaagc agactggtcc tgtttgcctg ctggcaacta cggcggctac aatccaccag 1020

gggacggagc ttgcggttac aggtgcttgg ccttcatgaa tggcgccact gttgtgtcgg 1080

ctggttgcag ttctgacttg tggtgtgatg atgagttggc ttatcgagtc tttcaattgt 1140

cacccacgtt cacggttacc atcccaggtg ggcgagtttg tccgaatgcc aagtacgcaa 1200

tgatttgtga caagcagcac tggcgcgtca aacgtgcaaa gggcgtcggc ctgtgtctcg 1260

atgaaagctg tttcaggggc atctgcaatt gccaacgcat gagtggacca ccacctgcac 1320

ccgtgtcagc cgccgtgtta gatcacatac tggaggcggc gacgtttggc aacgttcgcg 1380

tggttacacc tgaagggcag ccacgccccg taccagcgcc gcgagttcgt cccagcgcca 1440

actcttctgg agatgtcaaa gatccggcgc ccgttccgcc agtaccaaaa ccaaggacca 1500

agcttgccac accgaaccca actcaggcgc ccatcccagc accgcgcacg cgacttcaag 1560

gggcctcaac acaggagcca ctggcgagtg caggagttgc ttctgactcg gcacctaaat 1620

ggcgtgtggc caaaactgtg tacagctccg cggagcgctt tcggaccgaa ctggtacaac 1680

gtgctcggtc cgttggggac gttcttgttc aagcgctacc gctcaaaacc ccagcagtgc 1740

agcggtatac catgactctg aagatgatgc gttcacgctt cagttggcac tgcgacgtgt 1800

ggtacccttt ggctgtaatc gcttgtttgc tccctatatg gccatctctt gctttgctcc 1860

ttagctttgc cattgggttg atacccagtg tgggcaataa tgttgttctg acagcgcttc 1920

tggtttcatc agctaattat gttgcgtcaa tggaccatca atgtgaaggt gcggcttgct 1980

tagccttgct ggaagaagaa cactattata gagcggtccg ttggcgcccg attacaggcg 2040

cgctgtcgct tgtgctcaat ttactggggc aggtaggcta tgtagctcgt tccacctttg 2100

atgcagctta tgttccttgc actgtgttcg atctttgcag ctttgctatt ctgtacctct 2160

gccgcaatcg ttgctggaga tgcttcggac gctgtgtgcg agttgggcct gccacgcatg 2220

ttttgggctc caccgggcaa cgagtttcca aactggcgct cattgatttg tgtgaccact 2280

tttcaaagcc caccatcgat gttgtgggca tggcaactgg ttggagcgga tgttacacag 2340

gaaccgccgc aatggagcgt cagtgtgcct ctacggtgga ccctcactcg ttcgaccaga 2400

agaaggcagg agcgactgtt tacctcaccc cccctgtcaa cagcgggtca gcgctgcagt 2460

gcctcaatgt catgtggaag cgaccaattg ggtccactgt ccttggggaa caaacaggag 2520

ctgttgtgac ggcggtcaag agtatctctt tctcacctcc ctgctgcgtc tctaccactt 2580

tgcccacccg acccggtgtg accgttgtcg accatgctct ttacaaccgg ttgactgctt 2640

caggggtcga tcccgcttta ttgcgtgttg ggcaaggtga ttttctaaaa cttaatccgg 2700

ggttccggct gataggtgga tggatttatg ggatatgcta ttttgtgttg gtggttgtgt 2760

caacttttac ctgcttacct atcaaatgtg gcattggcac ccgcgaccct ttctgccgca 2820

gagtgttttc tgtacccgtc accaagaccc aagagcactg ccatgctgga atgtgtgcta 2880

gcgctgaagg catctctctg gactctctgg ggttaactca gttacaaagt tactggatcg 2940

cagccgtcac tagcggatta gtgatcttgt tggtctgcca ccgcctggcc atcagcgcct 3000

tggacttgtt gactctagct tccccttttag tgttgcttgt gttcccttgg gcatctgtgg 3060

ggcttttact tgcttgcagt ctcgctggtg ctgctgtgaa aatacagttg ttggcgacgc 3120

tttttgtgaa tctattcttt ccccaagcta cccttgtcac tatgggatac tgggcgtgcg 3180

tggcggcttt ggccgtttac agtttgatgg gcttgcgagt gaaagtgaat gtgcccatgt 3240

gtgtgacacc tgcccatttt ctgctgctgg cgaggtcagc tggacagtca agagagcaga 3300

tgctccgggt cagcgctgct gcccccacca attcactgct tggagtggct cgtgattgtt 3360

atgtcacagg cacaactcgg ctgtacatac ccaaggaagg cgggatggtg tttgaagggc 3420

tattcaggtc accgaaggcg cgcggcaacg tcggcttcgt ggctggtagc agctacggca 3480

cagggtcagt gtggaccagg aacaacgagg tcgtcgtact gacagcgtca cacgtggttg 3540

gccgcgctaa catggccact ctgaagatcg gtgacgcaat gctgactctg actttcaaaa 3600

agaatggcga cttcgccgag gcagtgacga cacagtccga gctcccaggc aattggccac 3660

agttgcattt cgcccaacca acaaccgggc ccgcttcatg gtgcactgcc acaggagatg 3720

aagaaggctt gctcagtggc gaggtttgtc tggcgtggac tactagtggc gactctggat 3780

ctgcagtggt tcagggtgac gctgtggtag gggtccacac cggttcgaac acaagtggtg 3840

ttgcctacgt gaccacccca agcggaaaac tccttggcgc cgacaccgtg actttgtcat 3900

cactgtcaaa gcatttcaca ggccctttga catcaatccc gaaggacatc cctgacaaca 3960

ttattgccga tgttgatgct gttcctcgtt ctctggccat gctgattgat ggcttatcca 4020

atagagagag cagcctttct ggacctcagt tgttgttaat tgcttgtttt atgtggtctt 4080

atcttaacca acctgcttac ttgccttatg tgctgggctt ctttgccgct aacttcttcc 4140

tgccaaaaag tgttggccgc cctgtggtca ctgggcttct atggttgtgc tgcctcttca 4200

caccgctttc catgcgcttg tgcttgttcc atctggtctg tgctaccgtc acgggaaacg 4260

tgatatcttt gtggttctac atcactgccg ctggcacgtc ttacctttct gagatgtggt 4320

tcggaggcta tcccaccatg ttgtttgtgc cacggttcct agtgtaccag ttccccggct 4380

gggctattgg cacagtacta gcggtatgca gcatcaccat gctggctgct gccctcggtc 4440

acaccctgtt actggatgtg ttctccgcct caggtcgctt tgacaggact ttcatgatga 4500

aatacttcct ggagggagga gtgaaagaga gtgtcaccgc ctcagtcacc cgcgcttatg 4560

gcaaaccaat tacccaggag agtctcactg caacattagc tgccctcact gatgatgact 4620

tccaattcct ctctgatgtg cttgactgtc gggccgtccg atcggcaatg aatctgcgtg 4680

ccgctctcac aagttttcaa gtggcgcagt atcgtaacat ccttaatgca tccttgcaag 4740

tcgatcgtga cgctgctcgt agtcgcagac taatggcaaa actggctgat tttgcggttg 4800

aacaagaagt aacagctgga gaccgtgttg tggttatcga cggtctggac cgcatggctc 4860

acttcaaaga cgatttggtg ctggttcctt tgaccaccaa agtagtaggc ggttctaggt 4920

gcaccatttg tgacgtcgtt aaggaagaag ccaatgacac cccagttaag ccaatgccca 4980

gcaggagacg ccgcaagggc ctgcctaaag gtgctcagtt ggagtgggac cgtcaccagg 5040

aagagaagag gaacgccggt gatgatgatt ttgcggtctc gaatgattat gtcaagagag 5100

tgccaaagta ctgggatccc agcgacaccc gaggcacgac agtgaaaatc gccggcacta 5160

cctatcagaa agtggttgac tattcaggca atgtgcatta cgtggagcat caggaagatc 5220

tgctagacta cgtgctgggc aaggggagct atgaaggcct agatcaggac aaagtgttgg 5280

acctcacaaa catgcttaaa gtggacccca cggagctctc ctccaaagac aaagccaagg 5340

cgcgtcagct tgctcatctg ctgttggatc tggctaaccc agttgaggca gtgaatcagt 5400

taaactgaga gcgccccaca tctttcccgg cgatgtgggg cgtcggacct ttgctgactc 5460

taaagacaag ggtttcgtgg ctctacacag tcgcacaatg tttttagctg cccgggactt 5520

tttatttaac atcaaatttg tgtgcgacga agagttcaca aagaccccaa aagacacact 5580

gcttgggtac gtacgcgcct gccctggtta ctggtttatt ttccgtcgta cgcaccggtc 5640

gctgattgat gcatactggg acagtatgga gtgcgtttac gcgcttccca ccatatctga 5700

ttttgatgtg agcccaggtg acgtcgcagt gacgggcgag cgatgggatt ttgaatctcc 5760

cggaggaggc cgtgcaaaac gtctcacagc tgatctggtg cacgcttttc aagggttcca 5820

cggagcctct tattcctatg atgacaaggt ggcagctgct gtcagtggtg acccgtatcg 5880

gtcggacggc gtcttgtata acacccgttg gggcaacatt ccatattctg tcccaaccaa 5940

tgctttggaa gccacagctt gctaccgtgc tggatgtgag gccgttaccg acgggaccaa 6000

cgtcatcgca acaattgggc ccttcccgga gcaacaaccc ataccggaca tcccaaagag 6060

cgtgcttgac aactgcgctg acatcagctg tgacgctttc atagcgcccg ctgcagagac 6120

agccctgtgt ggagatttag agaaatacaa cctatccacg cagggttttg tgttgcctag 6180

tgttttctcc atggtgcggg cgtacttaaa agaggagatt ggagacgctc caccactcta 6240

cttgccatct actgtaccat ctaaaaattc acaagccgga attaacggcg ctgagtttcc 6300

tacaaagtct ttacagagct actgtttgat tgatgacatg gtgtcacagt ccatgaaaag 6360

caatctacaa accgccacca tggcgacttg taaacggcaa tactgttcca aatacaagat 6420

taggagcatt ctgggcacca acaattacat tggcctaggt ttgcgtgcct gcctttcggg 6480

ggttacggcc gcattccaaa aagctggaaa ggatgggtca ccgatttatt tgggcaagtc 6540

aaaattcgac ccgataccag ctcctgacaa gtactgcctt gaaacagacc tggagagttg 6600

tgatcgctcc accccggctt tggtgcgttg gttcgctact aatcttattt ttgagctagc 6660

tggccagccc gagttggtgc acagctacgt gttgaattgc tgtcacgatc tagttgtggc 6720

gggtagtgta gcattcacca aacgcggggg tttgtcatct ggagacccta tcacttccat 6780

ttccaatacc atctattcat tggtgctgta cacccagcac atgttgctat gtggacttga 6840

aggctatttc ccagagattg cagaaaaata tcttgatggc agcctggagc tgcgggacat 6900

gttcaagtac gttcgagtgt acatctactc ggacgatgtg gttctaacca cacccaacca 6960

gcattacgcg gccagctttg accgctgggt cccccacctg caggcgctgc taggtttcaa 7020

ggttgaccca aagaaaactg tgaacaccag ctccccttcc tttttgggct gccggttcaa 7080

gcaagtggac ggcaagtgtt atctagccag tcttcaggac cgcgttacac gctctctgtt 7140

ataccacatt ggtgcaaaga atccctcaga gtactatgaa gctgctgttt ccatctttaa 7200

ggactccatt atctgctgtg atgaagactg gtggacggac ctccatcgac gtatcagtgg 7260

cgctgcgcgt accgacggag ttgagttccc caccattgaa atgttaacat ccttccgcac 7320

caagcagtat gagagtgccg tgtgcacagt ttgtggggcc gcccccgtgg ccaagtctgc 7380

ttgtggaggg tggttctgtg gcaattgtgt cccgtaccac gcgggtcatt gtcacacaac 7440

ctcgctcttc gccaactgcg ggcacgacat catgtaccgc tccacttact gcacaatgtg 7500

tgagggttcc ccaaaacaga tggtaccaaa agtgcctcac ccgatcctgg atcatttgct 7560

gtgccacatt gattacggca gtaaagagga actaactctg gtagtggcgg atggtcgaac 7620

aacatcaccg cccgggcgct acaaagtggg tcacaaggta gtcgccgtgg ttgcagatgt 7680

gggaggcaac attgtgtttg ggtgcggtcc tggatcacac atcgcagtac cacttcagga 7740

tacgctcaag ggcgtggtgg tgaataaagc tctgaagaac gccgccgcct ctgagtacgt 7800

ggaaggaccc cctgggagtg ggaagacttt tcacctggtc aaagatgtgc tagccgtggt 7860

cggtagcgcg accttggttg tgcccaccca cgcgtccatg ctggactgca tcaacaagct 7920

caaacaagcg ggcgccgatc catactttgt ggtgcccaag tatacagttc ttgactttcc 7980

ccggcctggc agtggaaaca tcacagtgcg actgccacag gtcggaacca gtgagggaga 8040

aacctttgtg gatgaggtgg cctacttctc accagtggat ctggcgcgca ttttaaccca 8100

gggtcgagtc aagggttacg gtgatttaaa tcagctcggg tgcgtcggac ccgcgagcgt 8160

gccacgtaac ctttggctcc gacattttgt cagcctggag cccttgcgag tgtgccatcg 8220

attcggcgct gctgtgtgtg atttgatcaa gggcatttat ccttattatg agccagctcc 8280

acataccact aaagtggtgt ttgtgccaaa tccagacttt gagaaaggtg tagtcatcac 8340

cgcctaccac aaagatcgcg gtcttggtca ccgcacaatt gattcaattc aaggctgtac 8400

attccctgtt gtgactcttc gactgcccac accccaatca ctgacgcgcc cgcgcgcagt 8460

tgtggcggtt actagggcgt ctcaggaatt atacatctac gacccctttg atcagcttag 8520

cgggttgttg aagttcacca aggaagcaga ggcgcaggac ttgatccatg gcccacctac 8580

agcatgccac ctgggccaag aaattgacct ttggtccaat gagggcctcg aatattacaa 8640

ggaagtcaac ctgctgtaca cacacgtccc catcaaggat ggtgtaatac acagttaccc 8700

taattgtggc cctgcctgtg gctgggaaaa gcaatccaac aaaatttcgt gcctcccgag 8760

agtggcacaa aatttgggct accactattc cccagactta ccaggatttt gccccatacc 8820

aaaagaactc gctgagcatt ggcccgtagt gtccaatgat agatacccga attgcttgca 8880

aattacctta cagcaagtat gtgaactcag taaaccgtgc tcagcgggct atatggttgg 8940

acaatcggtt ttcgtgcaga cgcctggtgt gacatcttac tggcttactg aatgggtcga 9000

cggcaaagcg cgtgctctac cagattcctt attctcgtcc ggtaggttcg agactaacag 9060

ccgcgctttc ctcgatgaag ccgaggaaaa gtttgccgcc gctcaccctc atgcctgttt 9120

gggagaaatt aataagtcca ccgtgggagg atcccacttc atcttttccc aatatttacc 9180

accattgcta cccgcagacg ctgttgccct ggtaggtgct tcattggctg ggaaagctgc 9240

taaagctgct tgcagcgttg ttgatgtcta tgctccatca tttgaacctt atctacaccc 9300

tgagacactg agtcgcgtgt acaagattat gatcgatttc aagccgtgta ggcttatggt 9360

gtggagaaac gcgacctttt atgtccaaga gggtgttgat gcagttacat cagcactagc 9420

agctgtgtcc aaactcatca aagtgccggc caatgagcct gtttcattcc atgtggcatc 9480

agggtacaga accaacgcgc tggtagcgcc ccaggctaaa atttcaattg gagcctacgc 9540

cgccgagtgg gcactgtcaa ctgaaccgcc acctgctggt tatgcgatcg tgcggcgata 9600

tattgtaaag aggctcctca gctcaacaga agtgttcttg tgccgcaggg gtgttgtgtc 9660

ttccacctca gtgcagacca tttgtgcact agagggatgt aaacctctgt tcaacttctt 9720

acaaattggt tcagtcattg ggcccgtgtg actctagagt ggacctgttc ccatcccccg 9780

ctcaactact caggtagtgg ttcgcggcaa cgggtacacc gcagttggta acaagcttgt 9840

cgatggaaaa tatggaaaac gacgagaaca tcgtggtggg ccccaagccc ttctacccca 9900

tcgaggaagg cagcgccggc acccagctgc ggaagtacat ggaaagatac gccaagctgg 9960

gcgccattgc cttcaccaac gccgtgaccg gcgtggacta cagctacgcc gagtacctgg 10020

aaaagagctg ctgcctgggc aaggctctgc agaactacgg cctggtggtg gacggccgga 10080

tcgccctgtg cagcgagaac tgcgaggaat tcttcatccc cgtgatcgcc ggcctgttca 10140

tcggcgtggg cgtggctccc accaacgaga tctacaccct gcgggagctg gtgcacagcc 10200

tgggcatcag caagcccacc atcgtgttca gcagcaagaa gggcctggac aaagtcatca 10260

ccgtgcagaa aaccgtgacc accatcaaga ccatcgtgat cctggacagc aaggtggact 10320

accggggcta ccagtgcctg gacaccttca tcaagcggaa cacccccccct ggcttccagg 10380

ccagcagctt caagaccgtg gaggtggacc ggaaagaaca ggtggccctg atcatgaaca 10440

gcagcggcag caccggcctg cccaagggcg tgcagctgac ccacgagaac accgtgaccc 10500

ggttcagcca cgccagggac cccatctacg gcaaccaggt gtcccccggc accgccgtgc 10560

tgaccgtggt gcccttccac cacggcttcg gcatgttcac caccctgggc tacctgatct 10620

gcggcttccg ggtggtgatg ctgaccaagt tcgacgagga aaccttcctg aaaaccctgc 10680

aggactacaa gtgcacctac gtgattctgg tgcccaccct gttcgccatc ctgaacaaga 10740

gcgagctgct gaacaagtac gacctgagca acctggtgga gatcgccagc ggcggagccc 10800

ccctgagcaa agaagtggga gaggccgtcg ccaggcggtt caatctgccc ggcgtgcggc 10860

agggctacgg cctgaccgag acaaccagcg ccatcatcat caccccgag ggcgacgaca 10920

agcctggagc cagcggcaag gtggtgcccc tgttcaaggc caaagtgatc gacctggaca 10980

ccaagaagag cctgggcccc aacagacggg gcgaagtgtg cgtgaagggc cccatgctga 11040

tgaagggcta cgtgaacaac cccgaggcca ccaaagagct gatcgacgaa gagggctggc 11100

tgcacaccgg cgacatcggc tactacgacg aagagaagca cttcttcatc gtggaccggc 11160

tgaagagcct gatcaagtac aagggctatc aggtgccccc tgccgagctg gaaagcgtcc 11220

tgctgcagca ccccagcatc ttcgacgccg gcgtggccgg ggtgccagat cctgtggccg 11280

gcgagctgcc tggcgccgtg gtggtgctgg aatccggcaa gaacatgacc gagaaagaag 11340

tgatggacta cgtcgccagc caggtgtcca acgccaagcg gctgagaggc ggcgtgagat 11400

tcgtggacga agtgccaaag ggcctgaccg gcaagatcga cggcagggcc atccgggaga 11460

tcctgaagaa acccgtggcc aagatgtgat tataactcga gggagccata gattcatttt 11520

gtggtgacgg gattttaggt gagtatctag attactttat tctgtccgtc ccactcttgc 11580

tgttgcttac taggtatgta gcatctgggt tagtgtatgt tttgactgcc ttgttctatt 11640

cctttgtatt agcagcttat atttggtttg ttatagttgg aagagccttt tctactgctt 11700

atgcttttgt gcttttggct gcttttctgt tattagtaat gaggatgatt gtgggtatga 11760

tgcctcgtct tcggtccatt ttcaaccatc gccaactggt ggtagctgat tttgtggaca 11820

cacctagtgg acctgttccc atccccccgcc caaccactca ggtagtggtt cgcggcaacg 11880

ggtacaccgc agttggtaac aagcttgtcg atggcgtcaa gacgatcacg tccgcaggcc 11940

gcctcttttc gaaacggacg gcggcgacag cctacaagct acaatgacct actgcgcatg 12000

tttggtcaga tgcgggtccg caaaccgccc gcgcaaccca ctcaggctat tattgcagag 12060

cctggagacc ttaggcatga tttaaatcaa caggagcgcg ccacccttttc gtcgaacgta 12120

caacggttct tcatgattgg gcatggttca ctcactgcag atgccggagg actcacgtac 12180

accgtcagtt gggttcctac caaacaaatc cagcgcaaag ttgcgcctcc agcagggccg 12240

taagacgtgg atattctcct gtgtggcgtc atgttgaagt agttattagc cacccaggaa 12300

ccaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa 12342

<210> 35
<211> 8554
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polypeptide

<220>
<221> misc_feature
<223> Construct Alpha-R-eGFP

<400> 35
ataggcggcg catgagagaa gcccagacca attacctacc caaaatggag aaagttcacg 60

ttgacatcga ggaagacagc ccattcctca gagctttgca gcggagcttc ccgcagtttg 120

aggtagaagc caagcaggtc actgataatg accatgctaa tgccagagcg ttttcgcatc 180

tggcttcaaa actgatcgaa acggaggtgg acccatccga cacgatcctt gacattggaa 240

gtgcgcccgc ccgcagaatg tattctaagc acaagtatca ttgtatctgt ccgatgagat 300

gtgcggaaga tccggacaga ttgtataagt atgcaactaa gctgaagaaa aactgtaagg 360

aaataactga taaggaattg gacaagaaaa tgaaggagct cgccgccgtc atgagcgacc 420

ctgacctgga aactgagact atgtgcctcc acgacgacga gtcgtgtcgc tacgaagggc 480

aagtcgctgt ttaccaggat gtatacgcgg ttgacggacc gacaagtctc tatcaccaag 540

ccaataaggg agttagagtc gcctactgga taggctttga caccacccct tttatgttta 600

agaacttggc tggagcatat ccatcatact ctaccaactg ggccgacgaa accgtgttaa 660

cggctcgtaa cataggccta tgcagctctg acgttatgga gcggtcacgt agagggatgt 720

ccattcttag aaagaagtat ttgaaaccat ccaacaatgt tctattctct gttggctcga 780

ccatctacca cgagaagagg gacttactga ggagctggca cctgccgtct gtatttcact 840

tacgtggcaa gcaaaattac acatgtcggt gtgagactat agttagttgc gacgggtacg 900

tcgttaaaag aatagctatc agtccaggcc tgtatgggaa gccttcaggc tatgctgcta 960

cgatgcaccg cgagggattc ttgtgctgca aagtgacaga cacattgaac ggggagaggg 1020

tctcttttcc cgtgtgcacg tatgtgccag ctacattgtg tgaccaaatg actggcatac 1080

tggcaacaga tgtcagtgcg gacgacgcgc aaaaactgct ggttgggctc aaccagcgta 1140

tagtcgtcaa cggtcgcacc cagagaaaca ccaataccat gaaaaattac cttttgcccg 1200

tagtggccca ggcatttgct aggtgggcaa aggaatataa ggaagatcaa gaagatgaaa 1260

ggccactagg actacgagat agacagttag tcatggggtg ttgttgggct tttagaaggc 1320

acaagataac atctatttat aagcgcccgg atacccaaac catcatcaaa gtgaacagcg 1380

atttccactc attcgtgctg cccaggatag gcagtaacac attggagatc gggctgagaa 1440

caagaatcag gaaaatgtta gaggagcaca aggagccgtc acctctcatt accgccgagg 1500

acgtacaaga agctaagtgc gcagccgatg aggctaagga ggtgcgtgaa gccgaggagt 1560

tgcgcgcagc tctaccacct ttggcagctg atgttgagga gcccactctg gaagccgatg 1620

tcgacttgat gttacaagag gctggggccg gctcagtgga gacacctcgt ggcttgataa 1680

aggttaccag ctacgatggc gaggacaaga tcggctctta cgctgtgctt tctccgcagg 1740

ctgtactcaa gagtgaaaaa ttatcttgca tccaccctct cgctgaacaa gtcatagtga 1800

taacacactc tggccgaaaa gggcgttatg ccgtggaacc ataccatggt aaagtagtgg 1860

tgccagaggg acatgcaata cccgtccagg actttcaagc tctgagtgaa agtgccacca 1920

ttgtgtacaa cgaacgtgag ttcgtaaaca ggtacctgca ccatattgcc acacatggag 1980

gagcgctgaa cactgatgaa gaatattaca aaactgtcaa gcccagcgag cacgacggcg 2040

aatacctgta cgacatcgac aggaaacagt gcgtcaagaa agaactagtc actgggctag 2100

ggctcacagg cgagctggtg gatcctccct tccatgaatt cgcctacgag agtctgagaa 2160

cacgaccagc cgctccttac caagtaccaa ccataggggt gtatggcgtg ccaggatcag 2220

gcaagtctgg catcattaaa agcgcagtca ccaaaaaaga tctagtggtg agcgccaaga 2280

aagaaaactg tgcagaaatt ataagggacg tcaagaaaat gaaagggctg gacgtcaatg 2340

ccagaactgt ggactcagtg ctcttgaatg gatgcaaaca ccccgtagag accctgtata 2400

ttgacgaagc ttttgcttgt catgcaggta ctctcagagc gctcatagcc attataagac 2460

ctaaaaaggc agtgctctgc ggggatccca aacagtgcgg ttttttaac atgatgtgcc 2520

tgaaagtgca ttttaaccac gagatttgca cacaagtctt ccacaaaagc atctctcgcc 2580

gttgcactaa atctgtgact tcggtcgtct caaccttgtt ttacgacaaa aaaatgagaa 2640

cgacgaatcc gaaagagact aagattgtga ttgacactac cggcagtacc aaacctaagc 2700

aggacgatct cattctcact tgtttcagag ggtgggtgaa gcagttgcaa atagattaca 2760

aaggcaacga aataatgacg gcagctgcct ctcaagggct gacccgtaaa ggtgtgtatg 2820

ccgttcggta caaggtgaat gaaaatcctc tgtacgcacc cacctctgaa catgtgaacg 2880

tcctactgac ccgcacggag gaccgcatcg tgtggaaaac actagccggc gacccatgga 2940

taaaaacact gactgccaag taccctggga atttcactgc cacgatagag gagtggcaag 3000

cagagcatga tgccatcatg aggcacatct tggagagacc ggaccctacc gacgtcttcc 3060

agaataaggc aaacgtgtgt tgggccaagg ctttagtgcc ggtgctgaag accgctggca 3120

tagacatgac cactgaacaa tggaacactg tggattattt tgaaacggac aaagctcact 3180

cagcagagat agtattgaac caactatgcg tgaggttctt tggactcgat ctggactccg 3240

gtctattttc tgcacccact gttccgttat ccattaggaa taatcactgg gataactccc 3300

cgtcgcctaa catgtacggg ctgaataaag aagtggtccg tcagctctct cgcaggtacc 3360

cacaactgcc tcgggcagtt gccactggaa gagtctatga catgaacact ggtacactgc 3420

gcaattatga tccgcgcata aacctagtac ctgtaaacag aagactgcct catgctttag 3480

tcctccacca taatgaacac ccacagagtg acttttcttc attcgtcagc aaattgaagg 3540

gcagaactgt cctggtggtc ggggaaaagt tgtccgtccc aggcaaaatg gttgactggt 3600

tgtcagaccg gcctgaggct accttcagag ctcggctgga tttaggcatc ccaggtgatg 3660

tgcccaaata tgacataata tttgttaatg tgaggacccc atataaatac catcactatc 3720

agcagtgtga agaccatgcc attaagctta gcatgttgac caagaaagct tgtctgcatc 3780

tgaatcccgg cggaacctgt gtcagcatag gttatggtta cgctgacagg gccagcgaaa 3840

gcatcattgg tgctatagcg cggcagttca agttttcccg ggtatgcaaa ccgaaatcct 3900

cacttgaaga gacggaagtt ctgtttgtat tcattgggta cgatcgcaag gcccgtacgc 3960

acaatcctta caagctttca tcaaccttga ccaacattta tacaggttcc agactccacg 4020

aagccggatg tgcaccctca tatcatgtgg tgcgagggga tattgccacg gccaccgaag 4080

gagtgattat aaatgctgct aacagcaaag gacaacctgg cggaggggtg tgcggagcgc 4140

tgtataagaa attcccggaa agcttcgatt tacagccgat cgaagtagga aaagcgcgac 4200

tggtcaaagg tgcagctaaa catatcattc atgccgtagg accaaacttc aacaaagttt 4260

cggaggttga aggtgacaaa cagttggcag aggcttatga gtccatcgct aagattgtca 4320

acgataacaa ttacaagtca gtagcgattc cactgttgtc caccggcatc ttttccggga 4380

acaaagatcg actaacccaa tcattgaacc atttgctgac agctttagac accactgatg 4440

cagatgtagc catatactgc agggacaaga aatgggaaat gactctcaag gaagcagtgg 4500

ctaggagaga agcagtggag gagatatgca tatccgacga ctcttcagtg acagaacctg 4560

atgcagagct ggtgagggtg catccgaaga gttctttggc tggaaggaag ggctacagca 4620

caagcgatgg caaaactttc tcatatttgg aagggaccaa gtttcaccag gcggccaagg 4680

atatagcaga aattaatgcc atgtggcccg ttgcaacgga ggccaatgag caggtatgca 4740

tgtatatcct cggagaaagc atgagcagta ttaggtcgaa atgccccgtc gaagagtcgg 4800

aagcctccac accacctagc acgctgcctt gcttgtgcat ccatgccatg actccagaaa 4860

gagtacagcg cctaaaagcc tcacgtccag aacaaattac tgtgtgctca tcctttccat 4920

tgccgaagta tagaatcact ggtgtgcaga agatccaatg ctcccagcct atattgttct 4980

caccgaaagt gcctgcgtat attcatccaa ggaagtatct cgtggaaaca ccaccggtag 5040

acgagactcc ggagccatcg gcagagaacc aatccacaga ggggacacct gaacaaccac 5100

cacttataac cgaggatgag accaggacta gaacgcctga gccgatcatc atcgaagagg 5160

aagaagagga tagcataagt ttgctgtcag atggcccgac ccaccaggtg ctgcaagtcg 5220

aggcagacat tcacgggccg ccctctgtat ctagctcatc ctggtccatt cctcatgcat 5280

ccgactttga tgtggacagt ttatccatac ttgacaccct ggagggagct agcgtgacca 5340

gcggggcaac gtcagccgag actaactctt acttcgcaaa gagtatggag tttctggcgc 5400

gaccggtgcc tgcgcctcga acagtattca ggaaccctcc acatcccgct ccgcgcacaa 5460

gaacaccgtc acttgcaccc agcagggcct gctcgagaac cagcctagtt tccacccccgc 5520

caggcgtgaa tagggtgatc actagagagg agctcgaggc gcttaccccg tcacgcactc 5580

ctagcaggtc ggtctcgaga accagcctgg tctccaaccc gccaggcgta aatagggtga 5640

ttacaagaga ggagtttgag gcgttcgtag cacaacaaca atgacggttt gatgcgggtg 5700

catacatctt ttcctccgac accggtcaag ggcatttaca acaaaaatca gtaaggcaaa 5760

cggtgctatc cgaagtggtg ttggagagga ccgaattgga gatttcgtat gccccgcgcc 5820

tcgaccaaga aaaagaagaa ttactacgca agaaattaca gttaaatccc acacctgcta 5880

acagaagcag ataccagtcc aggaaggtgg agaacatgaa agccataaca gctagacgta 5940

ttctgcaagg cctagggcat tatttgaagg cagaaggaaa agtggagtgc taccgaaccc 6000

tgcatcctgt tcctttgtat tcatctagtg tgaaccgtgc cttttcaagc cccaaggtcg 6060

cagtggaagc ctgtaacgcc atgttgaaag agaactttcc gactgtggct tcttactgta 6120

ttattccaga gtacgatgcc tatttggaca tggttgacgg agcttcatgc tgcttagaca 6180

ctgccagttt ttgccctgca aagctgcgca gctttccaaa gaaacactcc tatttggaac 6240

ccacaatacg atcggcagtg ccttcagcga tccagaacac gctccagaac gtcctggcag 6300

ctgccacaaa aagaaattgc aatgtcacgc aaatgagaga attgcccgta ttggattcgg 6360

cggcctttaa tgtggaatgc ttcaagaaat atgcgtgtaa taatgaatat tgggaaacgt 6420

ttaaagaaaa ccccatcagg cttactgaag aaaacgtggt aaattacatt accaaattaa 6480

aaggaccaaa agctgctgct ctttttgcga agacacataa tttgaatatg ttgcaggaca 6540

taccaatgga caggtttgta atggacttaa agagagacgt gaaagtgact ccaggaacaa 6600

aacatactga agaacggccc aaggtacagg tgatccaggc tgccgatccg ctagcaacag 6660

cgtatctgtg cggaatccac cgagagctgg ttaggagatt aaatgcggtc ctgcttccga 6720

acattcatac actgtttgat atgtcggctg aagactttga cgctattata gccgagcact 6780

tccagcctgg ggattgtgtt ctggaaactg acatcgcgtc gtttgataaa agtgaggacg 6840

acgccatggc tctgaccgcg ttaatgattc tggaagactt aggtgtggac gcagagctgt 6900

tgacgctgat tgaggcggct ttcggcgaaa tttcatcaat acatttgccc actaaaacta 6960

aatttaaatt cggagccatg atgaaatctg gaatgttcct cacactgttt gtgaacacag 7020

tcattaacat tgtaatcgca agcagagtgt tgagagaacg gctaaccgga tcaccatgtg 7080

cagcattcat tggagatgac aatatcgtga aaggagtcaa atcggacaaa ttaatggcag 7140

acaggtgcgc cacctggttg aatatggaag tcaagattat agatgctgtg gtgggcgaga 7200

aagcgcctta tttctgtgga gggtttattt tgtgtgactc cgtgaccggc acagcgtgcc 7260

gtgtggcaga ccccctaaaa aggctgttta agcttggcaa acctctggca gcagacgatg 7320

aacatgatga tgacaggaga agggcattgc atgaagagtc aacacgctgg aaccgagtgg 7380

gtattctttc agagctgtgc aaggcagtag aatcaaggta tgaaaccgta ggaacttcca 7440

tcatagttat ggccatgact actctagcta gcagtgttaa atcattcagc tacctgagag 7500

gggcccctat aactctctac ggctaacctg aatggactac gacatagtct agtccgccaa 7560

gatatcgcac catgggaaga gccggcgtga gcaagggcga ggagctgttc accggggtgg 7620

tgcccatcct ggtcgagctg gacggcgacg taaacggcca caagttcagc gtgtccggcg 7680

agggcgaggg cgatgccacc tacggcaagc tgaccctgaa gctgatctgc accaccggca 7740

agctgcccgt gccctggccc accctcgtga ccaccctggg ctacggcctg cagtgcttcg 7800

cccgctaccc cgaccacatg aagcagcacg acttcttcaa gtccgccatg cccgaaggct 7860

acgtccagga gcgcaccatc ttcttcaagg acgacggcaa ctacaagacc cgcgccgagg 7920

tgaagttcga gggcgacacc ctggtgaacc gcatcgagct gaagggcatc gacttcaagg 7980

aggacggcaa catcctgggg cacaagctgg agtacaacta caacagccac aacgtctata 8040

tcaccgccga caagcagaag aacggcatca aggccaactt caagatccgc cacaacatcg 8100

aggacggcgg cgtgcagctc gccgaccact accagcagaa cacccccatc ggcgacggcc 8160

ccgtgctgct gcccgacaac cactacctga gctaccagtc cgccctgagc aaagacccca 8220

acgagaagcg cgatcacatg gtcctgctgg agttcgtgac cgccgccggg atcactctcg 8280

gcatggacga gctgtacaag taggctcttc gtaattaatt gatcgataca gcagcaattg 8340

gcaagctgct tacatagaag gcgcgccgtt taaacggccg gccttaatta agtaacgata 8400

cagcagcaat tggcaagctg cttacataga actcgcggcg attggcatgc cgctttaaaa 8460

tttttatttt atttttcttt tcttttccga atcggatttt gtttttaata tttcaaaaaa 8520

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaa 8554

<210> 36
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> 5 human beta globin UTR

<400> 36
acatttgctt ctgacacaac tgtgttcact agcaacctca aacagacacc gccgccacc 59

<210> 37
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> T7 Promoter

<400> 37
taatacgact cactatag 18

<210> 38
<211> 142
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>

<221> misc_feature
<223> DLP

<400> 38
atagtcagca tagtacattt catctgacta atactacaac accaccacca tgaatagagg 60

attctttaac atgctcggcc gccgcccctt cccggccccc actgccatgt ggaggccgcg 120

gagaaggagg caggcggccc cg 142

<210> 39
<211> 66
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> P2A

<400> 39
ggaagcggag ctactaactt cagcctgctg aagcaggctg gagacgtgga ggagaaccct 60

ggacct 66

<210> 40
<211> 846
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> dsGFP

<400> 40
atggtgagca agggcgagga gctgttcacc ggggtggtgc ccatcctggt cgagctggac 60

ggcgacgtaa acggccacaa gttcagcgtg tccggcgagg gcgagggcga tgccacctac 120

ggcaagctga ccctgaagtt catctgcacc accggcaagc tgcccgtgcc ctggcccacc 180

ctcgtgacca ccctgaccta cggcgtgcag tgcttcagcc gctaccccga ccacatgaag 240

cagcacgact tcttcaagtc cgccatgccc gaaggctacg tccaggagcg caccatcttc 300

ttcaaggacg acggcaacta caagacccgc gccgaggtga agttcgaggg cgacaccctg 360

gtgaaccgca tcgagctgaa gggcatcgac ttcaaggagg acggcaacat cctggggcac 420

aagctggagt acaactacaa cagccacaac gtctatatca tggccgacaa gcagaagaac 480

ggcatcaagg tgaacttcaa gatccgccac aacatcgagg acggcagcgt gcagctcgcc 540

gaccactacc agcagaacac ccccatcggc gacggccccg tgctgctgcc cgacaaccac 600

tacctgagca cccagtccgc cctgagcaaa gaccccaacg agaagcgcga tcacatggtc 660

ctgctggagt tcgtgaccgc cgccgggatc actctcggca tggacgagct gtacaagaag 720

cttagccatg gcttcccgcc ggaggtggag gagcaggatg atggcacgct gcccatgtct 780

tgtgcccagg agagcgggat ggaccgtcac cctgcagcct gtgcttctgc taggatcaat 840

gtgtag 846

<210> 41
<211> 134
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> 3 Human beta globin UTR

<400> 41
gctcgctttc ttgctgtcca atttctatta aaggttcctt tgttccctaa gtccaactac 60

taaactgggg gatattatga agggccttga gcatctggat tctgcctaat aaaaaacatt 120

tattttcatt gcaa 134

<210> 42
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> T7 Terminator

<400> 42
aacccctctc taaacggagg ggttttttt 29

<210> 43
<211> 1412
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic polynucleotide

<220>
<221> misc_feature
<223> Sequence of DLP dsGFP Mrna

<400> 43
taatacgact cactatagac atttgcttct gacacaactg tgttcactag caacctcaaa 60

cagacaccgc cgccaccata gtcagcatag tacatttcat ctgactaata ctacaacacc 120

accaccatga atagaggatt ctttaacatg ctcggccgcc gccccttccc ggcccccact 180

gccatgtgga ggccgcggag aaggaggcag gcggccccgg gaagcggagc tactaacttc 240

agcctgctga agcaggctgg agacgtggag gagaaccctg gacctatggt gagcaagggc 300

gaggagctgt tcaccggggt ggtgcccatc ctggtcgagc tggacggcga cgtaaacggc 360

cacaagttca gcgtgtccgg cgagggcgag ggcgatgcca cctacggcaa gctgaccctg 420

aagttcatct gcaccaccgg caagctgccc gtgccctggc ccaccctcgt gaccaccctg 480

acctacggcg tgcagtgctt cagccgctac cccgaccaca tgaagcagca cgacttcttc 540

aagtccgcca tgcccgaagg ctacgtccag gagcgcacca tcttcttcaa ggacgacggc 600

aactacaaga cccgcgccga ggtgaagttc gagggcgaca ccctggtgaa ccgcatcgag 660

ctgaagggca tcgacttcaa ggaggacggc aacatcctgg ggcacaagct ggagtacaac 720

tacaacagcc acaacgtcta tatcatggcc gacaagcaga agaacggcat caaggtgaac 780

ttcaagatcc gccacaacat cgaggacggc agcgtgcagc tcgccgacca ctaccagcag 840

aacaccccca tcggcgacgg ccccgtgctg ctgcccgaca accactacct gagcacccag 900

tccgccctga gcaaagaccc caacgagaag cgcgatcaca tggtcctgct ggagttcgtg 960

accgccgccg ggatcactct cggcatggac gagctgtaca agaagcttag ccatggcttc 1020

ccgccggagg tggaggagca ggatgatggc acgctgccca tgtcttgtgc ccaggagagc 1080

gggatggacc gtcaccctgc agcctgtgct tctgctagga tcaatgtgta ggctcgcttt 1140

cttgctgtcc aatttctatt aaaggttcct ttgttcccta agtccaacta ctaaactggg 1200

ggatattatg aagggccttg agcatctgga ttctgcctaa taaaaaacat ttattttcat 1260

tgcaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1320

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1380

aaaaacccct ctctaaacgg aggggttttt tt 1412

<210> 44
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic polypeptide

<220>
<221> misc_feature
<223> H-2 Kd peptide

<400> 44
Ile Tyr Ser Thr Val Ala Ser Ser Leu
1 5

<210> 45
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic polypeptide

<220>
<221> misc_feature
<223> CD4 T cell epitope

<400> 45
Lys Ser Ser Phe Phe Arg Asn Val Val Trp Leu Ile Lys Lys Asn
1 5 10 15

<210> 46
<211> 104
<212> DNA
<213> Sindbis virus

<400> 46
atgaatagag gattctttaa catgctcggc cgccgcccct tcccggcccc cactgccatg 60

tggaggccgc ggagaaggag gcaggcggcc ccgatgcctg cccg 104

<210> 47
<211> 120
<212> DNA
<213> Aura virus

<400> 47
atgaactctg tcttttacaa tccgtttggc cgaggtgcct acgctcaacc tccaatagca 60

tggaggccaa gacgtagggc tgcacctgcg cctcgaccat ccgggttgac tacccagatc 120

<210> 48
<211> 71
<212> DNA
<213> Eastern Equine Encephalitis virus SA

<400> 48
atgtttccgt atccaacatt gaactacccg cctatggcac cggttaatcc gatggcatac 60

agggacccca a 71

<210> 49
<211> 91
<212> DNA
<213> O'Nyong-Nyong virus

<400> 49
atggagttca taccagcaca aacttactac aatagaagat accagcctag accctggact 60

caacgcccta ctatccaggt gatcaggcca a 91

<210> 50
<211> 67
<212> DNA
<213> Semliki Forest virus

<400> 50
atgaattaca tccctacgca aacgttttac ggccgccggt ggcgcccgcg cccggcggcc 60

cgtcctt 67

<210> 51
<211> 69
<212> DNA
<213> Ross River virus

<400> 51
atgaattaca taccaaccca gacttttac ggacgccgtt ggcggcctcg cccggcgttc 60

cgtccatgg 69

<210> 52
<211> 91
<212> DNA
<213> Mayaro virus

<400> 52
atggatttcc taccaacaca agtgttttat ggcaggcgat ggagaccacg aatgccgcca 60

cgcccttgga ggccacgccc acctacaatt c 91