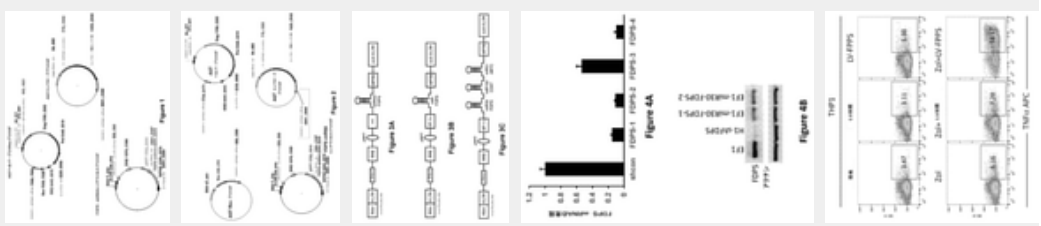
Combination Vectors and Methods for Treating Cancer

Images (11)



Classifications

C12N15/1135 Non-coding nucleic acids modulating the expression of genes, e.g. antisense oligonucleotides; Antisense DNA or RNA; Triplex- forming oligonucleotides; Catalytic nucleic acids, e.g. ribozymes; Nucleic acids used in co-suppression or gene silencing against oncogenes or tumor suppressor genes

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Other languages: Japanese

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Claims (14)

Hide Dependent

translated from Japanese

A viral vector containing a therapeutic cargo portion, wherein the therapeutic cargo portion is
The first small RNA sequence that, when expressed, is capable of binding to a first predetermined complementary mRNA sequence, said first predetermined complementary mRNA sequence is farnesyl dilin. It contains acid synthase (FDPS) mRNA, the binding results in inhibition of farnesyl diphosphate synthase (FDPS) , and the first small RNA sequence is the FDPS small RNA of SEQ ID NO: 1, 2, 3 or 4. A first small RNA sequence comprising a sequence having at least 90%, or at least 95%, or 100% identity percent with the sequence. A second small RNA that, when expressed, is capable of binding to a second predetermined complementary mRNA sequence, wherein the second predetermined complementary mRNA sequence is a CD47 mRNA sequence or A viral vector comprising a cMyc mRNA sequence and a second small RNA, wherein the binding results in inhibition of CD47 or cMyc. The viral vector of claim 1, wherein the at least one small RNA is under the control of a first promoter and the second small RNA sequence is under the control of a second promoter. The therapeutic cargo moiety further comprises a third small RNA sequence capable of binding to a third predetermined complementary mRNA sequence when expressed, the third predetermined complementary mRNA. The viral vector according to claim 1 , wherein the sequence comprises a CD47 mRNA sequence or a cMyc mRNA sequence, and the binding results in inhibition of CD47 or cMyc. The viral vector of claim 3, wherein the third small RNA sequence is under the control of a third promoter. The viral vector of claim 3, wherein the small RNA sequence is under the control of a single promoter. The viral vector according to claim 1, wherein the small RNA sequence comprises miRNA or shRNA. The second small RNA sequence is
Small RNA sequence of CD47 of SEQ ID NO: 5, 6, 7, 8 or 9 or cMyc small RNA sequence of SEQ ID NO: 10, 11, 12, 13 or 14
The viral vector of claim 1, comprising a sequence having at least 90%, or at least 95%, or 100% identity. The third small RNA sequence is
Small RNA sequence of CD47 of SEQ ID NO: 5, 6, 7, 8 or 9 or small RNA sequence of cMyc of SEQ ID NO: 10, 11, 12, 13 or 14
The viral vector of claim 3, comprising a sequence having at least 90%, or at least 95%, or 100% identity. The virus vector according to any one of claims 1 to 8 , which is a lentiviral vector. Lentiviral particles that can infect target cells
a. Envelope proteins optimized to infect the target cells, and b. A lentiviral particle comprising the viral vector according to any one of claims 1 to 9 . a. The lentiviral particle according to claim 10, and b. A composition comprising an aminobisphosphonate drug. The composition according to claim 11 , wherein the aminobisphosphonate drug is zoledronic acid. The composition according to claim 1 1 or 12 for use in the treatment of cancer. The composition according to claim 11 or 12 for use in the formulation of a pharmaceutical product for treating cancer.

Description

translated from Japanese

Cross-reference to related applications This application was filed on 9 March 2016 and is entitled "Combination Vectors and Uses Thereof" US Provisional Patent Application Nos. 62/305,944 (which is hereby for reference). Claim priority to (incorporated in the book).

Aspects of the present disclosure relate to treating cancer using vectors. More specifically, aspects of the present disclosure relate to treating cancer using vectors such as combination vectors.

Cancer is a serious health problem for people around the world. As an example, liver cancer is the fifth most commonly diagnosed cancer in the world in adult men and the second most common cause of cancer-related deaths worldwide. Numerous treatment strategies have been used in efforts to effectively treat cancer. Traditional therapeutic approaches have revolved around the use of chemotherapy and radiation therapy.

Chemotherapy refers to the administration of one or more anticancer drugs and / or other drugs to a cancer patient by various methods. Broadly speaking, most chemotherapeutic agents work by inhibiting mitosis (cell division) and effectively targeting rapidly dividing cells. However, other rapidly dividing cells, such as those involved in hair growth and those involved in the exchange of intestinal epithelium (lining), are also affected. Because chemotherapy affects cell division, both normal and cancerous cells are susceptible to the cytotoxic effects of chemotherapeutic agents.

Radiation therapy refers to exposing a patient to high-energy radiation such as X-rays, gamma rays, and neutrons. This type of therapy includes, but is not limited to, external beam therapy, internal radiation therapy, implant radiation therapy, proximity radiation therapy, systemic radiation therapy, and radiation therapy. Examples of the external beam irradiation include three-dimensional conformal irradiation, intensity-modulated radiotherapy, and proto-proton beam irradiation therapy. It is practically difficult to irradiate a therapeutic dose while shielding nearby normal tissue from the cytotoxic effects of radiation. A further problem associated with radiation is the

induction of radiation-resistant cells during the course of treatment. Therefore, even the best radiation therapy techniques often result in incomplete tumor reduction and subsequent recurrence.

More recently, immunotherapeutic approaches have been used that seek to treat cancer by harnessing the power of the host's immune system. For example, strategies have been used to target such antigens using host-based T cells that specifically recognize cancer-related antigens. For example, recent approaches have focused on the development and use of chimeric antigen receptor (CAR) T cells (also known as CAR-T cells). Possible side effects associated with CAR-T cell therapy include chemokine release syndrome, B cell aplasia, and tumor lysis syndrome. Despite the development of these approaches, cancer remains a serious health problem.

In one aspect of the present disclosure, a viral vector comprising a therapeutic cargo moiety is disclosed. The therapeutic cargo moiety comprises at least one small RNA sequence capable of binding to at least one predetermined complementary mRNA sequence, and at least one complementary mRNA sequence comprises an FDPS mRNA sequence. .. In embodiments, the therapeutic cargo moiety may further comprise a second small RNA sequence capable of binding to a second predetermined complementary mRNA sequence, the second predetermined complementary mRNA sequence. Contains the mRNA sequence of CD47 or cMyc mRNA sequence. In embodiments, the at least one small RNA sequence is under the control of the first promoter and the second small RNA sequence is under the control of the second promoter. In embodiments, the therapeutic cargo moiety may further comprise a third small RNA sequence capable of binding to a third predetermined complementary mRNA sequence, the third predetermined complementary mRNA sequence. Contains the mRNA sequence of CD47 or cMyc mRNA sequence. In embodiments, the at least one small RNA sequence is under the control of the first promoter, the second small RNA sequence is under the control of the second promoter, and the third small RNA sequence is the third. Is under the control of the promoter. In embodiments, the small RNA sequence is under the control of a single promoter. In embodiments, the small RNA sequence is microRNA (miRNA) or short hairpin RNA (SHRNA).

In another embodiment, the small RNA sequence is
GTCCTGGAGTACAATGCCATTCTCCGAGAATGGCATTGTACTCAGGACTTTTT (SEQ ID NO: 1);
GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTCTGCTTTTT (SEQ ID NO: 2);
GCCATGTACATGGCAGGGAATTCTTCGAATATCCGCCATTGTCATGGGTTTTT (SEQ ID NO: 3);
Includes a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% identity percent with a small RNA sequence of FDPS comprising. In embodiments, the small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, or 4.

In another embodiment, the second small RNA sequence is
GGTGAAACGATCATCGAGCCTCGAGGCTCGATGATCGTTTCACTTTTT (SEQ ID NO: 5);
GCTACTGGCCTTGGTTTAACTCGAGTTAAAACCAAGGCCAGTAGCTTTTT (SEQ ID NO: 6);
CCTCCTTCGTCATTGCCATTCGAGAGGCAATGAAGGAGGGTTTTT (SEQ ID NO: 7);
GCATGGCCCTTTCTGATTCTCCGAGAATCAGAAGAGGCCATGTTTTT (SEQ ID NO: 8);
Small RNA sequence of CD47 containing CD47 or GCTTCACCACAGGAACTATTGCTCGAGCATAGTTTCCTGTTGGTGAAGCTTTT (SEQ ID NO: 10);
GCGAACACACAAACGTCTTGGACTCGAGTCCAAAGACGTTGTGTGTGTTCGCTTTT (SEQ ID NO: 11);
GACATGGGTGAACCAGAGTTTCTCCGAGGAAACTCTGGGTTCACCATGTCTTTTT (SEQ ID NO: 12);
GAGAATGTCAAGAGGCGAACACTCGAGTGTTCGCCTTTGACATTTCTTTTT (SEQ ID NO: 13);
Includes a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% identity percent with a small RNA sequence of cMyc comprising. In embodiments, the second small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.

In another embodiment, the third small RNA sequence is a small RNA sequence of CD47 comprising SEQ ID NO: 5, 6, 7, 8 or 9, or a small cMyc comprising SEQ ID NO: 10, 11, 12, 13, or 14. Includes a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% identity percent with an RNA sequence. In embodiments, the third small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.

In another aspect, a viral vector comprising a therapeutic cargo moiety is disclosed. The therapeutic cargo moiety comprises at least one small RNA sequence capable of binding to at least one predetermined complementary mRNA sequence, and the at least one complementary mRNA sequence comprises the mRNA sequence of CD47. In embodiments, the therapeutic cargo moiety further comprises a second small RNA sequence capable of binding to a second predetermined complementary mRNA sequence, the second predetermined complementary mRNA sequence being FDPS. Includes mRNA sequence of or cMyc mRNA sequence. In embodiments, the at least one small RNA sequence is under the control of the first promoter and the second small RNA sequence is under the control of the second promoter. In embodiments, the therapeutic cargo moiety further comprises a third small RNA sequence capable of binding to a third predetermined complementary mRNA sequence, the third

predetermined complementary mRNA sequence being FDPS. Includes mRNA sequence of or cMyc mRNA sequence. The small RNA sequence can be miRNA or shRNA. In embodiments, the at least one small RNA sequence is under the control of the first promoter, the second small RNA sequence is under the control of the second promoter, and the third small RNA sequence is the third. Is under the control of the promoter. In embodiments, the small RNA sequence is under the control of a single promoter.

In another embodiment, the small RNA sequence is at least 80%, or at least 85%, or at least 90%, or at least 95% identical to the small RNA sequence of CD47 comprising SEQ ID NO: 5, 6, 7, 8, or 9. Includes sequences with sex percent. In embodiments, the small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, or 9.

In another embodiment, the second small RNA sequence is an FDPS small RNA sequence comprising SEQ ID NO: 1, 2, 3, or 4 or a cMyc small RNA sequence comprising SEQ ID NO: 10, 11, 12, 13, or 14. And include sequences having at least 80%, or at least 85%, or at least 90%, or at least 95% identity percent. In embodiments, the second small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, 4, 10, 11, 12, 13, or 14.

In another embodiment, the third small RNA is a small RNA sequence of FDPS comprising SEQ ID NO: 1, 2, 3, or 4 or a small RNA sequence of cMyc comprising SEQ ID NO: 10, 11, 12, 13, or 14. Contains sequences having at least 80%, or at least 85%, or at least 90%, or at least 95% identity percent. In embodiments, the third small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, 4, 10, 11, 12, 13, or 14.

In another aspect, a viral vector comprising a therapeutic cargo moiety is disclosed. The therapeutic cargo moiety is a first small RNA sequence capable of binding to a first predetermined complementary mRNA sequence, and at least one capable of binding to a second predetermined complementary mRNA sequence. The first predetermined complementary mRNA sequence contains an additional small RNA sequence, the second predetermined complementary mRNA sequence contains the cMyc mRNA sequence, and the second predetermined complementary sequence contains the FDPS mRNA sequence or the CD47 mRNA sequence. include.

In another embodiment, the therapeutic cargo moiety further comprises a third small RNA sequence capable of binding to a third predetermined complementary mRNA sequence, the third predetermined complementary mRNA sequence. Includes FDPS mRNA sequence or CD47 mRNA sequence. In embodiments, the small RNA sequence is a miRNA or shRNA. In embodiments, the first small RNA sequence is under the control of the first promoter, the second small RNA sequence is under the control of the second promoter, and the third small RNA sequence is the third. Is under the control of the promoter. In embodiments, the small RNA sequence is under the control of a single promoter.

In another embodiment, the first small RNA sequence is at least 80%, or at least 85%, or at least 90%, or at least 95% of the small RNA sequence of cMyc comprising SEQ ID NO: 10, 11, 12, 13, or 14. Includes sequences with% identity percent. In embodiments, the first small RNA sequence is selected from SEQ ID NOs: 10, 11, 12, 13, or 14.

In another embodiment, the at least one additional small RNA sequence is a small RNA sequence of FDPS containing SEQ ID NO: 1, 2, 3, or 4 or a small CD47 containing SEQ ID NO: 5, 6, 7, 8, or 9. Includes a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% identity percent with an RNA sequence. In embodiments, at least one additional small RNA is selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, or 9.

In another embodiment, the third small RNA sequence is an FDPS small RNA sequence comprising SEQ ID NO: 1, 2, 3, or 4 or a CD47 small RNA sequence comprising SEQ ID NO: 5, 6, 7, 8, or 9. And include sequences having at least 80%, or at least 85%, or at least 90%, or at least 95% identity percent. In embodiments, the third small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, or 9.

In another aspect, the viral vector is a lentiviral vector. In another aspect, lentiviral particles capable of infecting target cells are disclosed. Lentiviral particles include enveloped proteins optimized to infect target cells, and the viral vectors described herein. In embodiments, the target cell is a tumor cell.

In another aspect, a composition comprising the lentiviral particles described herein and an aminobisphosphonate drug is disclosed. In embodiments, the aminobisphosphonate drug is zoledronic acid.

In another aspect, a method of treating cancer in a subject is disclosed. The method comprises administering to the subject a therapeutically effective amount of the composition detailed herein.

In another aspect, a method of treating cancer in a subject is disclosed. The method comprises administering to the subject a therapeutically effective amount of lentiviral particles and a therapeutically effective amount of an aminobisphosphonate drug as detailed herein. In another aspect, a method of preventing cancer in a subject is disclosed. The method comprises administering to the subject a therapeutically effective amount of lentiviral particles and a therapeutically effective amount

of an aminobisphosphonate drug as detailed herein. In embodiments, the above steps are performed simultaneously. In embodiments, a period of defined length elapses during the steps described above. In embodiments, the aminobisphosphonate drug is zoledronic acid. In embodiments, the therapeutically effective amount of lentiviral particles comprises a plurality of single dose lentiviral particles. In embodiments, the therapeutically effective amount of the aminobisphosphonate drug comprises a single dose of the aminobisphosphonate drug.

Other aspects and advantages of the invention described herein will become apparent from the following detailed description, in conjunction with the accompanying drawings illustrating embodiments of the invention by way of example.

FIG. 1 shows an exemplary circular 3-vector lentiviral system.

FIG. 2 shows an exemplary circular 4-vector lentiviral system.

FIG. 3 shows (A) a linear map of a lentiviral vector encoding an FDPS shRNA targeting sequence, (B) a linear map of a lentiviral vector encoding a synthetic microRNA (miRNA) having an FDPS targeting sequence, and (C).) A linear map of a lentiviral combination vector encoding a synthetic microRNA (miRNA) having a target sequence for expression of cMyc, FDPS, and CD47 is shown.

FIG. 4 shows (A) relative expression levels of human FDPS mRNA in response to the various shRNA constructs described herein, and (B) RNA interference based on miR delivered by lentivirus to express FDPS. Shows that it inhibits.

FIG. 5 shows the cytokine expression levels of human peripheral blood gamma delta T cells after exposure to (A) THP1 cells transduced with lentivirus to suppress FDPS. FIG. 5 shows the cytokine expression levels of human peripheral blood gamma delta T cells after exposure to (B) HepG2 cells transduced with lentivirus to suppress FDPS.

FIG. 6 shows the percentage of THP-1 tumor cell lines mixed with normal human gamma delta T cells under various experimental conditions described herein after modification by transduction with lentivirus to suppress FDPS. Shows the percentage of dissolution.

FIG. 7 shows (A) relative expression levels of human CD47 mRNA in response to the various shRNA constructs described herein, and (B) RNA interference based on miR delivered with a lentivirus reveals CD47 expression. Indicates to inhibit.

FIG. 8 shows that (A) relative expression levels of human cMyc in response to the various shRNA constructs described herein, and (B) RNA interference based on miR delivered by lentivirus inhibits cMyc expression. Indicates to do.

FIG. 9 shows a linear map of the lentiviral vector encoding the FDPS shRNA targeting sequence used in Example 6 herein.

FIG. 10 shows the effect of zoledronic acid treatment on NOD / SCID mice transplanted with PC3 cells transduced with LV-shFDPS or control LV described herein. (A) shows the photo data of the 8th day, (B) shows the photon intensity data of the 8th day, (C) shows the photo data of the 22nd day, and (D) shows the photon of the 22nd day. Intensity data is shown.

Summary of Disclosure The present disclosure relates to therapeutic vectors and their delivery to cells. In embodiments, the therapeutic vector targets more than one mRNA target. In embodiments, the therapeutic vector comprises a small RNA, such as short-chain homologous RNA (SHRNA) or microRNA (miRNA), that reduces the expression level of this enzyme by targeting FDPS. Examples of the therapeutic vector include lentiviral vectors. The present disclosure demonstrates that cancer can be effectively treated by targeting FDPS in combination with treatment with aminobisphosphonate drugs.

Definitions and Interpretations Unless defined otherwise herein, scientific terms used in the context of this disclosure have meaning generally understood by one of ordinary skill in the art. Further, unless otherwise required by the context, a singular word embraces the plural and a plurality of words embraces the singular. In general, the academic terms and techniques used herein in the context of cell and tissue culture, molecular biology, immunology, microbiology, genetics, and protein and nucleic acid chemistry and hybridization are used. It is well known and commonly used in the art. Unless otherwise stated, the methods and techniques of the present disclosure refer to conventional methods well known in the art and described in various general and more specific references cited and discussed throughout this specification. Therefore, it is generally done. For example, Sambrook J. et al. And Russel D. , Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. et al. Y. (2000); Ausubel et al., Short Protocols in Molecular Biology: A Compendium of Methods from Clinical Biology, Wiley, Wiley, John. (2002);

Harlow and Lane, Using Antibodies: A Laboratory Manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. et al. Y. (1998); and Coligan et al., Short Protocols in Protein Science, Wiley, John & Sons, Inc. See (2003). Any enzymatic reaction or purification technique is performed according to the manufacturer's specifications as is generally performed in the art or as described herein. The academic terms used herein in the context of analytical chemistry, synthetic organic chemistry, medicinal chemistry, and medicinal chemistry, as well as experimental procedures and techniques, are well known and commonly used in the art. be.

As used herein and in the appended claims, the singular forms "one (a)", "one (an)", and "the" are used interchangeably and the context is clear. Unless indicated otherwise, the plural is also intended to be included and to be included within the scope of their respective meanings. Also, as used herein, "and / or" shall not be combined as any possible combination of one or more of the listed items, as well as when interpreted as an option ("or"). Refers to, and includes these.

For example, all numerical designations such as pH, temperature, time, concentration, and molecular weight, such as range, are approximations that vary from (+) or (−) in increments of 0.1. It should be understood that the term "about" precedes all numerical designations, although not necessarily explicitly stated. The term "about" includes a small increment of "X" such as "X + 0.1" or "X-0.1", as well as the exact value of "X". Although not necessarily explicitly stated, it should also be understood that the reagents described herein are merely exemplary and equivalents of such are known in the art.

As used herein, the term "about" is understood by one of ordinary skill in the art and will vary to some extent depending on the context in which it is used. "About" means up to plus or minus 10% of a particular term when a term that is not clear to one of ordinary skill in the art is used given the context in which it is used.

The term "administering" or "administering" an activator can introduce an activator into a subject in need of treatment in a therapeutically useful form and in a therapeutically effective amount. It should be understood that it means giving in form.

As used herein, the term "combination vector" means a therapeutic vector that targets more than one mRNA. For example, a therapeutic vector containing two shRNAs or two miRNAs targeting two different mRNAs may be referred to as a "combination vector".

As used herein, the term "contains" is intended to mean that the composition and method include the described elements but does not exclude others. When used to define a composition and method, "becoming essential" means excluding other elements that have some intrinsic significance to the composition or method. By "consisting" is meant excluding elements of other ingredients that are not minor to the claimed composition and substantive method steps. The embodiments defined by each of these transitional clauses are within the scope of this disclosure. Thus, the method and composition may (contains) additional steps and ingredients, or may (essentially consist of) unimportant steps and compositions, or are intended solely as described method steps or compositions. Is intended to be (consisting of).

As used herein, "expressing," "expressing," or "encoding" means the process by which a polynucleotide is transcribed into mRNA and / or the transcribed mRNA is subsequently peptide. The process of being translated into a polypeptide or protein. Expression can include splicing of mRNA in eukaryotic cells, or other forms of post-transcriptional or post-translational modification.

The term "farnesyl diphosphate synthase" may also be referred to herein as FDPS, and may also be referred to herein as farnesyl pyrophosphate synthase or FPPS.

The term "gamma delta T cell" can also be referred to herein as a γδ T cell or even a GDT cell. The term "gamma delta T cell activation" refers to any measurable biological phenomenon associated with such T cells, which is representative of activated gamma delta T cells. Non-limiting examples of such biological phenomena include increased cytokine production, altered qualitative or quantitative composition of cell surface proteins, increased T cell proliferation, and / or killing target cells. Alternatively, there is an increase in T cell effector function, such as helping another effector cell kill the target cell. The target cell can be a cancer cell.

The terms "individual," "subject," and "patient" are used interchangeably herein and are any individual mammalian subject (eg, bovine, dog, cat, horse, or human). Point to.

The term "LV" generally refers to "lentivirus". As an example, the reference to "LV-shFDPS" is a reference to a lentivirus that expresses an FDPS targeting shRNA.

The term "miRNA" refers to microRNA and may also be referred to herein as "miR".

The term "packaging cell line" refers to any cell line that can be used to express lentiviral particles.

The term "percent identity" in the context of two or more nucleic acid or polypeptide sequences is one of the following sequence comparison algorithms (eg, BLASTP and BLASTN, or other algorithms available to those of skill in the art). Two or more sequences having a particular percentage of nucleotide or amino acid residues that are identical when compared and aligned for maximum match, either using one or as measured by visual inspection. Refers to a partial array. Depending on the application,

the "percent identity" can be present over one region of the sequence being compared (eg, across the functional domain) or over the full length of the two sequences being compared. For sequence comparison, usually one sequence serves as a reference sequence to which the test sequence is compared. When using the sequence comparison algorithm, enter the test and reference sequences into the computer, specify the partial array coordinates, and specify the parameters of the sequence algorithm program if necessary. The sequence comparison algorithm then calculates the percent sequence identity of the test sequence to the reference sequence based on the specified program parameters.

Optimal alignment of sequences for comparison is described, for example, in Smith and Waterman, Adv. Apple. Math. Volume 2: Page 482 (1981) Local Homology Algorithm, Needleman and Wunsch, J. Mol. Mol. Biol. 48: 443 (1970) Homology Alignment Algorithm, Pearson and Lipman, Proc. Nat'l. Acad. Sci. USA Vol. 85: p. 2444 (1988), Computerized Execution of These Algorithms (GAP, BESTFIT, FASTA, and TFASTA; Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science.), Or by visual inspection (generally see Ausube et al., See below).

Examples of suitable algorithms for determining percent sequence identity and percent sequence similarity are described in Altschul et al., J. Mol. Mol. Biol. 215: The BLAST algorithm described in pp. 403-410 (1990). Software for performing BLAST analysis is available through the website of the National Center for Biotechnology Information.

Percent identities between the two nucleotide sequences were determined using the GAP program in the GCG software package (available at http://www.ggcg.com) and NWSgapdna. It can be determined using a CMP matrix and a gap weighting of 40, 50, 60, 70, or 80, and a length weighting of 1, 2, 3, 4, 5, or 6. Also, the percent identity between two nucleotide sequences or between amino acid sequences was incorporated into the ALIGN program (version 2.0). Meyers and W.M. It can also be determined using the algorithm of Miller (CABIOS, Vol. 4, pp. 11-17, (1989)) using the PAM120 weighted residue table, 12 as the gap length penalty, and 4 as the gap penalty. .. In addition, the percent identity between the two amino acid sequences is included in the GAP program in the GCG software package (available at http://www.ggcg.com) for Needleman and Wunsch (J. Mol. Biol. 48): using the algorithm of pages 444-453 (1970)), either the Blossum 62 matrix or the PAM250 matrix, and the gap weighting of 16, 14, 12, 10, 8, 6, or 4, and 1. It can be determined using a length weight of 2, 3, 4, 5, or 6.

The nucleic acid and protein sequences of the present disclosure can be further used as "query sequences" for searching public databases, eg, to identify related sequences. Such a search was performed by Altschul et al. (1990), J. Mol. Mol. Biol. Volume 215: This can be done using the NBLAST and XBLAST programs (version 2.0) on pages 403-10. BLAST nucleotide searches can be performed using the NBLAST program with a score of 100 and a word length of 12 to obtain nucleotide sequences that are homologous to the nucleic acid molecules provided in the present disclosure. The BLAST protein search can be performed using the XBLAST program with a score of 50 and a word length of 3 in order to obtain an amino acid sequence homologous to the protein molecule of the present disclosure. To obtain a gapped alignment for comparison purposes, Altschul et al. (1997) Nucleic Acids Res. Volume 25 (No. 17): Gapped BLAST can be used as described on pages 3389-3402. When using BLAST and Gapped BLAST programs, the default parameters of each program (eg, XBLAST and NBLAST) can be used. http: // www. ncbi. nlm. nih. gov. See.

As used herein, "pharmaceutically acceptable" is, within reasonable medical judgment, excessive toxicity, irritation, allergic reactions commensurate with a reasonable benefit / risk ratio. , Or a compound, material, composition, and / or dosage form suitable for use in contact with human and animal tissues, organs, and / or body fluids without causing any other problems or complications.

As used herein, "pharmaceutically acceptable carrier" is any physiologically compatible solvent, dispersion medium, coating, antibacterial and antifungal agent, isotonic agent, and absorption. Refers to, and includes, retarders and the like. The composition may comprise a pharmaceutically acceptable salt, such as an acid or base addition salt (see, eg, Berge et al., (1977) J Pharm Sci Vol. 66, pp. 1-19).

As used herein, the term "SEQ ID NO" is synonymous with the term "Sequence ID No.".

As used herein, "small RNA" refers to non-coding RNA, generally about 200 nucleotides or less in length, with silencing or interfering function. In other embodiments, the small RNA is about 175 nucleotides or less, about 150 nucleotides or less, about 125 nucleotides or less, about 100 nucleotides or less, or about 75 nucleotides or less in length. .. Such RNAs include microRNAs (miRNAs), small interfering RNAs (siRNAs), double-stranded RNAs (dsRNAs), and short-stranded hairpin RNAs (SHRNAs). The "small RNA" of the present disclosure should generally be able to inhibit or knock down gene expression of the target gene through a pathway that causes disruption of the mRNA of the target gene.

The term "therapeutically effective amount" is used in a composition suitable for treating or preventing the development of symptoms, progressions, or complications found in a patient suffering from a given disease, injury, disease, or condition. And refers to a sufficient amount of the active agent of the present disclosure in a suitable

dosage form. The therapeutically effective amount varies depending on the condition of the patient or its severity, and the age, weight, etc. of the subject to be treated. The therapeutically effective amount may vary depending on any of several factors, such as the route of administration, the condition of the subject, and other factors understood by those of skill in the art.

As used herein, the term "therapeutic vector" includes, without limitation, reference to a lentiviral vector or an adeno-associated virus (AAV) vector. Further, as used herein with respect to the lentiviral vector system, the term "vector" is synonymous with the term "plasmid". For example, a 3-vector system and a 4-vector system including a 2-vector and 3-vector packaging system can also be referred to as a 3-plasmid system and a 4-plasmid system.

"Treatment" is intended to target and combat the disease state, i.e., to improve or prevent the disease state. Thus, the particular treatment depends on the diseased condition being targeted, as well as the current or future situation of pharmaceutical therapy and therapeutic approaches. Treatment may have associated toxicity.

The term "treatment" or "treatment" generally refers to interventions that attempt to change the natural course of the subject being treated and may be performed prophylactically or during the course of clinical pathology. Desirable effects include prevention of disease onset or recurrence, relief of symptoms, suppression, reduction or inhibition of any direct or indirect pathological consequences of the disease, improvement or alleviation of the disease state, and remission or prognosis. Induction of improvement can be mentioned, but is not limited to these.

Description of Embodiments of the Disclosure In one aspect of the disclosure, a viral vector comprising a therapeutic cargo moiety is disclosed. The therapeutic cargo moiety comprises at least one small RNA sequence capable of binding to at least one predetermined complementary mRNA sequence, and at least one complementary mRNA sequence comprises an FDPS mRNA sequence. In embodiments, the therapeutic cargo moiety may further comprise a second small RNA sequence capable of binding to a second predetermined complementary mRNA sequence, the second predetermined complementary mRNA sequence. Contains the mRNA sequence of CD47 or cMyc mRNA sequence. In embodiments, the therapeutic cargo moiety may further comprise a third small RNA sequence capable of binding to a third predetermined complementary mRNA sequence, the third predetermined complementary mRNA sequence. Contains the mRNA sequence of CD47 or cMyc mRNA sequence. The small RNA sequence can be microRNA (miRNA) or short hairpin RNA (SHRNA).

In another embodiment, the small RNA sequence is at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84 of the FDPS small RNA sequence comprising SEQ ID NO: 1, 2, 3, or 4. %, Or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94. %, Or at least 95%, or higher. In embodiments, the small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, or 4.

In another embodiment, the second small RNA sequence is a small RNA sequence of CD47 comprising SEQ ID NO: 5, 6, 7, 8 or 9, or a small cMyc comprising SEQ ID NO: 10, 11, 12, 13, or 14. At least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89 of the RNA sequence. %, Or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or a sequence having higher identity. In embodiments, the second small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.

In another embodiment, the third small RNA sequence is a small RNA sequence of CD47 comprising SEQ ID NO: 5, 6, 7, 8 or 9, or a small cMyc comprising SEQ ID NO: 10, 11, 12, 13, or 14. At least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89 of the RNA sequence. %, Or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or a sequence having higher identity. In embodiments, the third small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.

In another aspect, a viral vector comprising a therapeutic cargo moiety is disclosed. The therapeutic cargo moiety comprises at least one small RNA sequence capable of binding to at least one predetermined complementary mRNA sequence, and the at least one complementary mRNA sequence comprises the mRNA sequence of CD47. In embodiments, the therapeutic cargo moiety further comprises a second small RNA sequence capable of binding to a second predetermined complementary mRNA sequence, the second predetermined complementary mRNA sequence being FDPS. Includes mRNA sequence of or cMyc mRNA sequence. In embodiments, the therapeutic cargo moiety further comprises a third small RNA sequence capable of binding to a third predetermined complementary mRNA sequence, the third predetermined complementary mRNA sequence being FDPS. Includes mRNA sequence of or cMyc mRNA sequence. In embodiments, the small RNA sequence is a miRNA or shRNA.

In another embodiment, the small RNA sequence is at least 80%, or at least 85%, or at least 90%, or at least 95% identical to the small RNA sequence of CD47 comprising SEQ ID NO: 5, 6, 7, 8, or 9. Includes sequences with sex percent. In embodiments, the small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, or 9.

In another embodiment, the second small RNA sequence is an FDPS small RNA sequence comprising SEQ ID NO: 1, 2, 3, or 4 or a cMyc small RNA sequence comprising SEQ ID NO: 10, 11, 12, 13, or 14. And include sequences having at least 80%, or at least 85%, or at least 90%, or at least 95% identity percent. In embodiments, the second small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, 4, 10, 11, 12, 13, or 14.

In another embodiment, the third small RNA is a small RNA sequence of FDPS comprising SEQ ID NO: 1, 2, 3, or 4 or a small RNA sequence of cMyc comprising SEQ ID NO: 10, 11, 12, 13, or 14. At least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or Includes sequences with at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or higher identity. In embodiments, the third small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, 4, 10, 11, 12, 13, or 14.

In another aspect, a viral vector comprising a therapeutic cargo moiety is disclosed. The therapeutic cargo moiety is a first small RNA sequence capable of binding to a first predetermined complementary mRNA sequence, and at least one capable of binding to a second predetermined complementary mRNA sequence. The first predetermined complementary mRNA sequence contains an additional small RNA sequence, the second predetermined complementary mRNA sequence contains the cMyc mRNA sequence, and the second predetermined complementary sequence contains the FDPS mRNA sequence or the CD47 mRNA sequence. include.

In another embodiment, the therapeutic cargo moiety further comprises a third small RNA sequence capable of binding to a third predetermined complementary mRNA sequence, the third predetermined complementary mRNA sequence. Includes FDPS mRNA sequence or CD47 mRNA sequence. In embodiments, the small RNA sequence is a miRNA or shRNA.

In another embodiment, the first small RNA sequence is at least 80%, or at least 81%, or at least 82%, or at least 83 with the cMyc small RNA sequence comprising SEQ ID NO: 10, 11, 12, 13, or 14. %, Or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93. %, Or at least 94%, or at least 95%, or higher. In embodiments, the first small RNA sequence is selected from SEQ ID NOs: 10, 11, 12, 13, or 14.

In another embodiment, the at least one additional small RNA sequence is a small RNA sequence of FDPS containing SEQ ID NO: 1, 2, 3, or 4 or a small CD47 containing SEQ ID NO: 5, 6, 7, 8, or 9. At least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89 of the RNA sequence. %, Or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 95%, or higher. In embodiments, at least one additional small RNA is selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, or 9.

In another embodiment, the third small RNA sequence is an FDPS small RNA sequence comprising SEQ ID NO: 1, 2, 3, or 4 or a CD47 small RNA sequence comprising SEQ ID NO: 5, 6, 7, 8, or 9. And at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, Or include sequences having at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or higher identity. In embodiments, the third small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, or 9.

In another aspect, the small RNA sequences referred to herein are miR30 FDPS sequence # 1 (SEQ ID NO: 53), miR30 FDPS sequence # 2 (SEQ ID NO: 54), miR30 FDPS sequence # 3 (SEQ ID NO: 55), and the like. miR155 FDPS sequence # 1 (SEQ ID NO: 56), miR21 FDPS sequence # 1 (SEQ ID NO: 57), miR185 FDPS sequence # 1 (SEQ ID NO: 58), miR155 CD47 sequence # 1 (SEQ ID NO: 82; miR155 CD47 target sequence # 2 (SEQ ID NO: 82; miR155 CD47 target sequence # 2) Books such as SEQ ID NO: 66), miR155 CD47 target sequence # 3 (SEQ ID NO: 67), miR155 CD47 target sequence # 4 (SEQ ID NO: 68), miR21 cMyc sequence (SEQ ID NO: 83), or miR155 cMyc sequence (SEQ ID NO: 70). At least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87 of any of the miRNA sequences detailed herein. %, Or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or higher. May include an array.

In embodiments, the small RNA sequences are miR30 FDPS sequence # 1 (SEQ ID NO: 53), miR30 FDPS sequence # 2 (SEQ ID NO: 54), miR30 FDPS sequence # 3 (SEQ ID NO: 55), miR155 FDPS sequence # 1 (SEQ ID NO:). 56), miR21 FDPS sequence # 1 (SEQ ID NO: 57), miR185 FDPS sequence # 1 (SEQ ID NO: 58), miR155 CD47 sequence # 1 (SEQ ID NO: 82; miR155 CD47 target sequence # 2 (SEQ ID NO: 66), miR155 CD47 target. The miRNA detailed herein, such as SEQ ID NO: 3 (SEQ ID NO: 67), miR155 CD47 target sequence # 4 (SEQ ID NO: 68), miR21 cMyc sequence (SEQ ID NO: 83), or miR155 cMyc sequence (SEQ ID NO: 70). It may contain any of the sequences.

In another aspect, the viral vector is a lentiviral vector. In another aspect of the present disclosure, lentiviral particles capable of infecting target cells are disclosed. Lentiviral particles include enveloped proteins optimized to infect target cells, and the viral vectors described herein. In embodiments, the target cell is a tumor cell.

In another aspect, a composition comprising the lentiviral particles described herein and an aminobisphosphonate drug is disclosed. In embodiments, the aminobisphosphonate drug is zoledronic acid.

In another aspect of the present disclosure, a method of treating cancer in a subject is disclosed. The method comprises administering to the subject a therapeutically effective amount of the composition detailed herein.

In another aspect, a method of treating cancer in a subject is disclosed. The method comprises administering to the subject a therapeutically effective amount of lentiviral particles and a therapeutically effective amount of an aminobisphosphonate drug as detailed herein. In embodiments, the above steps are performed simultaneously. In embodiments, a period of defined length elapses during the steps described above. In embodiments, the aminobisphosphonate drug is zoledronic acid. In embodiments, the therapeutically effective amount of lentiviral particles comprises a plurality of single dose lentiviral particles. In embodiments, the therapeutically effective amount of the aminobisphosphonate drug comprises a single dose of the aminobisphosphonate drug.

A further aspect of the invention describes the development of a multi-gene targeting vector for the treatment of cancer, by which, as a non-limiting example, is for the treatment of hepatocellular carcinoma (“HCC”). These vectors address three concerns regarding the therapy of HCC. First, the therapeutic vector may contain an inhibitory RNA construct for reducing the expression of the cMyc oncogene protein. The cMyc oncogene protein is involved in tumorigenesis, tumor growth, and antigenic escape. Therapeutic vectors may contain more than one inhibitory RNA construct for reducing expression of cMyc. For example, in embodiments, combinatorial vectors are specifically envisioned where cMyc is the target of the vector. Second, a vector was developed to reduce the expression of farnesyl diphosphate synthase ("FDPS") (eg, through an inhibitory RNA construct). By reducing the level of FDPS, the tumor cells are modified to stimulate, for example, gamma delta T cells. These gamma delta T cells are capable of cytotoxic killing of tumor cells. Third, a vector was developed to reduce the expression of at least one other gene product (eg, through an inhibitory RNA construct). In certain embodiments, the at least one other gene product can be an immune checkpoint regulator. Examples of immune checkpoint regulators are programmed cell death ligand 1 (PD-L1), galactosidase-binding soluble lectin 9 (LGALS9A), tumor necrosis factor receptor superfamily member 14 (HVEM), V-set domain-containing T cells. Examples include, but are not limited to, activation inhibitor 1 (B7-H4), CD276 molecule (B7-H3), CD80 molecule (CD28LG1), and CD86 molecule (CD28LG2). In embodiments, the immune checkpoint regulator is PD-L1. Since cMyc is a positive regulator of PD-L1 expression and the expression of other antigenic escape genes such as CD47 expressed in tumor cells, reducing cMyc expression results in PD-L1. The level decreases. By reducing the level of CD47, tumor phagocytosis is increased, leading to improved T cell response through cross-presentation of tumor antigens on antigen-presenting cells. By reducing PD-L1 and potentially other immune checkpoint inhibitory molecules, the efficiency of T cell immune stimulation, such as gamma delta T cell stimulation, can be improved. cMyc regulates PD-L1 levels, but uses the therapeutic vectors described herein by producing shRNA or miRNA specifically targeted for PD-L1 or other immune checkpoint regulators. PD-L1 or other immune checkpoint regulators can be directly targeted.

In certain embodiments, the at least one other gene product can be a gene product that affects phagocytosis. For example, at least one other gene product that affects phagocytosis can be CD47. Reducing the expression of CD47 removes the impaired macrophage phagocytosis of tumor cells. These two mechanisms combine to increase the efficiency and activity of adaptive or innate immunity required for treatment or elimination of HCC.

The combinational vectors disclosed herein are optimized to select the correct promoter that best meets the requirements of the RNA processing system. Further, the therapeutic cargo portion is designed such that miRNAs (s) are clustered, processing of the first miRNA promotes processing of the second miRNA, and the like. The order of miRNAs is not so rapid that processing genomic RNA for packaging into lentiviral particles is processed to reduce lentivirus production efficiency by improving processing fidelity and associated speed. It can be important to ensure that. In addition, the combination vector can be designed such that the therapeutic cargo moiety contains multiple shRNAs under the control of a separate promoter.

Cancer The compositions and methods provided herein are used to treat cancer. The cell, tissue, or target can be a cancer cell, can be a cancerous tissue, can have a cancerous tissue, or has been diagnosed with or is at risk of developing a disease or condition. It can be a subject or a patient. In certain embodiments, the cell can be an epithelial cell, an endothelial cell, an epithelial cell, a glial cell, a stromal cell, or a mucosal cell. Cancer cell populations include brain cells, nerve cells, blood cells, endometrial cells, meningeal cells, esophageal cells, lung cells, cardiovascular cells, hepatocytes, lymph cells, breast cells, bone cells, connective tissue cells, Fat cells, retinal cells, thyroid cells, glandular cells, adrenal cells, pancreatic cells, gastric cells, intestinal cells, kidney cells, bladder cells, colon cells, prostate cells, uterine cells, ovarian cells, cervical cells, testis cells, spleen It may include, but is not limited to, cells, skin cells, smooth muscle cells, myocardial cells, or rhombic muscle cells. In another further aspect, the cancers include stellate cell tumor, acute myeloid leukemia, undifferentiated large cell lymphoma, acute lymphoblastic leukemia, angiosarcoma, B cell lymphoma, Berkit lymphoma, breast cancer, bladder cancer, Head and neck cancer, cervical cancer, chronic lymphoblastic leukemia, chronic myeloid leukemia, colorectal cancer, endometrial cancer, esophageal squamous epithelial cancer, Ewing sarcoma, fibrosarcoma, glioma, glioblastoma, gastrinoma, Gastric cancer,

germ tumor, hepatocellular carcinoma, capoic sarcoma, hodgkin lymphoma, laryngeal squamous epithelial cancer, laryngeal cancer, leukemia, smooth muscle tumor, lipoma, liposarcoma, melanoma, mantle cell lymphoma, medullary carcinoma, mesopharyngeal tumor, Mucous fibrosarcoma, myeloid leukemia, mucosal-related lymphoid tissue B-cell lymphoma, multiple myeloma, high-risk myelodystrophy syndrome, nasopharyngeal cancer, neuroblastoma, neurofibroma, high-grade non-hodgkin lymphoma, non-hodgkin Lymphoma, lung cancer, non-small cell lung cancer, ovarian cancer, esophageal cancer, osteosarcoma, pancreatic cancer, brown cell tumor, prostate cancer, renal cell cancer, retinal blastoma, rhizome myoma, salivary gland tumor, Schwanomma , Small cell lung cancer, squamous epithelial cancer of the head and neck, testis tumor, thyroid cancer, urinary tract epithelial cancer, and Wilms tumor.

The compositions and methods provided herein include NSCLC (non-small cell lung cancer), childhood malignancies, cervical and other tumors induced or promoted by human papillomavirus (HPV), melanoma, Barrett's esophagus (It is also used to treat premalignant syndrome), adrenal cancer, and skin cancer, as well as autoimmune and neoplastic skin diseases.

Therapeutic Vectors Therapeutic vectors are, but are not limited to, known transfection vectors and / or transduction vectors, protein and / or lipid complexes, such as, but not limited to, lentiviral vectors, adeno-associated virus vectors, poxvirus vectors, herpesvirus vectors, and the like. It can be delivered via liposomes, micelles, etc.

Viral vectors can be preferentially targeted to cell types useful for the methods of the present disclosure (ie, tumor cells or myeloid cells). Viral vectors can be used to transduce genes into target cells by specific viral envelope-host cell receptor interaction and viral gene expression mechanisms. As a result, viral vectors have been used as vehicles for introducing genes into many different cell types such as whole embryos, fertilized eggs, isolated tissue samples, tissue targets in situ, and cultured cell lines. There is. The ability to introduce and express foreign genes in cells provides the possibility of studying gene expression and elucidating cell lineages, as well as therapeutic interventions such as gene therapy, somatic reprogramming of artificial pluripotent stem cells, and It is useful in various types of immunotherapy. Like a pox vector such as the papovavirus family (eg, bovine papillomavirus or BPV), or the herpesvirus family (eg, Epsteinver virus or EBV), or the hepadonavirus family (eg, hepatitis B virus or HBV), or vaccinia. Viral components derived from the virus can be used in the vectors of the present disclosure.

The present disclosure is not particularly limited to lentiviral vectors, but lentiviral vectors are the preferred type of vector for the compositions and methods of the present disclosure. Lentiviruses are a genus of viruses that can deliver significant amounts of viral nucleic acids into host cells. Lentiviruses are characterized as having a unique ability to infect / transduce non-dividing cells, after transduction the lentivirus integrates its nucleic acid into the chromosomes of the host cell.

Infectious lentiviruses have three major genes encoding toxic proteins, gag, pol, and env, as well as two regulatory genes, including tat and rev. Depending on the particular serotype and virus, there may be additional accessory genes encoding proteins involved in the regulation, synthesis, and / or processing of viral nucleic acids, as well as other replication functions.

Furthermore, the lentivirus contains a terminal repeat sequence (LTR) region, which can be about 600 nt in length. The LTR can be divided into a U3 region, an R region, and a U5 region. The LTR can mediate the integration of retroviral DNA into the host chromosome through the action of integrase. Alternatively, without the function of integrase, the LTR can be used to cyclize viral nucleic acids.

Viral proteins involved in the early stages of lentiviral replication include reverse transcriptase and integrase. Reverse transcriptase is a virus-encoded RNA-dependent DNA polymerase. This enzyme uses the viral RNA genome as a template for the synthesis of complementary DNA copies. Reverse transcriptase also has RNase H activity due to the disruption of the RNA template. Integrase binds to both viral cDNA and host DNA produced by reverse transcriptase. The integrase processes the LTR before inserting the viral genome into the host DNA. Tat acts as a transactivator that promotes initiation and elongation during transcription. Rev response elements act post-transcriptional to regulate mRNA splicing and cytoplasmic transport.

Viral vectors generally contain glycoproteins, and various glycoproteins may provide specific affinities. For example, VSVG peptides can increase transfection into bone marrow cells. Alternatively, the viral vector may also have a targeted moiety, such as an antibody, attached to its shell peptide. Targeted antibodies can be specific for antigens that are overexpressed in tumors, such as, for example, HER-2, PSA, CEA, M2-PK, and CA19-9. Other specificities of viral vectors are also known in the art and can be used to target specific cell populations. For example, the poxvirus vector targets macrophages and dendritic cells.

With respect to the therapeutic vectors detailed herein, in aspects of the present disclosure, the miRNA or shRNA is under the control of a single promoter. In an embodiment, when multiple miRNAs are present in the same therapeutic vector, the miRNAs are under the control of a single promoter (eg, Pol II promoter). In embodiments, the Pol II promoter is the EF1-alpha promoter or CMV promoter.

In an embodiment, when multiple shRNAs are present in the same therapeutic vector, the shRNA is under the control of multiple promoters. For example, the first shRNA is under the control of the first promoter, the second shRNA is under the control of the second promoter, the third shRNA is under the control of the third promoter, and so on. In a non-limiting embodiment, the promoter can be selected from H1 (SEQ ID NO: 15), U6 (SEQ ID NO: 16), or 7SK (SEQ ID NO: 17).

表 1. miRNA 配列の組み合わせ

ベクター1		miR155FDPS	
ベクター2			miR21CD47
ベクター3	miR30cMyc		
ベクター4	miR30cMyc	miR155FDPS	
ベクター5	miR30cMyc		miR21CD47
ベクター6		miR155FDPS	miR21CD47
ベクター7	miR30cMyc		miR21CD47
ベクター8	miR30cMyc	miR155FDPS	miR21CD47

As shown in FIG. 3C, non-limiting examples of therapeutic vectors include therapeutic cargoes of three miRNAs that target mRNA for cMyc, FDPS, and CD47. As shown in Table 1 herein, alternating combinations of one to three miRNA sequences can be used in the final form of the therapeutic vector such that the therapeutic vector is a combination vector. Combinations of 1 to 3 miRNA sequences can be used in the final therapeutic vector, but up to 4, up to 5, or up to 6, or up to 7, or up to 8, or It is specifically envisioned that more miRNA sequences could be used in the final therapeutic vector. In addition, miRNA sequences can be sequenced sequentially or randomly (ie, the first miRNA does not have to precede the second miRNA, etc.). In addition to the selected combination, all possible miRNA sequences from the 5'end to the 3'end of the sense strand can be utilized for these lentiviral vectors. The vector constituents are not repeated for each combination of miRNAs. In the development of vectors containing miRNAs, if the shRNA for the gene of interest is first used to prove that the gene of interest functions in the lentiviral construct, and then the shRNA is proven to function. (Described below), eg, assembled into a miRNA cluster, as shown in FIG. 3C of the present application. MiRNAs conserve targeting sequences, but have changes in overall structure to better suit the miRNA processing pathway.

表 1. miRNA 配列の組み合わせ

ベクター1		miR155FDPS	
ベクター2			miR21CD47
ベクター3	miR30cMyc		
ベクター4	miR30cMyc	miR155FDPS	
ベクター5	miR30cMyc		miR21CD47
ベクター6		miR155FDPS	miR21CD47
ベクター7	miR30cMyc		miR21CD47
ベクター8	miR30cMyc	miR155FDPS	miR21CD47

Combination vectors can also be generated using shRNA. However, in these situations, it is necessary to utilize a separate promoter for each target sequence, as described herein.

Lentiviral vector system Lentiviral virions (particles) are expressed by a vector system that encodes the viral protein required to produce virions (viral particles). There is at least one vector containing a nucleic acid sequence operably linked to a promoter and encoding a lentiviral pol protein required for reverse transcription and integration. In another embodiment, the pol protein is expressed by multiple vectors. There is also a vector containing a nucleic acid sequence operably linked to a promoter, encoding a lentiviral gag protein required to form a viral capsid. In one embodiment, the gag nucleic acid sequence is on a vector separate from at least a portion of the pol nucleic acid sequence. In another embodiment, the gag nucleic acid is on a vector separate from all pol nucleic acid sequences encoding the pol protein.

Numerous modifications used to make particles can be made to the vector to further minimize the chances of obtaining wild-type reversion variants. These include, but are not limited to, deletions of the U3 region of the LTR, deletions of the tat, and deletions of the matrix (MA).

The gag, pol, and envelope vectors do not contain nucleotides from the lentiviral genome that package the lentiviral RNA, referred to as the lentiviral packaging sequence.

The vector forming the particles preferably does not contain a nucleic acid sequence derived from the lentiviral genome expressing the enveloped protein. Preferably, another vector containing a nucleic acid sequence encoding an enveloped protein operably linked to the promoter is used. This env vector also contains no lentivirus packaging sequence. In one embodiment, the nucleic acid sequence of env encodes a lentiviral envelope protein.

In another embodiment, the enveloped protein is not derived from a lentivirus, but from a different virus. The resulting particles are referred to as pseudotyped particles. With proper selection of envelopes, virtually any cell can be "infected". For example, the envelope genes encoding envelope proteins that target endocytosis compartments can be used, including influenza virus, VSV-G, alpha virus (Semriki forest virus, Sindbis virus), arenavirus (lymph). Spheroidal choriomeningitis virus), flavivirus (dani-mediated encephalitis virus, dengue virus, hepatitis C virus, GB virus), rabdovirus (bullet stomatitis virus, mad dog disease virus), paramixovirus (epidemic parotid gland) (Flame or measles), and orthomixovirus (influenza virus). Other envelopes that may be preferably used include those derived from Moloney leukemia virus such as MLV-E, MLV-A, and GALV. These latter envelopes are particularly preferred when the host cell is a primary cell. Other enveloped proteins may be selected depending on the desired host cell. Targeting of specific receptors, such as dopamine receptors, can be used for delivery to the brain. Another target can be the vascular endothelium. These cells can be targeted using the envelope of filovirus. For example, Ebola's GP, which becomes GP by post ˍ transcriptional modification, and GP2 glycoprotein. In another embodiment, different lentiviral capsids with a pseudotyped envelope can be used (eg, FIV or SHIV [US Pat. No. 5,654,195]). The SHIV pseudotyped vector can be readily used in animal models such as monkeys.

As detailed herein, a lentiviral vector system usually comprises at least one helper plasmid containing at least one of a gag gene, a pol gene, or a rev gene. Each of the gag gene, pol gene, and rev gene may be provided on an individual plasmid, or one or more genes may be provided together on the same plasmid. In one embodiment, the gag gene, pol gene, and rev gene are provided on the same plasmid (eg, FIG. 1). In another embodiment, the gag and pol genes are provided on the first plasmid and the rev gene is provided on the second plasmid (eg, FIG. 2). Therefore, both 3-vector and 4-vector systems can be used to produce lentiviruses as described in the Examples section and elsewhere herein. The therapeutic vector, envelope plasmid, and at least one helper plasmid are transfected into the packaging cell line. A non-limiting example of a packaging cell line is the 293T / 17 HEK cell line. Lentivirus particles are finally produced when the therapeutic vector, envelope plasmid, and at least one helper plasmid are transfected into the packaging cell line.

In another aspect, a lentiviral vector system for expressing lentiviral particles is disclosed. The system is the lentiviral vector described herein, an envelope plasmid for expressing an envelope protein optimized for infecting cells, and at least for expressing the gag, pol, and rev genes. When the lentiviral vector, envelope plasmid, and at least one helper plasmid are transfected into the packaging cell line, the lentiviral particles are produced by the packaging cell line and the lentiviral particles are: Genes targeted by shRNA or miRNA can be inhibited.

In another embodiment, the therapeutic vector may comprise the following elements: hybrid 5'end repeat sequence (RSV / 5'LTR) (SEQ ID NOs: 74-75), psai sequence (RNA packaging site) (SEQ ID NO: 76). , RRE (Rev Response Element) (SEQ ID NO: 77), cPPT (Polyprintlacto) (SEQ ID NO: 78), H1 Promoter (SEQ ID NO: 15), FDPS shRNA (eg, SEQ ID NO: 1, 2, 3, 4, or Variants thereof), Woodchuck Post-Transfer Modulation Element (WPRE) (SEQ ID NO: 79), and 3'Delta LTR (SEQ ID NO: 80). In another aspect, sequence changes due to substitutions, deletions, additions, or mutations can be used to modify the sequences referred to herein.

In another aspect, as detailed herein, the helper plasmid is designed to include the following elements: CAG promoter (SEQ ID NO: 19), HIV component gag (SEQ ID NO: 21),. HIV component pol (SEQ ID NO: 22), HIV Int (SEQ ID NO: 23), HIV RRE (SEQ ID NO: 24), and HIV Rev (SEQ ID NO: 25). In another embodiment, the helper plasmid can be modified to include a first helper plasmid for expressing the gag and pol genes, and a second alternative plasmid for expressing the rev gene. In another aspect, sequence changes due to substitutions, deletions, additions, or mutations can be used to modify the sequences referred to herein.

In another aspect, as detailed herein, the enveloped plasmid is designed to contain the following elements from left to right: RNA polymerase II promoter (CMV) (SEQ ID NO: 27) and blisters. Stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 29). In another aspect, sequence changes due to substitutions, deletions, additions, or mutations can be used to modify the sequences referred to herein.

In another aspect, the plasmid used for lentivirus packaging can be modified with similar elements, and the intron sequence can be removed without loss of vector function. .. For example, the following elements can replace similar elements in the plasmids that make up the packaging system: Elongation Factor-1 (EF-1), Phosphoglycerate Kinase (PGK), and Ubiquitin C (UbC) Promoters. , Can replace the CMV promoter or CAG promoter. SV40 poly A and bGH poly A can replace rabbit beta globin poly A. The HIV sequence in the helper plasmid can be constructed from different HIV strains or clades. VSV-G glycoproteins include feline endogenous virus (RD114), tenagazal leukemia virus (GALV), mad dog disease (FUG), lymphocytic choriomyelitis virus (LCMV), influenza A poultry pestovirus (influenza A foll plague). It can be replaced with a membrane glycoprotein derived from virus (FPV), loss river alpha virus (RRV), mouse leukemia virus 10A1 (MLV), or Ebola virus (EboV).

Commercially available lentivirus packaging systems are available (eg, Lenti-vpak packing kit, OriGene Technologies, Inc., Rockville, MD) and can also be designed as described herein. Further, it is within the technical scope of those skilled in the art to replace or modify aspects of the lentiviral packaging system to improve a number of related factors such as the efficiency of lentiviral particle production.

Dosages and Dosage Forms The vector compositions of the present disclosure allow short-term, medium-term, or long-term expression of the gene or sequence of interest, as well as maintenance of the episomes of the vectors of the present disclosure. Therefore, the dosing regimen can vary based on the condition being treated and the method of administration.

In embodiments, the vector composition can be administered to the subject in need at various doses. Specifically, the subject may be administered an infectious dose of about ^{106} or greater (an average of 1 dose required for transduction into 1 target cell). More specifically, the subject may be administered an infectious dose of about ^{107} or higher, about 108 or higher, about 109 or higher, or about 10 10 or higher , or any number of doses between these values. .. Dosing limits are determined for each disease indication, such as a particular cancer type, and depend on the toxicity / safety profile of each individual product and product lot.

In addition, the vector compositions of the present disclosure may be administered once or twice daily, or periodically for any other suitable period. For example, the vector composition is once a week, once every two weeks, once every three weeks, once a month, every two months, every three months, every six months, every nine months, every one year. It can be administered to the subject in need every 18 months, every 2 years, every 30 months, or every 3 years.

In embodiments, the vector compositions of the present disclosure are administered as pharmaceutical compositions. In embodiments, the pharmaceutical composition can be formulated into a variety of dosage forms, including nasal, pulmonary, oral, topical, or parenteral dosage forms for clinical application. However, it is not limited to these. Each dosage form may include various solubilizers, disintegrants, surfactants, fillers, thickeners, binders, diluents such as wetting agents, or other pharmaceutically acceptable excipients. .. The pharmaceutical composition may also be formulated for injection, insufflation, infusion, or intradermal exposure. For example, an injectable formulation may contain the vector of the present disclosure in an aqueous or non-aqueous solution of suitable pH and tonicity.

The vector compositions of the present disclosure can be administered to a subject either into the tumor site or via direct injection at the site of infection. In some embodiments, the vector can be administered systemically. In some embodiments, the vector composition may be administered via guided cannulation into the tissue immediately surrounding the tumor or site of infection.

The vector composition of the present disclosure is, for example, intranasal administration, oral administration, sublingual administration, oral administration, rectal administration, ocular administration, parenteral (intravenous, intradermal, intramuscular, subcutaneous, intraperitoneal) administration, Transpulmonary administration, intravaginal administration, topical administration, local administration, local administration after random infusion, mucosal administration, via aerosol, in a semi-solid medium such as agarose or gelatin, or via oral or nasal spray preparation. It can be administered using any pharmaceutically acceptable method, such as.

Further, the vector compositions of the present disclosure include, for example, solid dosage forms, tablets, rounds, lozenges, capsules, liquid dispersions, gels, aerosols, lung aerosols, nasal aerosols, ointments, creams, and the like. It can be formulated into any pharmaceutically acceptable dosage form, such as semi-solid dosage forms, liquids, emulsions, and suspending agents. Further, the pharmaceutical composition may be a controlled release preparation, a sustained release preparation, an immediate release preparation, or any combination thereof. In addition, the pharmaceutical composition can be a transdermal delivery system.

In embodiments, the pharmaceutical composition may be formulated as a solid dosage form for oral administration, which may be a powder, granule, capsule, tablet, or pill. In embodiments, the solid dosage form may comprise one or more excipients such as, for example, calcium carbonate, starch, sucrose, lactose, microcrystalline cellulose, or gelatin. In addition, solid dosage forms may include lubricants such as talc or magnesium stearate in addition to excipients. In some embodiments, the oral dosage form can be an immediate release form or a controlled release form. Controlled release dosage forms include controlled or sustained release, intestinal release, and the like. Excipients used in controlled release dosage forms are generally known to those of skill in the art.

In embodiments, the pharmaceutical composition can be formulated as a sublingual or oral dosage form. Such dosage forms include sublingual tablets or sublingual solution compositions administered sublingually, and oral tablets placed between the cheeks and gums.

In embodiments, the pharmaceutical composition can be formulated as a nasal dosage form. Such dosage forms of the invention include solution compositions, suspension compositions, and gel compositions for nasal delivery.

In embodiments, the pharmaceutical composition can be formulated as a liquid dosage form for oral administration, such as a suspension, emulsion, or syrup. In embodiments, the liquid dosage form may include various excipients such as moisturizers, sweeteners, fragrances, or preservatives, in addition to commonly used simple diluents such as water and liquid paraffin. In embodiments, the composition can be formulated to be suitable for administration to a pediatric patient.

In embodiments, the pharmaceutical composition can be formulated as a parenteral dosage form such as a sterile aqueous solution, suspension, emulsion, non-aqueous solution, or suppository. In embodiments, the liquid or suspension may include vegetable oils such as propylene glycol, polyethylene glycol, olive oil, and injectable esters such as ethyl oleate.

The dosage of the pharmaceutical composition may vary depending on the patient's weight, age, gender, time and mode of administration, rate of excretion, and severity of the disease.

In embodiments, treatment of the cancer is accomplished by direct injection under the guidance of the vector constructs of the present disclosure into the tumor using a needle, or by intravascular cannulation. In embodiments, the vector composition is cerebrospinal fluid, blood by cannulation or injection into a vein or artery, intradermal delivery, intramuscular delivery, or injection into a draining organ near the diseased site. , Or during lymphatic circulation.

The following examples are given to give examples of embodiments of the present invention. However, it should be understood that the invention is not limited to the particular conditions or details described in these examples. All publications referenced herein are specifically incorporated by reference.

(Example 1: Development of lentiviral vector system)
As summarized in FIG. 1, a lentiviral vector system was developed (cyclical form). Lentivirus particles were produced in 293T / 17 HEK cells (purchased from American Type Culture Collection, Manassas, VA) after transfection of therapeutic vectors, envelope plasmids, and helper plasmids. Transfection of 293T / 17 HEK cells produced functional viral particles. For this transfection, the reagent poly (ethyleneimine) (PEI) was used to increase the efficiency of plasmid DNA uptake. First, the plasmid and DNA were added separately in a serum-free culture medium in a 3: 1 ratio (mass ratio of PEI to DNA). After 2-3 days, cell medium was harvested and lentivirus particles were purified by high speed centrifugation and / or filtration followed by anion exchange chromatography. The concentration of lentivirus particles can be expressed in transduction units / ml (TU / ml). TU determination can be determined by measuring HIV p24 levels in culture (p24 protein is incorporated into lentivirus particles), measuring the number of viral DNA copies per transduced cell by quantitative PCR, or cell infection and light. Achieved by the use of (if the vector encodes a luciferase or fluorescent protein marker).

As mentioned above, a 3-vector system (ie, including a 2-vector lentivirus packaging system) was designed for the production of lentiviral particles. A schematic diagram of the three-vector system is shown in FIG. Briefly with respect to FIG. 1, the top vector is a helper plasmid, which in this case contains Rev. The vector found in the center of FIG. 1 is an enveloped plasmid. The bottom vector is the therapeutic vector described herein.

With respect to FIG. 1, the helper plus Rev plasmids are CAG enhancer (SEQ ID NO: 18), CAG promoter (SEQ ID NO: 19), chicken beta actinintron (SEQ ID NO: 20), HIV gag (SEQ ID NO: 21), HIV Pol (SEQ ID NO: 22).), HIV Int (SEQ ID NO: 23), HIV RRE (SEQ ID NO: 24), HIV Rev (SEQ ID NO: 25), and Rabbit Betaglobinpoly A (SEQ ID NO: 26).

Envelope plasmids include the CMV promoter (SEQ ID NO: 27), betaglobin intron (SEQ ID NO: 28), VSV-G (SEQ ID NO: 29), and rabbit betaglobin poly A (SEQ ID NO: 30).

Synthesis of 3-vector system including 2-vector lentivirus packaging system consisting of helper (plus Rev) plasmid and envelope plasmid

material and method:
Construction of helper plasmids: Helper plasmids were constructed by initial PCR amplification of DNA fragments from the pNL4-3 HIV plasmid (NIH Aids Reagent Program) containing the Gag, Pol, and integrase genes. Primers were designed to amplify fragments with EcoRI and NotI restriction sites that could be used for insertion into the same site in the pCDNA3 plasmid (Invitrogen). The forward primer was (5'-TAAGCAGAATTCATCATGAATTTGCCAGGAAGAT-3') (SEQ ID NO: 31) and the reverse primer was (5'-CCATACAATGAATGGACACTAGGCGGCCGCACGAAT-3') (SEQ ID NO: 32).

GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTGGA
GGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGCGGACATA
AAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAA
ATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCCCATTAGTCCTATTGAGACT
GTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAAGTTAAACAATGGCCA
TTGACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAG
GAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAGTATTT
GCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTCAGAGAA
CTTAATAAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCTG
CAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATGTGGGCGATGCATATT
TTTCAGTTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAG
TATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGG
ATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAAATCTTAGAGCC
TTTTAGAAAACAAAATCCAGACATAGTCATCTATCAATACATGGATGATTTGTAT
GTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAACTGAG
ACAACATCTGTTGAGGTGGGGATTTACCACACCAGACAAAAAACATCAGAAAGA
ACCTCCATTCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAG
CCTATAGTGCTGCCAGAAAAGGACAGCTGGACTGTCAATGACATACAGAAATTA
GTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGGATTAAAGTAAGGCAA
TTATGTAAACTTCTTAGGGGAACCAAAGCACTAACAGAAGTAGTACCACTAACA
GAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGGAGATTCTAAAAGAACCGGT
ACATGGAGTGTATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCA
GGGGCAAGGCCAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAA
AACAGGAAAGTATGCAAGAATGAAGGGTGCCCACACTAATGATGTGAAACAATT
AACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAATATGGGGAAAGA
CTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGGAAGCATGGTGGACAG

AGTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGTTTGTCAATACCCCTCCCTT
AGTGAAGTTATGGTACCAGTTAGAGAAAGAACCCATAATAGGAGCAGAAACTTT
CTATGTAGATGGGGCAGCCAATAGGGAAACTAAATTAGGAAAAGCAGGATATGT
AACTGACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGACACAACAAATCAGAA
GACTGAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAAC
ATAGTGACAGACTCACAATATGCATTGGGAATCATTCAAGCACAACCAGATAAG
AGTGAATCAGAGTTAGTCAGTCAAATAATAGAGCAGTTAATAAAAAAGGAAAAA
GTCTACCTGGCATGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTA
GATAAATTGGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAGATGGAATAGATA
AGGCCCAAGAAGAACATGAGAAATATCACAGTAATTGGAGAGCAATGGCTAGTG
ATTTTAACCTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATG
TCAGCTAAAAGGGGAAGCCATGCATGGACAAGTAGACTGTAGCCCAGGAATATG
GCAGCTAGATTGTACACATTTAGAAGGAAAAGTTATCTTGGTAGCAGTTCATGTA
GCCAGTGGATATATAGAAGCAGAAGTAATTCCAGCAGAGACAGGGCAAGAAAC
AGCATACTTCCTCTTAAAATTAGCAGGAAGATGGCCAGTAAAAACAGTACATAC
AGACAATGGCAGCAATTTCACCAGTACTACAGTTAAGGCCGCCTGTTGGTGGGC
GGGGATCAAGCAGGAATTTGGCATTCCCTACAATCCCCAAAGTCAAGGAGTAAT
AGAATCTATGAATAAAGAATTAAAGAAAATTATAGGACAGGTAAGAGATCAGGC
TGAACATCTTAAGACAGCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGA
AAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGC
AACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTT
TCGGGTTTATTACAGGGACAGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGCT
CCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAAAAGT
AGTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTATGGAAAACAGATGGCAG
GTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA (配列番号　　33)

The sequences of the Gag, Pol, and integrase fragments were as follows:

GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTGGA
GGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGCGGACATA
AAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAA
ATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCCCATTAGTCCTATTGAGACT
GTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAAGTTAAACAATGGCCA
TTGACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAG
GAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAGTATTT
GCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTCAGAGAA
CTTAATAAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCTG
CAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATGTGGGCGATGCATATT
TTTCAGTTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAG
TATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGG
ATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAAATCTTAGAGCC
TTTTAGAAAACAAAATCCAGACATAGTCATCTATCAATACATGGATGATTTGTAT
GTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAACTGAG
ACAACATCTGTTGAGGTGGGGATTTACCACACCAGACAAAAAACATCAGAAAGA
ACCTCCATTCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAG
CCTATAGTGCTGCCAGAAAAGGACAGCTGGACTGTCAATGACATACAGAAATTA
GTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGGATTAAAGTAAGGCAA
TTATGTAAACTTCTTAGGGGAACCAAAGCACTAACAGAAGTAGTACCACTAACA
GAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGGAGATTCTAAAAGAACCGGT
ACATGGAGTGTATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCA
GGGGCAAGGCCAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAA
AACAGGAAAGTATGCAAGAATGAAGGGTGCCCACACTAATGATGTGAAACAATT
AACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAATATGGGGAAAGA
CTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGGAAGCATGGTGGACAG

AGTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGTTTGTCAATACCCCTCCCTT
AGTGAAGTTATGGTACCAGTTAGAGAAAGAACCCATAATAGGAGCAGAAACTTT
CTATGTAGATGGGGCAGCCAATAGGGAAACTAAATTAGGAAAAGCAGGATATGT
AACTGACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGACACAACAAATCAGAA
GACTGAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAAC
ATAGTGACAGACTCACAATATGCATTGGGAATCATTCAAGCACAACCAGATAAG
AGTGAATCAGAGTTAGTCAGTCAAATAATAGAGCAGTTAATAAAAAAGGAAAAA
GTCTACCTGGCATGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTA
GATAAATTGGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAGATGGAATAGATA
AGGCCCAAGAAGAACATGAGAAATATCACAGTAATTGGAGAGCAATGGCTAGTG
ATTTTAACCTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATG
TCAGCTAAAAGGGGAAGCCATGCATGGACAAGTAGACTGTAGCCCAGGAATATG
GCAGCTAGATTGTACACATTTAGAAGGAAAAGTTATCTTGGTAGCAGTTCATGTA
GCCAGTGGATATATAGAAGCAGAAGTAATTCCAGCAGAGACAGGGCAAGAAAC
AGCATACTTCCTCTTAAAATTAGCAGGAAGATGGCCAGTAAAAACAGTACATAC
AGACAATGGCAGCAATTTCACCAGTACTACAGTTAAGGCCGCCTGTTGGTGGGC
GGGGATCAAGCAGGAATTTGGCATTCCCTACAATCCCCAAAGTCAAGGAGTAAT
AGAATCTATGAATAAAGAATTAAAGAAAATTATAGGACAGGTAAGAGATCAGGC
TGAACATCTTAAGACAGCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGA
AAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGC
AACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTT
TCGGGTTTATTACAGGGACAGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGCT
CCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAAAAGT
AGTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTATGGAAAACAGATGGCAG
GTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA (配列番号　　33)

TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAACAGT
CAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACC
CGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGAT
CCATTCGATTAGTGAACGGATCCTTGGCACTTATCTGGGACGATCTGCGGAGCCT
GTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATT
GTGGAACTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTC
CTACAATATTGGAGTCAGGAGCTAAAGAATAGAGGAGCTTTGTTCCTTGGGTTCT
TGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGG
CCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTAT
TGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCA
GGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGATCT
TTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACT
TCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTG
TCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGA
GTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAG
GTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTA
TTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTT
ATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTT
CCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCC
TCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAA
ATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAA
AGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTG
CCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTC
AGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGT
TCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGA
GGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGC
CTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCTTATAATGGTTACAAATA
AAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGT
TGTGGTTTGTCCAAACTCATCAATGTATCTTATCAGCGGCCGCCCCGGG（配列番号
34)

Next, DNA fragments containing the sequences of Rev, RRE, and rabbit beta-globin poly A with XbaI and XmaI flanking restriction sites were synthesized by MWG Operon. DNA fragments were then inserted into the plasmid at the XbaI and XmaI restriction sites. The DNA sequence was as follows:

TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAACAGT
CAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACC
CGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGAT
CCATTCGATTAGTGAACGGATCCTTGGCACTTATCTGGGACGATCTGCGGAGCCT
GTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATT
GTGGAACTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTC
CTACAATATTGGAGTCAGGAGCTAAAGAATAGAGGAGCTTTGTTCCTTGGGTTCT
TGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGG
CCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTAT
TGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCA
GGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGATCT
TTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACT
TCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTG
TCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGA
GTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAG
GTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTA
TTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTT
ATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTT
CCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCC
TCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAA
ATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAA
AGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTG
CCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTC
AGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGT
TCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGA
GGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGC
CTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCTTATAATGGTTACAAATA
AAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGT
TGTGGTTTGTCCAAACTCATCAATGTATCTTATCAGCGGCCGCCCCGGG（配列番号
34)

ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCA
ACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
AGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTG
GCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG
GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTAC
TTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCC
CACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTT
ATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGC
GCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTG
CGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGC
GGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCT
GCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGC
TCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCC
GGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTG
AAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGG
GGTGCGTGCGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCG
GCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCG
CGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGG
GAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGG
GCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGC
ACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCG
TGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCT
CGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGGAGCGCCGGCGGC
TGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGG
GCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGCGCC
GCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGA
AATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCATCTC
CAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGG
CGGGGTTCGGCTTCTGGCGTGTGACCGGCGGGAATTC（配列番号　35)

Finally, the CMV promoter of pCDNA3.1 was replaced with the CAG enhancer / promoter plus chicken beta actin intron sequence. DNA fragments containing the CAG enhancer / promoter / intron sequence with MluI and EcoRI flanking sites were synthesized by MWG Operon. DNA fragments were then inserted into the plasmid at the MluI and EcoRI restriction sites. The DNA sequence was as follows:

ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCA
ACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
AGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTG
GCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG
GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTAC
TTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCC
CACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTT
ATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGC
GCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTG
CGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGC
GGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCT
GCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGC
TCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCC
GGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTG
AAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGG
GGTGCGTGCGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCG
GCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCG
CGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGG
GAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGG
GCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGC
ACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCG
TGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCT
CGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGGAGCGCCGGCGGC
TGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGG
GCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGCGCC
GCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGA
AATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCATCTC
CAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGG
CGGGGTTCGGCTTCTGGCGTGTGACCGGCGGGAATTC（配列番号　35)

GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATTGGGGTGAATTGCAA
GTTCACCATAGTTTTTCCACACAACCAAAAAGGAAACTGGAAAAATGTTCCTTCT
AATTACCATTATTGCCCGTCAAGCTCAGATTTAAATTGGCATAATGACTTAATAG
GCACAGCCTTACAAGTCAAAATGCCCAAGAGTCACAAGGCTATTCAAGCAGACG
GTTGGATGTGTCATGCTTCCAAATGGGTCACTACTTGTGATTTCCGCTGGTATGG
ACCGAAGTATATAACACATTCCATCCGATCCTTCACTCCATCTGTAGAACAATGC
AAGGAAAGCATTGAACAAACGAAACAAGGAACTTGGCTGAATCCAGGCTTCCCT
CCTCAAAGTTGTGGATATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCCAG
GTGACTCCTCACCATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGATTCAC
AGTTCATCAACGGAAAATGCAGCAATTACATATGCCCCACTGTCCATAACTCTAC
AACCTGGCATTCTGACTATAAGGTCAAAGGGCTATGTGATTCTAACCTCATTTCC
ATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCCCTGGGAAAGGAG
GGCACAGGGTTCAGAAGTAACTACTTTGCTTATGAAACTGGAGGCAAGGCCTGC
AAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCATCAGGTGTCTGGTTCG
AGATGGCTGATAAGGATCTCTTTGCTGCAGCCAGATTCCCTGAATGCCCAGAAGG
GTCAAGTATCTCTGCTCCATCTCAGACCTCAGTGGATGTAAGTCTAATTCAGGAC
GTTGAGAGGATCTTGGATTATTCCCTCTGCCAAGAAACCTGGAGCAAAATCAGA
GCGGGTCTTCCAATCTCTCCAGTGGATCTCAGCTATCTTGCTCCTAAAAACCCAG
GAACCGGTCCTGCTTTCACCATAATCAATGGTACCCTAAAATACTTTGAGACCAG
ATACATCAGAGTCGATATTGCTGCTCCAATCCTCTCAAGAATGGTCGGAATGATC
AGTGGAACTACCACAGAAAGGGAACTGTGGGATGACTGGGCACCATATGAAGAC
GTGGAAATTGGACCCAATGGAGTTCTGAGGACCAGTTCAGGATATAAGTTTCCTT
TATACATGATTGGACATGGTATGTTGGACTCCGATCTTCATCTTAGCTCAAAGGC
TCAGGTGTTCGAACATCCTCACATTCAAGACGCTGCTTCGCAACTTCCTGATGAT
GAGAGTTTATTTTTTGGTGATACTGGGCTATCCAAAAATCCAATCGAGCTTGTAG
AAGGTTGGTTCAGTAGTTGGAAAAGCTCTATTGCCTCTTTTTTCTTTATCATAGGG
TTAATCATTGGACTATTCTTGGTTCTCCGAGTTGGTATCCATCTTTGCATTAAATT
AAAGCACACCAAGAAAAGACAGATTTATACAGACATAGAGATGAGAATTC (

配列番号 29)

Construction of VSV-G envelope plasmid:

The vesicular stomatitis Indiana virus glycoprotein (VSV-G) sequence was synthesized by the MWG Operon using adjacent EcoRI restriction sites. DNA fragments were then inserted into the pCDNA3.1 plasmid (Invitrogen) at the EcoRI restriction site and sequenced using CMV-specific primers to determine correct orientation. The DNA sequence was as follows:

GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATTGGGGTGAATTGCAA
GTTCACCATAGTTTTTCCACACAACCAAAAAGGAAACTGGAAAAATGTTCCTTCT
AATTACCATTATTGCCCGTCAAGCTCAGATTTAAATTGGCATAATGACTTAATAG
GCACAGCCTTACAAGTCAAAATGCCCAAGAGTCACAAGGCTATTCAAGCAGACG
GTTGGATGTGTCATGCTTCCAAATGGGTCACTACTTGTGATTTCCGCTGGTATGG
ACCGAAGTATATAACACATTCCATCCGATCCTTCACTCCATCTGTAGAACAATGC
AAGGAAAGCATTGAACAAACGAAACAAGGAACTTGGCTGAATCCAGGCTTCCCT
CCTCAAAGTTGTGGATATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCCAG
GTGACTCCTCACCATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGATTCAC
AGTTCATCAACGGAAAATGCAGCAATTACATATGCCCCACTGTCCATAACTCTAC
AACCTGGCATTCTGACTATAAGGTCAAAGGGCTATGTGATTCTAACCTCATTTCC
ATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCCCTGGGAAAGGAG
GGCACAGGGTTCAGAAGTAACTACTTTGCTTATGAAACTGGAGGCAAGGCCTGC
AAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCATCAGGTGTCTGGTTCG
AGATGGCTGATAAGGATCTCTTTGCTGCAGCCAGATTCCCTGAATGCCCAGAAGG
GTCAAGTATCTCTGCTCCATCTCAGACCTCAGTGGATGTAAGTCTAATTCAGGAC
GTTGAGAGGATCTTGGATTATTCCCTCTGCCAAGAAACCTGGAGCAAAATCAGA
GCGGGTCTTCCAATCTCTCCAGTGGATCTCAGCTATCTTGCTCCTAAAAACCCAG
GAACCGGTCCTGCTTTCACCATAATCAATGGTACCCTAAAATACTTTGAGACCAG
ATACATCAGAGTCGATATTGCTGCTCCAATCCTCTCAAGAATGGTCGGAATGATC
AGTGGAACTACCACAGAAAGGGAACTGTGGGATGACTGGGCACCATATGAAGAC
GTGGAAATTGGACCCAATGGAGTTCTGAGGACCAGTTCAGGATATAAGTTTCCTT
TATACATGATTGGACATGGTATGTTGGACTCCGATCTTCATCTTAGCTCAAAGGC
TCAGGTGTTCGAACATCCTCACATTCAAGACGCTGCTTCGCAACTTCCTGATGAT
GAGAGTTTATTTTTTGGTGATACTGGGCTATCCAAAAATCCAATCGAGCTTGTAG
AAGGTTGGTTCAGTAGTTGGAAAAGCTCTATTGCCTCTTTTTTCTTTATCATAGGG
TTAATCATTGGACTATTCTTGGTTCTCCGAGTTGGTATCCATCTTTGCATTAAATT
AAAGCACACCAAGAAAAGACAGATTTATACAGACATAGAGATGAGAATTC (

配列番号 29)

A 4-vector system, including a 3-vector lentivirus packaging system, was also designed and produced using the methods and materials described herein. A schematic diagram of the 4-vector system is shown in FIG. Briefly with respect to FIG. 2, the top vector is a helper plasmid, which in this case does not contain Rev. The second vector from the top is a separate Rev plasmid. The second vector from the bottom is the envelope plasmid. The bottom vector is the therapeutic vector described herein.

With respect to FIG. 2, helper plasmids include CAG enhancer (SEQ ID NO: 18), CAG promoter (SEQ ID NO: 19), chicken beta actinintron (SEQ ID NO: 20), HIV gag (SEQ ID NO: 21), HIV Pol (SEQ ID NO: 22), and the like. Includes HIV Int (SEQ ID NO: 23), HIV RRE (SEQ ID NO: 24), and Rabbit Betaglobinpoly A (SEQ ID NO: 26).

The Rev plasmid contains the RSV promoter (SEQ ID NO: 80), HIV Rev (SEQ ID NO: 25), and rabbit beta globinpoly A (SEQ ID NO: 26).

Envelope plasmids include the CMV promoter (SEQ ID NO: 27), betaglobin intron (SEQ ID NO: 28), VSV-G (SEQ ID NO: 29), and rabbit betaglobin poly A (SEQ ID NO: 30).

Synthesis of 4-vector system including 3-vector lentivirus packaging system consisting of helper plasmid, Rev plasmid, and envelope plasmid

TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGC
GCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGC
AGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAAC
TCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGAT
ACCTAAAGGATCAACAGCTCCTAGATCTTTTTCCCTCTGCCAAAAATTATGGGGA
CATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTC
ATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGA
GGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATA
TGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATAT
GAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGT
TAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTT
CCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATA
GCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAAT
CATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAAC
ATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAA
CTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGT
GCCAGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCG
CCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACT
AATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAG
AAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTT
TATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAAT
AAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATC
TTATCACCCGGG (配列番号 34)

material and method:
Construction of Rev-free helper plasmid:
A Rev-free helper plasmid was constructed by inserting a DNA fragment containing the RRE and rabbit beta globin poly A sequences. This sequence was synthesized by MWG Operon using adjacent XbaI and XmaI restriction sites. The RRE / rabbit polyA beta globin sequence was then inserted into the helper plasmid at the XbaI and XmaI restriction sites. The DNA sequence is as follows:

TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGC
GCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGC
AGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAAC
TCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGAT
ACCTAAAGGATCAACAGCTCCTAGATCTTTTTCCCTCTGCCAAAAATTATGGGGA
CATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTC
ATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGA
GGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATA
TGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATAT
GAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGT
TAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTT
CCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATA
GCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAAT
CATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAAC
ATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAA
CTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGT
GCCAGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCG
CCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACT
AATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAG
AAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTT
TATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAAT
AAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATC
TTATCACCCGGG (配列番号　34)

CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAGGGTGTGTTT

AGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTCAGGATATAG

TAGTTTCGCTTTTGCATAGGGAGGGGGAAATGTAGTCTTATGCAATACACTTGTA

GTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAA

GCACCGTGCATGCCGATTGGTGGAAGTAAGGTGGTACGATCGTGCCTTATTAGGA

AGGCAACAGACAGGTCTGACATGGATTGGACGAACCACTGAATTCCGCATTGCA

GAGATAATTGTATTTAAGTGCCTAGCTCGATACAATAAACGCCATTTGACCATTC

ACCACATTGGTGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACCGTCAGATCGC

CTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCC

AGCCTCCCCTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAG

ACAGCGACGAAGAACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAA

GCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAA

GAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCCTTA

GCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGA

GAGACTTACTCTTGATTGTAACGAGGATTGTGGAACTTCTGGGACGCAGGGGGTG

GGAAGCCCTCAAATATTGGTGGAATCTCCTACAATATTGGAGTCAGGAGCTAAA

GAATAGTCTAGA (配列番号　36)

Construction of Rev plasmid:

The RSV promoter and HIV Rev sequence were synthesized by MWG Operon as a single DNA fragment using adjacent MfeI and XbaI restriction sites. DNA fragments were then inserted into the pCDNA3.1 plasmid (Invitrogen) at the MfeI and XbaI restriction sites where the CMV promoter was replaced with the RSV promoter. The DNA sequence was as follows:

CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAGGGTGTGTTT
AGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTCAGGATATAG
TAGTTTCGCTTTTGCATAGGGAGGGGGAAATGTAGTCTTATGCAATACACTTGTA
GTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAA
GCACCGTGCATGCCGATTGGTGGAAGTAAGGTGGTACGATCGTGCCTTATTAGGA
AGGCAACAGACAGGTCTGACATGGATTGGACGAACCACTGAATTCCGCATTGCA
GAGATAATTGTATTTAAGTGCCTAGCTCGATACAATAAACGCCATTTGACCATTC
ACCACATTGGTGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACCGTCAGATCGC
CTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCC
AGCCTCCCCTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAG
ACAGCGACGAAGAACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAA
GCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAA
GAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCCTTA
GCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGA
GAGACTTACTCTTGATTGTAACGAGGATTGTGGAACTTCTGGGACGCAGGGGGTG
GGAAGCCCTCAAATATTGGTGGAATCTCCTACAATATTGGAGTCAGGAGCTAAA
GAATAGTCTAGA (配列番号 36)

The plasmids used in the packaging system can be modified with similar elements and the intron sequence can be removed without loss of vector function. For example, the following elements can replace similar elements in the packaging system:

Promoters: Elongation Factor-1 (EF-1) (SEQ ID NO: 37), Phosphoglycerate Kinase (PGK) (SEQ ID NO: 38), and Ubiquitin C (UbC) (SEQ ID NO: 39) are CMV promoters (SEQ ID NO: 27). Or it can be a substitute for the CAG promoter (SEQ ID NO: 19). These sequences can also be further altered by additions, substitutions, deletions, or mutations.

Poly A sequence: SV40 poly A (SEQ ID NO: 40) and bGH poly A (SEQ ID NO: 41) can replace rabbit beta globin poly A (SEQ ID NO: 26). These sequences can also be further altered by additions, substitutions, deletions, or mutations.

HIV Gag, Pol, and Integrase Sequences: HIV sequences in helper plasmids can be constructed from different HIV strains or clades. For example, HIV Gag (SEQ ID NO: 21), HIV Pol (SEQ ID NO: 22), and HIV Int (SEQ ID NO: 23) from the Bal strain have been incorporated into the helper / helper plus Rev plasmid as outlined herein. It can be exchanged for the contained gag, pol, and int sequences. These sequences can also be further altered by additions, substitutions, deletions, or mutations.

Envelope: VSV-G glycoproteins include feline endogenous virus (RD114) (SEQ ID NO: 42), tenagazal leukemia virus (GALV) (SEQ ID NO: 43), mad dog disease (FUG) (SEQ ID NO: 44), lymphocytic choroidal medulla. Flame virus (LCMV) (SEQ ID NO: 45), influenza A poultry pestovirus (FPV) (SEQ ID NO: 46), Ross River alpha virus (RRV) (SEQ ID NO: 47), murine leukemia virus 10A1 (MLV) (SEQ ID NO: 81)), Or a membrane sugar protein derived from Ebola virus (EboV) (SEQ ID NO: 48). The sequences of these envelopes are specified as part of the sequence herein. In addition, these sequences can be further altered by additions, substitutions, deletions, or mutations.

In summary, the 3-vector system and the 4-vector system can be compared and contrasted as follows. The 3-vector wrench viral vector system contains: 1. Helper plasmids: HIV Gag, Pol, integrase, and Rev / Tat; 2. Envelope plasmids: VSV-G / FUG envelopes; and 3. Therapeutic vectors: RSV 5'LTR, psi packaging signal, Gag fragment, RRE, Env fragment, cPPT, WPRE, and 3'δ LTR. The 4-vector wrench viral vector system contains: 1. Helper plasmids: HIV Gag, Pol, and integrase; 2. Rev plasmid:

Rev; 3. Envelope plasmids: VSV-G / FUG envelopes; and 4. Therapeutic vectors: RSV 5'LTR, psi packaging signal, Gag fragment, RRE, env fragment, cPPT, WPRE, and 3'delta LTR. The sequences corresponding to the above elements are specified as part of the sequence listing herein.

(Example 2. Treatment vector)
For example, as shown in FIG. 3, an exemplary treatment vector was designed and developed.

First, from left to right with respect to FIG. 3A, the key genetic elements are: hybrid 5'end repeat sequence (RSV / LTR), psy sequence (RNA packaging site), RRE (Rev response element). , CPPT (polyprint lacto), H1 promoter, FDPS shRNA sequence including the FDPS shRNA sequence detailed herein, Woodchuck post-transcription regulatory element (WPRE), and LTR with deletions in the U3 region.

Next, with respect to FIG. 3B, from left to right, the key genetic elements are: hybrid 5'end repeat sequence (RSV / LTR), psai sequence (RNA packaging site), RRE (Rev response element). , CPPT (Polyprint Lact), EF-1 Alpha (EF-1 Alpha Promoter for Gene Transcription), FDPS miR (miRNA) containing FDPS miRNA sequences detailed herein, Woodchuck post-transcription regulatory element (WPRE).), And an LTR with a deletion in the U3 region.

The following methods and materials were used to produce the vectors outlined in FIGS. 3A and 3B.

Inhibitory RNA Design: Homo sapiens farnesyl diphosphate synthase (FDPS) mRNA sequence (NM_002004.3) is used to search for potential siRNA or shRNA candidates for knocking down FDPS levels in human cells. did. GPP Web Portal (http://portals.broadinstation.org/gpp/public/) sponsored by Broad Institute or BLOCK-iT RNAi Designs / rmithrside / signer (fasts. Alternatively, potential RNA interference sequences were selected from the candidates selected by the shRNA design program. To regulate shRNA expression, individual selected shRNA sequences were inserted into a lentiviral vector immediately 3'on the RNA polymerase III promoter H1 (SEQ ID NO: 15). These lentiviral shRNA constructs were used for transduction into cells and changes in specific mRNA levels were measured. Individual implantation of the most potent shRNAs into the microRNA backbone for reduced mRNA levels allowed expression by either the EF-1alpha or CMV RNA polymerase II promoter. The microRNA skeleton is mirbase. Selected from org. RNA sequences were also synthesized as synthetic siRNA oligonucleotides and introduced directly into cells without the use of lentiviral vectors.

Vector Construction: For FDPS ŠRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins MWG Operon. Overlapping sense and antisense oligonucleotide sequences were mixed and annealed while cooling from 70 ° C to room temperature. The lentiviral vector was digested with restriction enzymes BamHI and EcoRI for 1 hour at 37 ° C. The digested lentiviral vector was purified by agarose gel electrophoresis and extracted from the gel using Thermo Scientific's DNA gel extraction kit. DNA concentrations were determined and the vectors were mixed with oligos (3: 1 ratio), annealed and ligated. The ligation reaction was carried out at room temperature for 30 minutes using T4 DNA ligase. A 2.5 microliter ligation mixture was added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after heat shock at 42 ° C. Bacterial cells were spread on agar plates containing ampicillin, drug-resistant colonies (indicating the presence of ampicillin-resistant plasmids) were harvested and grown in LB medium. To confirm the insertion of the oligo sequence, plasmid DNA was extracted from the recovered bacterial culture using the Thermo Scientific DNA miniprep kit. Insertion of the shRNA sequence into the lentiviral vector was validated by DNA sequencing using specific primers for the promoter used to regulate shRNA expression. The following target sequences were used to determine exemplary shRNA sequences for knocking down FDPS:
GTCCTGGAGTACAATGCCATT (FDPS target sequence; SEQ ID NO: 49);
GTCCTGGAGTACAATGCCATTCTCGAGAGAGCATTGTACTCAGGACTTTTT (FDPS shRNA sequence # 1; SEQ ID NO: 1);
GCAGGATTTCGTTCAGCACTT (FDPS target sequence # 2; SEQ ID NO: 50);
GCAGGATTTCGTTCAGCACTTCTACGAGAAGTGCTGAACGAAATTCCTGCTTTTT (FDPS shRNA sequence # 2; SEQ ID NO: 2);
GCCATGTACATGGCAGGAATT (FDPS target sequence # 3; SEQ ID NO: 51);
GCCATGTCATGGCAGGAAATTCTCGAGATATCCGCCATTGTCATGGGTTTTT (FDPS shRNA sequence # 3; SEQ ID NO: 3);
GCAGAAGGAGGCTGAGAAAGT (FDPS target sequence # 4; SEQ ID NO: 52); and GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTTCTTCGCTTTTT (FDPS shRNA sequence # 4; SEQ ID NO: 4).

The shRNA sequence was then assembled into synthetic microRNA (miR) under the control of the EF-1 alpha promoter. Briefly, miR hairpin sequences such as miR30, miR21, or miR185 as detailed below are mirbase. Obtained from org. Synthetic miR sequences were constructed using 19-22 mer shRNA target sequences. The miR sequence was aligned with the antisense-target sequence-hairpin loop sequence (specific for each microRNA) -sense target sequence.

The following miR sequence was developed:
AAGGTATTTGCTGTTGACAGTGGAGCGACACTTTCTCAGCCTCCTTCTGCGGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAGTGCTGCTACTGCCTCCGACTTCAAGGGCT (miR30 FDPS sequence # 1; SEQ ID NO: 53)
AAGGTATTTGCTGTTGACAGTGGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAGTGCTGCTACTGCCTCCGACTTCAGGGCT (miR30 FDPS sequence # 2; SEQ ID NO: 54)
TGCTGTTGACAGTGGAGCGACTTTCTCAGCCTCCTTCGCGGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGGA (miR30 FDPS sequence # 3; SEQ ID NO: 55)
CCTGGAGGCTTGCTGAAGGCTGTTAGCTGACTTTCTCAGCCCTTCTCTGCTTTTGCCACTGACTGAGCAAGGGCTGAGAAGTCAGGACACAAGGCCTGTTACTGCACTCA (miR155 FDPS sequence # 1; SEQ ID NO: 56)
CATCTCCATTGGCTGTACCACCTCTGTCCGGGGACTTTCTCAGCCCTTCTCTGCCTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTTGGTATTTTCATCTGACCA (miR21 FDPS sequence # 1; SEQ ID NO: 57)
GGGCTCGGCTCGAGCAGGGGGGGCGAGGGATACTTTTCTCAGCCTCCTCTCTGCTGGTCCCCTCCCCCGCAGAAGGAGGCTGAGAAGTCCTTCTCCCCAATGACCGGTCTTCCGTCG (miR185 FDPS sequence # 1; sequence number 58)

Combination vectors as generally shown in FIG. 3C can also be produced based on the development of the single target vector outlined above. An exemplary therapeutic combination vector is shown in FIG. 3C, which includes the following from left to right: hybrid 5'end repeat sequence (RSV / LTR), psai sequence (RNA packaging site), RRE (Rev response). Elements), cPPT (polyprint lacto), EF-1alpha (EF-1alpha promoter for gene transcription), miR30-FDPS, miR155-CD47, miR21-cMyc, Woodchuck post-transcriptional regulatory element (WPRE), and U3 region. LTR with a deletion in. The therapeutic vectors detailed in FIG. 3C can be produced using the materials and methods described using the following target sequences:
miR30 FDPS sequence # 1:
AAGGTATTTGCTGTTGACAGGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAGTGCTGCTACTGCCTCCGACTTCAGGGCT (SEQ ID NO: 53)
miR155 CD47 target sequence # 1:
CCTGGAGGCTTGCTGAAGGCTGTATGCTGTTATCCACTTCAAAGAGGCAGTTTTGCGCCACTGACTGACTGCCTCTTAAGCATGGATAACAGGACACAAGGCCTGTTACTGCACTCA (SEQ ID NO: 82)
miR21 cMyc sequence:
CATCTCCATTGGCTGTACCACCTTGTCCGGGGTGTTCCGCCTTGACATTTCCTGTTAGGGTCATGATACTGGAATTCATCAAGGTGAACACTGACATTTTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 83)

(Example 3. Materials and methods for FDPS)
Inhibitory RNA Design: Potential siRNA or potential siRNA for knocking down FDPS levels in human cells using the mRNA sequence of Homo sapiens farnesyl diphosphate synthase (FDPS) transcript variant 1 (NM_002004.3). She RNA candidates were searched. Potential RNA interference sequences were selected from candidates selected by the Broad Institute or siRNA or shRNA design programs such as Thermo Scientific's BLOCK-iT ™ RNAi Designer. To regulate shRNA expression, the shRNA sequence can be inserted into a lentiviral vector behind an RNA polymerase III promoter such as H1, U6, or 7SK. Also, RNA sequences can be embedded within the microRNA backbone to allow expression by RNA polymerase II promoters such as CMV or EF-1alpha. The RNA sequence may also be synthesized as a siRNA oligonucleotide and used independently of the lentiviral vector.

Vector Construction: For FDPS shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by the MWG operon. Oligonucleotide sequences were annealed by incubation at 70 ° C. and cooling to room temperature. After digesting the annealed oligonucleotide with the restriction enzymes BamHI and EcoRI for 1 hour at 37 ° C., the enzyme was heat inactivated for 20 minutes at 70 ° C. In parallel, the lentiviral vector was digested with the restriction enzymes BamHI and EcoRI at 37 ° C. for 1 hour. The digested lentiviral vector was purified by agarose gel electrophoresis and extracted from the gel using Invitrogen's DNA gel extraction kit. DNA concentration was determined and the vector was ligated to the oligo sequence with a 3: 1 insert-to-vector ratio. The ligation reaction was carried out at room temperature for 30 minutes using T4 DNA ligase. A 2.5 microliter ligation mixture was added to 25 microliters of STBL3 competent bacterial cells. Transformation was performed by heat shock at 42 ° C. Bacterial cells were smeared onto an agar plate containing ampicillin and then colonies were grown in LB medium. To confirm the insertion of the oligo sequence, plasmid DNA was extracted from the recovered bacterial culture using the Invitrogen DNA miniprep kit. Insertion of the shRNA sequence into the lentiviral vector was validated by DNA sequencing using specific primers in which any promoter is used to regulate shRNA expression. Lentiviral vectors containing the correct FDPS sequence were then used to package lentiviral particles to test their ability to knock down FDPS. Transduction into mammalian cells was

performed using lentiviral particles either in the presence or absence of polybrene. After 2-4 days, cells were harvested and proteins and RNA analyzed for FDPS expression.

Functional assay for mRNA reduction: The effect of different FDPS short-chain homologous RNA (SHRNA) targeting sequences on FDPS expression was determined by measuring mRNA expression. Transduction into HepG2 hepatocellular carcinoma cells was performed using a lentiviral vector containing an FDPS shRNA sequence. After 48 hours, cells were lysed and RNA was extracted using Qiagen's RNeasy minikit. Invitrogen's SuperScript VILO was then used to synthesize cDNA from RNA. Samples were then analyzed by quantitative RT-PCR using the Applied Biosystems StepOne PCR device. Using a forward primer (5'-AGGAATTGATGGCGAGAAGG-3') (SEQ ID NO: 59) and a reverse primer (5'-CCCAAAGGGTCAAGGTAATCA-3') (SEQ ID NO: 60) under standard conditions for analysis of the polymerase chain reaction, Expression of FDPS was detected using SYBR Green of Invitrogen. Using a forward primer (5'-AGCGCGACTACAGCTTCA-3') (SEQ ID NO: 61) and a reverse primer (5'-GGCGACGTAGCAACCTTCT-3') (SEQ ID NO: 62) under standard conditions for analysis of the polymerase chain reaction, Samples were normalized to mRNA for beta-actin gene expression. The relative expression of FDPS was determined by the Ct value normalized to the actin level of each sample.

Tumor cell functional assay modified by LV-FDPS and used to activate cytokine production in human gamma delta T cells: Tumor cells are also treated using the LV-FDPS vector, which is then used as a healthy donor. It was exposed to the primary human gamma delta T cells of origin. Combination treatment of tumor cell lines with both aminobisphosphonates and vectors that suppress farnesyl pyrophosphate synthase (FDPS) has a synergistic effect on TNF-alpha production in gamma delta T cells. THP1 monocytic tumor cell line (A) or HepG2 monocytic tumor cell line (B) is a lentiviral control vector (LV control), and a shRNA virus vector (LV-FDPS) expressing shRNA for downward regulation of FDPS. , Zolredoronic acid (Zol), Zoledronic acid plus lentiviral control (Zol + LV control), or shRNA vector for down-regulating Zoledronic acid plus FDPS (Zol + LV-FDPS). Treated cells were mixed with gamma delta T cells in a 1: 1 ratio for 4 hours. TNF-alpha production by gamma delta T cells was detected by intracellular staining and flow cytometry.

Tumor cell function assay modified by LV-FDPS and used to activate tumor cell killing by human gamma delta T cells: monocytic tumor cells using a lentiviral vector that suppresses FDPS mRNA. After transduction into (THP-1), the cells were used to activate tumor cytotoxicity in normal human gamma delta T cells. After 4 hours of exposure to transduced THP-1 cells, activated gamma delta T cells were harvested and then used in a cytotoxic assay to kill unmodified THP-1. When gamma delta T cells were stimulated with a combination of transduced THP-1 cells and 10 micromolar zoledronic acid, the ratio of 4 gamma delta T cells to 1 THP-1 cell was 70. More than% killing of THP-1 was observed.

表 2. FDPS shRNA の配列

説明	shRNA オリゴヌクレオチド(センス配列-ループ-アンチセンス配列	配列番号
FDPS-1	GTCCTGGAGTACAATGCCATT**CTCGAG**AATGGCATTGTACTCCAGGACTTTTT	1
FDPS-2	GCAGGATTTCGTTCAGCACTT**CTCGAG**AAGTGCTGAACGAAATCCTGCTTTTT	2
FDPS-3	GCCATGTACATGGCAGGAATT**CTCGAG**AATTCCTGCCATGTACATGGCTTTTT	3
FDPS-4	GCAGAAGGAGGCTGAGAAAGT**CTCGAG**ACTTTCTCAGCCTCCTTCTGCTTTTT	4

FDPS Experimental Data The FDPS shRNA sequences shown in Table 2 were used in the experiments described herein. In addition, the sequences detailed in Table 2 can be used in the therapeutic vectors detailed herein.

表 2. FDPS shRNA の配列

説明	shRNA オリゴヌクレオチド(センス配列-ループ-アンチセンス配列	配列番号
FDPS-1	GTCCTGGAGTACAATGCCATT**CTCGAG**AATGGCATTGTACTCCAGGACTTTTT	1
FDPS-2	GCAGGATTTCGTTCAGCACTT**CTCGAG**AAGTGCTGAACGAAATCCTGCTTTTT	2
FDPS-3	GCCATGTACATGGCAGGAATT**CTCGAG**AATTCCTGCCATGTACATGGCTTTTT	3
FDPS-4	GCAGAAGGAGGCTGAGAAAGT**CTCGAG**ACTTTCTCAGCCTCCTTCTGCTTTTT	4

As shown in FIG. 4A, the relative expression levels of human FDPS after administration of four different FDPS shRNA sequences were determined. The most significant inhibition of human FDPS expression was found in FDPS-2 and FDPS-4 samples (shown in FIG. 4A of the present application).

In addition, as shown in FIG. 4B, a lentivirus-based delivery system was used to target FDPS expression. HepG2 human hepatocellular carcinoma cells were infected with a lentiviral vector containing either the H1 promoter and FDPS shRNA (SEQ ID NO: 4) sequence or the EF-1 alpha promoter and the FDPS sequence based on miR30 below:
miR30 FDPS sequence # 1:
AAGGTATTTGCTGTTGACAGGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAGTGCTGCTACTGCCTCCGACTTCAGGGCT (SEQ ID NO: 53)
miR30 FDPS sequence # 2:
AAGGTATTTGCTGTTGACAGTGGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAGTGCTGCTACTGCCTCCGACTTCAAGGGCT (SEQ ID NO: 54)

After 48 hours, cells were lysed and immunoblots were performed using an anti-FDPS antibody (Thermo Scientific) and an anti-actin antibody (Sigma) for protein loading control. As shown in FIG. 4B, treatment with FDPS shRNA significantly reduced the expression of FDPS protein. Treatment with FDPS sequences based on miR30 reduced FDPS expression.

As shown in FIG. 5, monocyte-like (THP-1) (FIG. 5A) or hepatocyte (HepG2) (FIG. 5B) transduced with a lentivirus containing FDPS mRNA capable of suppressing mRNA. The cells activated cytokine expression in human gamma delta T cells.

This portion of this example is THP1 monoclonal leukemia due to FDPS shRNA expressed by lentivirus (LV) (SEQ ID NO: 4; also referred to herein as LV-FDPS RHRNA # 4), as shown in FIG. 5A. Knockdown of FDPS in cells indicates that it stimulates TNF-α expression in gamma delta T cells.

Transduction into THP1 cells (1×10^5 cells) was performed using LV controls or LV-FDPS shRNA # 4 for 3 days. Two days after transduction, cells were treated with or without 1 μM zoledronic acid. After 24 hours, transduced THP-1 cells were co-cultured with $^5 \times 105$ PBMC cells and IL-2 in a round-bottomed 96-well plate for 4 hours. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to proliferate Vγ9Vδ2 T cells. Vγ9Vδ2 and TNF-α were stained with fluorophore-conjugated anti-TCR-Vδ2 and anti-TNF-α antibodies, and then cells were analyzed by flow cytometry. Live cells were gated and Vδ2 + and TNF-α + cells were selected on dot blots. Activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytogram. In the absence of zoledronic acid, the LV control stimulated 3.11% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS ushRNA # 4 stimulated 5%. With zoledronic acid treatment, the LV control stimulated 7.2% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS ushRNA # 4 stimulated 56.17%.

The following data were obtained using the same conditions for HepG2 cells. In the absence of zoledronic acid, the LV control stimulated 2.5% of TNF-α expressing Vγ9Vδ2 T cells and the LV-FDPS shRNA # 4 stimulated 3.33%. With zoledronic acid treatment, the LV control stimulated 9.1% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS ushRNA # 4 stimulated 45.7%.

Further, as shown in FIG. 6, monocyte-like (THP-1) tumor cells transduced with a lentivirus capable of suppressing FDPS mRNA activate tumor cytotoxicity in normal human gamma delta T cells.

This portion of this example demonstrates the results of mixing treated THP-1 monocyte-like tumor cells with cultured human GD T cells, as shown in FIG.

The monocyte-like cell line THP-1 was treated with a control lentiviral vector (LV), LV (LV-FDPS) that suppresses gene expression of farnesyl diphosphate synthase, zoledronic acid (Zol), or a combination. As shown in FIG. 6, the description was as follows: lentiviral control vector (LV control), lentiviral vector (LV-FDPS) expressing microRNA that downregulates FDPS, Zometa (Zol),. Zometaplus lentiviral control (Zol + LV control), or lentiviral vector (Zol + LV-FDPS) expressing microRNA that downregulates Zometaplus FDPS.

Human GD T cells from anonymous donors were cultured and added to THP-1 cells treated at a 4: 1, 2: 1 or 1: 1 ratio (GD T: THP-1) for 4 hours. Cell killing was measured by fluorescence assay. Treatment of THP-1 cells with a combination of LV-FDPS and Zol significantly increased cytotoxic T cell killing by GD T cells compared to treatment with either alone. LV-FDPS alone led to greater killing, but less than one-third of tumor cell killing after combination treatment compared to treatment with LV-FDPS alone compared to treatment with Zol alone. The combination treatment of LV-FDPS plus Zol caused nearly 70% tumor cell killing in a 4: 1 ratio, which was more than three times that of the second best treatment (LV-FDPS alone).

(Example 4. Materials and methods for CD47)

Inhibitory RNA Selection: Homo sapiens CD47 molecule (CD47) mRNA sequence (NM_001777) was used to search for potential siRNA or shRNA candidates capable of lowering CD47 levels in human cells. Potential RNA interference sequences were selected from candidates selected by the Broad Institute or siRNA or shRNA design programs such as Thermo Scientific's BLOCK-iT ™ RNAi Designer. First, individual selected shRNA sequences were inserted into a lentiviral vector immediately 3'on the RNA polymerase III promoter such as H1, U6, or 7SK to regulate shRNA expression. These lentiviral shRNA constructs were used for transduction into cells and changes in specific mRNA levels were measured. Individual implantation of the most potent shRNAs into the microRNA backbone for reduced mRNA levels allowed expression by either the CMV or the EF-1 alpha RNA polymerase II promoter. RNA sequences were also synthesized as synthetic siRNA oligonucleotides and introduced directly into cells without the use of lentiviral vectors.

Vector Construction: For CD47 shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins MWG Operon, LLC. Overlapping sense and antisense oligonucleotide sequences are mixed and annealed during incubation at 70 ° C., then cooled to room temperature, the unpaired ends extended with DNA polymerase and then to room temperature. And cooled. The extension reaction produced a double-stranded sequence at each terminal of the oligonucleotide containing the restriction enzyme sites BamHI and EcoRI. Double-stranded oligonucleotides were digested with restriction enzymes BamHI and EcoRI for 1 hour at 37 ° C. and the enzyme was heat inactivated for 20 minutes at 70 ° C. In parallel, the lentiviral vector was digested with the restriction enzymes BamHI and EcoRI at 37 ° C. for 1 hour. The digested lentiviral vector was purified by agarose gel electrophoresis and extracted from the gel using Invitrogen's DNA gel extraction kit. DNA concentrations were determined and the vectors were mixed with oligos (3: 1 ratio), annealed and ligated. The ligation reaction was carried out at room temperature for 30 minutes using T4 DNA ligase. A 2.5 microliter ligation mixture was added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after heat shock at 42 ° C. Bacterial cells were spread on agar plates containing ampicillin, drug-resistant colonies (indicating the presence of ampicillin-resistant plasmids) were harvested, purified and grown in LB medium. To confirm the insertion of the oligo sequence, plasmid DNA was extracted from the recovered bacterial culture using the Invitrogen DNA miniprep kit. Insertion of the shRNA sequence into the lentiviral vector was validated by DNA sequencing using specific primers for the promoter used to regulate shRNA expression.

Functional Assay: The effect of different CD47 shRNA targeting sequences on CD47 expression was determined by measuring mRNA expression. Transduction into Hep3B hepatocellular carcinoma cells was performed using a lentiviral vector containing a CD47 shRNA sequence. After 48 hours, cells were lysed and RNA was extracted using Qiagen's RNeasy minikit. Invitrogen's SuperScript VILO was then used to synthesize cDNA from RNA. Samples were then analyzed by quantitative RT-PCR using the Applied Biosystems StepOne PCR device. Expression of CD47 was detected using Invitrogen's SYBR Green using a forward primer (5'-CACTGTCGTCATTCCATGCT-3') (SEQ ID NO: 63) and a reverse primer (5'-GCCTTTGACATTCTCTC-3') (SEQ ID NO: 64). did. Samples are normalized by measuring actin expression using forward primers (5'-AGCGCGCGCTACAGCTTCA-3') (SEQ ID NO: 61) and reverse primers (5'-AAAGTCAGTGGGGACAGTGG-3') (SEQ ID NO: 65). did. The relative expression of CD47 was determined by the Ct value normalized to the actin level of each sample.

表 3. CD47 shRNA の配列

説明	shRNA オリゴヌクレオチド(センス配列-**ループ**-アンチセンス配列	配列番号
CD47 配列 1	GGTGAAACGATCATCGAGC**CTCGAG**GCTCGATGATCGTTTCAC CTTTTT	5
CD47 配列 2	GCTACTGGCCTTGGTTTAA**CTCGAG**TTAAACCAAGGCCAGTAG CTTTTT	6
CD47 配列 3	CCTCCTTCGTCATTGCCAT**CTCGAG**ATGGCAATGACGAAGGAG GTTTTT	7
CD47 配列 4	GCATGGCCCTCTTCTGATT**CTCGAG**AATCAGAAGAGGGCCATG CTTTTT	8
CD47 配列 5	GGTGAAACGATCATCGAGCTA**CTCGAG**TAGCTCGATGATCGTT TCACCTTTTT	9

Experimental Data for CD47 Non-limiting examples of CD47 shRNA target sequences shown in Table 3 were used in the experiments described herein. In addition, the sequences detailed in Table 3 can be used in the therapeutic vectors detailed herein.

表 3. CD47 shRNA の配列

説明	shRNA オリゴヌクレオチド(センス配列-**ループ**-アンチセンス配列	配列番号
CD47 配列 1	GGTGAAACGATCATCGAGC**CTCGAG**GCTCGATGATCGTTTCAC CTTTTT	5
CD47 配列 2	GCTACTGGCCTTGGTTTAA**CTCGAG**TTAAACCAAGGCCAGTAG CTTTTT	6
CD47 配列 3	CCTCCTTCGTCATTGCCAT**CTCGAG**ATGGCAATGACGAAGGAG GTTTTT	7
CD47 配列 4	GCATGGCCCTCTTCTGATT**CTCGAG**AATCAGAAGAGGGCCATG CTTTTT	8
CD47 配列 5	GGTGAAACGATCATCGAGCTA**CTCGAG**TAGCTCGATGATCGTT TCACCTTTTT	9

As shown in FIG. 7A, the relative expression levels of human CD47 after administration of four different CD47 shRNA sequences were determined. The most significant inhibition of human CD47 expression was found in the shCD47-1 and shCD47-3 samples (shown in FIG. 7A of the present application).

In addition, as shown in FIG. 7B, a lentivirus-based delivery system was used to target the expression of CD47. SNU449 human hepatocellular carcinoma cells were infected with a lentiviral vector containing a CD47 sequence based on miR155 below:
miR155 CD47 target sequence # 1:
CCTGGAGGCTTGCTGAAGGCTGTATGCTGTTATCCACTTCAAAGAGGCAGTTTTGCGCCACTGACTGACTGCCTCTTAAGCATGGATAACAGGACACAAGGCCTGTTACTGCACTCA (SEQ ID NO: 82)
miR155 CD47 target sequence # 2:
CCTGGAGGCTTGCTGAAGGCTGTTAGCTGTTAGCTCGATGATCGTTTCACGTTTTGGGCCACTGACTGACGGTGAAAAGCATCCGAGCTAACAGGACACAAGGCTGTTACTAGCACTCA (SEQ ID NO: 66)
miR155 CD47 target sequence # 3:
CCTGGAGGCTTGCTGAAGGCTGTATTGAAGAATGGCTCCACAAATTGACGTTTTGGCCACTGACTGACGTCATTGTGAGCCATTCTTCAGGGACACAAGGCCTGTTACTAGCACTCA (SEQ ID NO: 67)
miR155 CD47 target sequence # 4:
CCTGGAGGCTTGCTGAAGGCTGTTAGCTGTATACACGCCGCCAATACAGAGGGTTTTGCCACTGACTGACCTTGCATCGCGCGTGTAGTACAGGACACAAGGCCTGTTACTAGCACTCA (SEQ ID NO: 68)

As shown in FIG. 7B, treatment with CD47 shRNA significantly reduced the expression of FDPS protein. Treatment with the CD47 sequence based on miR155 significantly reduced the expression of CD47.

(Materials and methods for Example 5.cMyc)
Inhibitory RNA Design: v-myc avian myeloid oncogene homosapiens homolog (MYC) mRNA sequence (NM_002467.4) is used to knock on MYC expression in hepatocellular cell lines. Potential shRNA candidates to go down were screened. Five MYC SHRNA sequences capable of reducing MYC expression were obtained. Potential RNA interference sequences were selected from candidates selected by the Broad Institute or siRNA or shRNA design programs such as Thermo Scientific's BLOCK-iT ™ RNAi Designer. To regulate shRNA expression, the shRNA sequence can be inserted into a lentiviral vector behind an RNA polymerase III promoter such as H1, U6, or 7SK. Also, RNA sequences can be embedded within the microRNA backbone to allow expression by RNA polymerase II promoters such as CMV or EF-1alpha. The RNA sequence may also be synthesized as a siRNA oligonucleotide and used independently of the lentiviral vector.

Vector Construction: For cMyc shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by the MWG operon. Oligonucleotide sequences were annealed by incubation at 70 ° C. and cooling to room temperature. After digesting the annealed oligonucleotide with the restriction enzymes BamHI and EcoRI for 1 hour at 37 ° C., the enzyme was heat inactivated for 20 minutes at 70 ° C. In parallel, the lentiviral vector was digested with the restriction enzymes BamHI and EcoRI at 37 ° C. for 1 hour. The digested lentiviral vector was purified by agarose gel electrophoresis and extracted from the gel using Invitrogen's DNA gel extraction kit. DNA concentration was determined and the vector was ligated to the oligo sequence in a 3: 1 insert-to-vector ratio. The ligation reaction was carried out at room temperature for 30 minutes using T4 DNA ligase. A 2.5 microliter ligation mixture was added to 25 microliters of STBL3 competent bacterial cells. Transformation was

performed by heat shock at 42 ° C. Bacterial cells were smeared onto an agar plate containing ampicillin and then colonies were grown in LB medium. To confirm the insertion of the oligo sequence, plasmid DNA was extracted from the recovered bacterial culture using the Invitrogen DNA miniprep kit. Insertion of the shRNA sequence into the lentiviral vector was validated by DNA sequencing using specific primers in which any promoter is used to regulate shRNA expression. Lentiviral vectors containing the correct cMyc sequence were then used to package lentiviral particles to test their ability to knock down FDPS. Transduction into mammalian cells was performed using lentiviral particles either in the presence or absence of polybrene. After 2-4 days, cells were harvested and proteins and RNA analyzed for cMyc expression.

Functional Assay: The effect of different cMyc hana target sequences on cMyc expression was determined by measuring mRNA expression. Transduction into HepG2 hepatocellular carcinoma cells was performed using a lentiviral vector containing the cMyc shRNA sequence. After 48 hours, cells were lysed and RNA was extracted using Qiagen's RNeasy minikit. Invitrogen's SuperScript VILO was then used to synthesize cDNA from RNA. Samples were then analyzed by quantitative PCR using the Applied Biosystems StepOne PCR device. Expression of cMyc was detected using Invitrogen's SYBR Green using a forward primer (5'-GGACTATCCTGCTGCCAA-3') (SEQ ID NO: 69) and a reverse primer (5'-GCCTTTGACATTCTCTC-3') (SEQ ID NO: 64). did. Samples are normalized by measuring actin expression using forward primers (5'-AGCGCGACTACAGCTTCA-3') (SEQ ID NO: 61) and reverse primers (5'-GGCGACGTAGCAGACGCTCT-3') (SEQ ID NO: 62). did. The relative expression of cMyc was determined by the Ct value normalized to the actin level of each sample.

表 4. cMyc shRNA の配列

説明	shRNA オリゴヌクレオチド(センス配列-**ループ**-アンチセンス配列	配列番号
cMyc shRNA 配列 1	GCTTCACCAACAGGAACTATG**CTCGAG**CATAGTTCCTGTTGGTGAAG CTTTT	10
cMyc shRNA 配列 2	GCGAACACACAACGTCTTGGA**CTCGAG**TCCAAGACGTTGTGTGTTCG CTTTT	11
cMyc shRNA 配列 3	GACATGGTGAACCAGAGTTTC**CTCGAG**GAAACTCTGGTTCACCATGT CTTTTT	12
cMyc shRNA 配列 4	GAGAATGTCAAGAGGCGAACA**CTCGAG**TGTTCGCCTCTTGACATTCT CTTTTT	13
cMyc shRNA 配列 5	GCTCATTTCTGAAGAGGACTT**CTCGAG**AAGTCCTCTTCAGAAATGAG CTTTTT	14

Experimental Data for cMyc Non-limiting examples of cMyc shRNA sequences shown in Table 4 below were used in the experiments described herein.

表 4. cMyc shRNA の配列

説明	shRNA オリゴヌクレオチド(センス配列-**ループ**-アンチセンス配列	配列番号
cMyc shRNA 配列 1	GCTTCACCAACAGGAACTATG**CTCGAG**CATAGTTCCTGTTGGTGAAG CTTTT	10
cMyc shRNA 配列 2	GCGAACACACAACGTCTTGGA**CTCGAG**TCCAAGACGTTGTGTGTTCG CTTTT	11
cMyc shRNA 配列 3	GACATGGTGAACCAGAGTTTC**CTCGAG**GAAACTCTGGTTCACCATGT CTTTTT	12
cMyc shRNA 配列 4	GAGAATGTCAAGAGGCGAACA**CTCGAG**TGTTCGCCTCTTGACATTCT CTTTTT	13
cMyc shRNA 配列 5	GCTCATTTCTGAAGAGGACTT**CTCGAG**AAGTCCTCTTCAGAAATGAG CTTTTT	14

As shown in FIG. 8A, the relative expression levels of human cMyc after administration of 5 different cMyc shRNA sequences were determined. The most significant inhibition of human cMyc expression was found in the myc-2 sample (shown in FIG. 8A of the present application).

In addition, as shown in FIG. 8B, SNU449 human hepatocellular carcinoma cells were infected with a lentiviral vector containing either the cMYC sequence or cMyc shRNA based on the following miR:
miR155 cMyc sequence:

CCTGGAGGCTTGCTGAAGGCTGTATTGCTGTGTTCGGCCTTGACATTTTCTTTTGCGCCACTGACTGAGAAGATAGAGGCCGAACACAGGACACAAGGCCTTAGCACTCA (SEQ ID NO: 70)
miR21 cMyc sequence:
CATCTCCATTGGCTGTACCACCTTGTCCGGGGTGTTCCGCCTTGACATTTCCTGTTAGGGTCATGATACTGGAATTCATCAAGGTGAACACTGACATTTTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 83)

The above two cMyc sequences were generated using the following target sequences:
cMyc target sequence:
GAGAATGTCAAGAGGCGAACA (SEQ ID NO: 71)
cMyc shRNA sequence:
GAGAATGTCAAGAGGCGAACACTCGAGTGTTCGCCTTTGACATTCTCTTTT
(SEQ ID NO: 13)

After 48 hours, cells were lysed and immunoblots were performed using anti-cMyc antibody (Santa Cruz) and anti-actin antibody (Sigma) for protein loading control. As shown in FIG. 8B, treatment with cMyc shRNA significantly reduced the expression of cMyc protein. Treatment with the cMyc sequence based on miR also reduced the expression of cMyc.

(Example 6. In vivo treatment with FDPS-SHRNA and zoledronic acid)
Summary of protocol for co-administration of LV-SHRNA-FDPS (farnesyl diphosphate synthase) with or without zoledronic acid in mice transplanted with human prostate cancer cell line PC3. After culturing tumor cells in vitro, a lenticular vector control with a scrambled sequence (non-functional) shRNA insert and an expression cassette for firefly luciferase, or an expression cassette for shRNA and firefly luciferase that can reduce the expression of FDPS mRNA. Transduction was performed using LV-FDPS having. Transduced tumor cells were transplanted into the flank of immunodeficient mice by subcutaneous injection. When the tumor reaches a volume of about 200 mm^3, all mice are given a single dose of zoledronic acid in saline (100 micrograms per kilogram body weight; this is similar to the standard human dose). Seven days after zoledronic acid injection, imaging was repeated to measure individual tumor volume and photon intensity.

CMV プロモーター配列 :
ATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGT
ATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT
GGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTTTATATAAG
CAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTT
(配列番号 72)

GFP T2A ルシフェラーゼ配列 :
ATGCCCGCCATGAAGATCGAGTGCCGCATCACCGGCACCCTGAACGGCGTGGAG
TTCGAGCTGGTGGGCGGCGGAGAGGGCACCCCCGAGCAGGGCCGCATGACCAAC
AAGATGAAGAGCACCAAAGGCGCCCTGACCTTCAGCCCCTACCTGCTGAGCCAC

GTGATGGGCTACGGCTTCTACCACTTCGGCACCTACCCCAGCGGCTACGAGAACC
CCTTCCTGCACGCCATCAACAACGGCGGCTACACCAACACCCGCATCGAGAAGT
ACGAGGACGGCGGCGTGCTGCACGTGAGCTTCAGCTACCGCTACGAGGCCGGCC
GCGTGATCGGCGACTTCAAGGTGGTGGGCACCGGCTTCCCCGAGGACAGCGTGA
TCTTCACCGACAAGATCATCCGCAGCAACGCCACCGTGGAGCACCTGCACCCCAT
GGGCGATAACGTGCTGGTGGGCAGCTTCGCCCGCACCTTCAGCCTGCGCGACGG
CGGCTACTACAGCTTCGTGGTGGACAGCCACATGCACTTCAAGAGCGCCATCCAC
CCCAGCATCCTGCAGAACGGGGGCCCCATGTTCGCCTTCCGCCGCGTGGAGGAG
CTGCACAGCAACACCGAGCTGGGCATCGTGGAGTACCAGCACGCCTTCAAGACC
CCCATCGCCTTCGCCAGATCTCGAGATATCAGCCATGGCTTCCCGCCGGCGGTGG
CGGCGCAGGATGATGGCACGCTGCCCATGTCTTGTGCCCAGGAGAGCGGGATGG
ACCGTCACCCTGCAGCCTGTGCTTCTGCTAGGATCAATGTGACCGGTGAGGGCAG
AGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCTTCCGGTAT
GGAAGACGCCAAAAACATAAAGAAAGGCCCGGCGCCATTCTATCCGCTAGAGGA
TGGAACCGCTGGAGAGCAACTGCATAAGGCTATGAAGAGATACGCCCTGGTTCC
TGGAACAATTGCTTTTACAGATGCACATATCGAGGTGAACATCACGTACGCGGA
ATACTTCGAAATGTCCGTTCGGTTGGCAGAAGCTATGAAACGATATGGGCTGAAT
ACAAATCACAGAATCGTCGTATGCAGTGAAAACTCTCTTCAATTCTTTATGCCGG
TGTTGGGCGCGTTATTTATCGGAGTTGCAGTTGCGCCCGCGAACGACATTTATAA
TGAACGTGAATTGCTCAACAGTATGAACATTTCGCAGCCTACCGTAGTGTTTGTT
TCCAAAAAGGGGTTGCAAAAAATTTTGAACGTGCAAAAAAAATTACCAATAATC
CAGAAAATTATTATCATGGATTCTAAAACGGATTACCAGGGATTTCAGTCGATGT
ACACGTTCGTCACATCTCATCTACCTCCCGGTTTTAATGAATACGATTTTGTACCA
GAGTCCTTTGATCGTGACAAAACAATTGCACTGATAATGAACTCCTCTGGATCTA
CTGGGTTACCTAAGGGTGTGGCCCTTCCGCATAGAACTGCCTGCGTCAGATTCTC
GCATGCCAGAGATCCTATTTTTGGCAATCAAATCATTCCGGATACTGCGATTTTA
AGTGTTGTTCCATTCCATCACGGTTTTGGAATGTTTACTACACTCGGATATTTGAT
ATGTGGATTTCGAGTCGTCTTAATGTATAGATTTGAAGAAGAGCTGTTTTTACGA
TCCCTTCAGGATTACAAAATTCAAAGTGCGTTGCTAGTACCAACCCTATTTTCATT
CTTCGCCAAAAGCACTCTGATTGACAAATACGATTTATCTAATTTACACGAAATT
GCTTCTGGGGGCGCACCTCTTTCGAAAGAAGTCGGGGAAGCGGTTGCAAAACGC
TTCCATCTTCCAGGGATACGACAAGGATATGGGCTCACTGAGACTACATCAGCTA
TTCTGATTACACCCGAGGGGGATGATAAACCGGGCGCGGTCGGTAAAGTTGTTCC
ATTTTTTGAAGCGAAGGTTGTGGATCTGGATACCGGGAAAACGCTGGGCGTTAAT

CAGAGAGGCGAATTATGTGTCAGAGGACCTATGATTATGTCCGGTTATGTAAACA
ATCCGGAAGCGACCAACGCCTTGATTGACAAGGATGGATGGCTACATTCTGGAG
ACATAGCTTACTGGGACGAAGACGAACACTTCTTCATAGTTGACCGCTTGAAGTC
TTTAATTAAATACAAAGGATACCAGGTGGCCCCCGCTGAATTGGAGTCGATATTG
TTACAACACCCCAACATCTTCGACGCGGGCGTGGCAGGTCTTCCCGACGATGACG
CCGGTGAACTTCCCGCCGCCGTTGTTGTTTTGGAGCACGGAAAGACGATGACGGA
AAAAGAGATCGTGGATTACGTCGCCAGTCAAGTAACAACCGCGAAAAAGTTGCG
CGGAGGAGTTGTGTTTGTGGACGAAGTACCGAAAGGTCTTACCGGAAAACTCGA
CGCAAGAAAAATCAGAGAGATCCTCATAAAGGCCAAGAAGGGCGGAAAGTCCA
AATTGTAA (配列番号　　73)

H1 プロモーター配列　:
GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAGTGTCACTAGG
CGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAGG
GGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCATA
AACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACTT
(配列番号　　15)

The LV-FDPS vector designed, developed and utilized in this example is graphically shown in FIG. The LV-FDPS vector was developed using the methods and materials described herein. The following sequences were used to generate the CMV GFP T2A luciferase sequence and introduced into the therapeutic vector as described below.

CMV プロモーター配列　:
ATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGT
ATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT
GGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTTTATATAAG
CAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTT
(配列番号　　72)

GFP T2A ルシフェラーゼ配列　:
ATGCCCGCCATGAAGATCGAGTGCCGCATCACCGGCACCCTGAACGGCGTGGAG
TTCGAGCTGGTGGGCGGCGGAGAGGGCACCCCCGAGCAGGGCCGCATGACCAAC
AAGATGAAGAGCACCAAAGGCGCCCTGACCTTCAGCCCCTACCTGCTGAGCCAC

GTGATGGGCTACGGCTTCTACCACTTCGGCACCTACCCCAGCGGCTACGAGAACC
CCTTCCTGCACGCCATCAACAACGGCGGCTACACCAACACCCGCATCGAGAAGT
ACGAGGACGGCGGCGTGCTGCACGTGAGCTTCAGCTACCGCTACGAGGCCGGCC
GCGTGATCGGCGACTTCAAGGTGGTGGGCACCGGCTTCCCCGAGGACAGCGTGA
TCTTCACCGACAAGATCATCCGCAGCAACGCCACCGTGGAGCACCTGCACCCCAT
GGGCGATAACGTGCTGGTGGGCAGCTTCGCCCGCACCTTCAGCCTGCGCGACGG
CGGCTACTACAGCTTCGTGGTGGACAGCCACATGCACTTCAAGAGCGCCATCCAC
CCCAGCATCCTGCAGAACGGGGGCCCCATGTTCGCCTTCCGCCGCGTGGAGGAG
CTGCACAGCAACACCGAGCTGGGCATCGTGGAGTACCAGCACGCCTTCAAGACC
CCCATCGCCTTCGCCAGATCTCGAGATATCAGCCATGGCTTCCCGCCGGCGGTGG
CGGCGCAGGATGATGGCACGCTGCCCATGTCTTGTGCCCAGGAGAGCGGGATGG
ACCGTCACCCTGCAGCCTGTGCTTCTGCTAGGATCAATGTGACCGGTGAGGGCAG
AGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCTTCCGGTAT
GGAAGACGCCAAAAACATAAAGAAAGGCCCGGCGCCATTCTATCCGCTAGAGGA
TGGAACCGCTGGAGAGCAACTGCATAAGGCTATGAAGAGATACGCCCTGGTTCC
TGGAACAATTGCTTTTACAGATGCACATATCGAGGTGAACATCACGTACGCGGA
ATACTTCGAAATGTCCGTTCGGTTGGCAGAAGCTATGAAACGATATGGGCTGAAT
ACAAATCACAGAATCGTCGTATGCAGTGAAAACTCTCTTCAATTCTTTATGCCGG
TGTTGGGCGCGTTATTTATCGGAGTTGCAGTTGCGCCCGCGAACGACATTTATAA
TGAACGTGAATTGCTCAACAGTATGAACATTTCGCAGCCTACCGTAGTGTTTGTT
TCCAAAAAGGGGTTGCAAAAAATTTTGAACGTGCAAAAAAAATTACCAATAATC
CAGAAAATTATTATCATGGATTCTAAAACGGATTACCAGGGATTTCAGTCGATGT
ACACGTTCGTCACATCTCATCTACCTCCCGGTTTTAATGAATACGATTTTGTACCA
GAGTCCTTTGATCGTGACAAAACAATTGCACTGATAATGAACTCCTCTGGATCTA
CTGGGTTACCTAAGGGTGTGGCCCTTCCGCATAGAACTGCCTGCGTCAGATTCTC
GCATGCCAGAGATCCTATTTTTGGCAATCAAATCATTCCGGATACTGCGATTTTA
AGTGTTGTTCCATTCCATCACGGTTTTGGAATGTTTACTACACTCGGATATTTGAT
ATGTGGATTTCGAGTCGTCTTAATGTATAGATTTGAAGAAGAGCTGTTTTTACGA
TCCCTTCAGGATTACAAAATTCAAAGTGCGTTGCTAGTACCAACCCTATTTTCATT
CTTCGCCAAAAGCACTCTGATTGACAAATACGATTTATCTAATTTACACGAAATT
GCTTCTGGGGGCGCACCTCTTTCGAAAGAAGTCGGGGAAGCGGTTGCAAAACGC
TTCCATCTTCCAGGGATACGACAAGGATATGGGCTCACTGAGACTACATCAGCTA
TTCTGATTACACCCGAGGGGGATGATAAACCGGGCGCGGTCGGTAAAGTTGTTCC
ATTTTTTGAAGCGAAGGTTGTGGATCTGGATACCGGGAAAACGCTGGGCGTTAAT

CAGAGAGGCGAATTATGTGTCAGAGGACCTATGATTATGTCCGGTTATGTAAACA
ATCCGGAAGCGACCAACGCCTTGATTGACAAGGATGGATGGCTACATTCTGGAG
ACATAGCTTACTGGGACGAAGACGAACACTTCTTCATAGTTGACCGCTTGAAGTC
TTTAATTAAATACAAAGGATACCAGGTGGCCCCCGCTGAATTGGAGTCGATATTG
TTACAACACCCCAACATCTTCGACGCGGGCGTGGCAGGTCTTCCCGACGATGACG
CCGGTGAACTTCCCGCCGCCGTTGTTGTTTTGGAGCACGGAAAGACGATGACGGA
AAAAGAGATCGTGGATTACGTCGCCAGTCAAGTAACAACCGCGAAAAAGTTGCG
CGGAGGAGTTGTGTTTGTGGACGAAGTACCGAAAGGTCTTACCGGAAAACTCGA
CGCAAGAAAAATCAGAGAGATCCTCATAAAGGCCAAGAAGGGCGGAAAGTCCA
AATTGTAA (配列番号 73)

H1 プロモーター配列 :
GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAGTGTCACTAGG
CGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAGG
GGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCATA
AACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACTT
(配列番号 15)

Construction of LV FDPS GFP T2A Luc:
The pGF-1 plasmid (System Biosciences) containing the CMV GFP T2A luciferase sequence was digested with ClaI and KPN1 and the LV-H1-shFDPS plasmid was digested with BstBI and KpnI restriction enzymes (NEB). DNA was electrophoresed on a 1% agarose gel and DNA fragments were extracted using a DNA gel extraction kit (Thermo Scientific). Two fragments were ligated using T4 DNA ligase (NEB) to transform STBL3 bacteria (Thermo Scientific). The plasmid DNA was extracted from the bacterium using a plasmid DNA miniprep kit (Thermo Scientific), and the sequence was verified by DNA sequencing (Eurofins Genomics).
Detailed experimental protocol:
-Day 19: Confluent growth in a 175 ml flask yields 1.87 x ^{107} ml PC3 cells, and confluent growth in a 75 ml flask yields 7.5 x ^{106} ml PC3 cells.
-Day 7: Thaw PC3 cells and grow.
-Day-4: Preparation and delivery of material. PC3 cells transduced with wrench vector control and wrench-SHRNA-FDPS are prepared.
1. 1. In a 75 ml flask, add 12 µl wrench control + 8 µl polybrene to 50% confluent PC3 cells, incubate for 5 minutes, then mix with 4 ml RPMI-10 to cover the surface of the PC3 cells.
2. 2. In a 75 ml flask, add 20 µl Wrench-FDPS + 8 µl of polybrene to 50% confluent PC3 cells, incubate for 5 minutes, then mix with 4 ml RPMI-10 to cover the surface of the PC3 cells.
3. 3. Transducted cells are incubated at 37 ° C. for 8 hours. Add 6 ml RPMI-10 for overnight culture.
-Day-2: 75 ml transduced PC3 cells (7.5 × ^{106} cells of confluent) are trypsinized and transferred to a 175 ml flask.
Day 0: Preparation and delivery of materials 1. PC3 cells transduced with an 80% confluent wrench vector and PC3 cells transduced with a wrench-FDPS are separately trypsinized and the cells are counted.
Wrench vector: 1.5 × 10 ^{8} cells (50 × 3 × 10 $^{6/5}$ ml) 15 flask
Wrench-FDPS: 1.5 × 10 ^{8} cells (50 × 3 × 10 $^{6/5}$ ml) 20 flasks 2. PC3 cells transduced with a wrench vector and PC3 cells transduced with a wrench-FDPS are resuspended in RPMI without FBS to a final concentration of 3 × 10 ^{6} cells / 100 µl.

Materials: I) 5 ml PC3-wrench vector cells in RPMI without FBS (150 x ^{106} cells total), II) 5 ml PC3-wrench-FDPS cells in RPMI without FBS (150 x ^{106} cells total) cell).

Day 0: Subcutaneous injection of PC3 cells. Group I (2 NOD / SCID mice): 0.15 ml PC3-wrench vector cells (0.1 mL 3 × ^{106} lentivector in RPMI without FBS + 0.05 mL Matrigel) to the right of the mouse Alternatively, subcutaneously inject one of the left flanks (a total of 5 ml is sufficient for 50 mice). Group II (3 NOD / SCID mice): 0.15 ml PC3-wrench-FDPS KD (0.1 mL 3 × ^{106} wrench vector in DMEM without FBS + 0.05 mL Matrigel) of mice. Subcutaneously inoculate either the right or left flank (a total of 5 ml is sufficient for 50 mice).

Day 8: Monitor the tumor. The tumor is palpable for the first few days after transplantation. Tumor size is determined by measuring the orthogonal diameter of the tumor with a caliper. Tumor size is calculated by the following measurement method: tumor volume (mm ^{3}) = d ^{2} (d = shortest diameter) x D / 2 (D = longest diameter). Bioluminescence imaging is performed to demonstrate tumor location, size, and photon intensity as a measure of lentiviral expression of the firefly luciferase gene.

Day 14: When the tumor size reaches 200-300 mm ^{3} , mice are injected intraperitoneally with 100 µg / ml zoledronic acid (Zol) or PBS.

Day 22: Tumor size is measured by imaging.

Effect of LV-SHRNA-FDPS with or without zoledronic acid on the growth of PC3 tumors in NOD / SCID mice. Mice are referred to as Scr (for scrambled vector controls), or KO for LV-SHRNA-FDPS. All LVs used for this study express the bioluminescent marker firefly luciferase to allow direct visualization of transduced cells and their growth. Bioluminescence imaging on day 8 determined the mean tumor size prior to zoledronic acid treatment (FIG. 10A). Tumor photon intensities were measured using a CCD light capture system. The average tumor size in Scr animals was slightly larger than that found in KO animals (Fig. 10B), but the difference was not significant.

Imaging was repeated 6 days after treatment with zoledronic acid (all animals were given zoledronic acid by intraperitoneal injection). Tumor size and location in Scr animals (FIG. 10C) were similar to previous observations, but there were significant differences in tumor size for animals in the KO group. Tumor volume was significantly reduced in KO # 1 and KO # 3, and no tumor was present in KO # 2. A comparison of the average photon intensities for the Scr and KO groups revealed a significant difference, with the largest changes being seen in the KO group.

These data show that LV-SHRNA-FDPS has a small but detectable effect on the growth of PC3 tumors in NOD / SCID mice. The effect was increased when combined with a single dose of zoledronic acid, and eradication of LV-SHRNA-FDPS transduced cells was achieved in one case. Thus, luminescent transduced cells were reduced by zoledronic acid only when LV expressed shRNA-FDPS. The reduction in tumor volume was not attributed to zoledronic acid treatment, as animals with tumors transduced with scrambled control LV showed little or no change in tumor volume after zoledronic acid treatment.

Key to tumor reduction was the combined effect of LV-SHRNA-FDPS, which reduces the expression level of the FDPS enzyme, with zoledronic acid, which inhibits all remaining FDPS activity. As expected, zoledronic acid was not toxic to mice and appeared to have no effect other than reducing tumor mass when combined with LV-SHRNA-FDPS. Zoledronic acid is a safe and effective treatment in humans when given at high bolus doses or as a long-term therapy for impaired bone mineral loss such as osteoporosis.

The disclosure of embodiments of the examples is intended to be exemplary and does not limit the scope of the invention as set forth by the following claims and their equivalents. Although embodiments of the present invention have been described in some detail for the purpose of providing a clear understanding, it will be clear that certain modifications and improvements may be made within the scope of the following claims. .. In the following claims, the elements and / or steps do not imply any particular operating sequence unless expressly stated in the claims or implicitly required by the present disclosure. ..

配列番号	説明	配列
1	FDPS shRNA 配列#1	GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACT CCAGGACTTTTT
2	FDPS shRNA 配列#2	GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGA AATCCTGCTTTTT
3	FDPS shRNA 配列#3	GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGT ACATGGCTTTTT
4	FDPS shRNA 配列#4	GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCT CCTTCTGCTTTTT
5	CD47 shRNA 配列#1	GGTGAAACGATCATCGAGCCTCGAGGCTCGATGATCGTTTC ACCTTTTT
6	CD47 shRNA 配列#2	GCTACTGGCCTTGGTTTAACTCGAGTTAAACCAAGGCCAGT AGCTTTTT
7	CD47 shRNA 配列#3	CCTCCTTCGTCATTGCCATCTCGAGATGGCAATGACGAAGG AGGTTTTT
8	CD47 shRNA 配列#4	GCATGGCCCTCTTCTGATTCTCGAGAATCAGAAGAGGGCC ATGCTTTTT
9	CD47 shRNA 配列#5	GGTGAAACGATCATCGAGCTACTCGAGTAGCTCGATGATC GTTTCACCTTTTT
10	cMyc shRNA 配列#1	GCTTCACCAACAGGAACTATGCTCGAGCATAGTTCCTGTTG GTGAAGCTTTT
11	cMyc shRNA 配列#2	GCGAACACACAACGTCTTGGACTCGAGTCCAAGACGTTGT GTGTTCGCTTTT
12	cMyc shRNA 配列#3	GACATGGTGAACCAGAGTTTCCTCGAGGAAACTCTGGTTC ACCATGTCTTTTT
13	cMyc shRNA 配列#4	GAGAATGTCAAGAGGCGAACACTCGAGTGTTCGCCTCTTG ACATTCTCTTTTT
14	cMyc shRNA 配列#5	GCTCATTTCTGAAGAGGACTTCTCGAGAAGTCCTCTTCAGA AATGAGCTTTTT
15	H1 プロモーター	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGC CCAGTGTCACTAGGCGGGAACACCCAGCGCGCGTGCGCCC TGGCAGGAAGATGGCTGTGAGGGACAGGGGAGTGGCGCCC

		TGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCA TAAACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGTTC TGTATGAGACCACTT
16	U6プロモーター	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACG ATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACT GTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAA AGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGT TTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTA TTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAA ACACC
17	7SKプロモーター	CTGCAGTATTTAGCATGCCCCACCCATCTGCAAGGCATTCT GGATAGTGTCAAAACAGCCGGAAATCAAGTCCGTTTATCT CAAACTTTAGCATTTTGGGAATAAATGATATTTGCTATGCT GGTTAAATTAGATTTTAGTTAAATTTCCTGCTGAAGCTCTA GTACGATAAGCAACTTGACCTAAGTGTAAAGTTGAGATTTC CTTCAGGTTTATATAGCTTGTGCGCCGCCTGGCTACCTC
18	CAGエンハンサー	TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAAT GGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGA CGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGG GACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAA CTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTG GCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTT GGCAGTACATCTACGTATTAGTCATC
19	CAGプロモーター	GCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCA CTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATT TATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGG GGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGA GGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGC CAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGA GGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGC GGCGGGCG
20	ニワトリベー	GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGC

	ダアクチンイントロン	CGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTAC TCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGG

		CTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCT GTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCT TTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGT GTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGG CGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCG CTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGC CCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGC GTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGG GCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCC CCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTC CGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGG GGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGC CTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCC CCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGC CATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACT TCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGC GCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGC GGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGT GCGTCGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCG GGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGG GCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGG
21	HIV gag	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAG ATCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAGA AAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGA GCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACAT CAGAAGGCTGTAGACAAATACTGGGACAGCTACAACCATC CCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAAT ACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGA TAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAG AGCAAAACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAG CTGACACAGGACACAGCAATCAGGTCAGCCAAAATTACCC TATAGTGCAGAACATCCAGGGGCAAATGGTACATCAGGCC ATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAG AAGAGAAGGCTTTCAGCCCAGAAGTGATACCCATGTTTTC AGCATTATCAGAAGGAGCCACCCCACAAGATTTAAACACC

		ATGCTAAACACAGTGGGGGGACATCAAGCAGCCATGCAAA

		TGTTAAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGA TAGAGTGCATCCAGTGCATGCAGGGCCTATTGCACCAGGC CAGATGAGAGAACCAAGGGGAAGTGACATAGCAGGAACT ACTAGTACCCTTCAGGAACAAATAGGATGGATGACACATA ATCCACCTATCCCAGTAGGAGAAATCTATAAAAGATGGAT AATCCTGGGATTAAATAAAATAGTAAGAATGTATAGCCCT ACCAGCATTCTGGACATAAGACAAGGACCAAAGGAACCCT TTAGAGACTATGTAGACCGATTCTATAAAACTCTAAGAGCC GAGCAAGCTTCACAAGAGGTAAAAAATTGGATGACAGAAA CCTTGTTGGTCCAAAATGCGAACCCAGATTGTAAGACTATT TTAAAAGCATTGGGACCAGGAGCGACACTAGAAGAAATGA TGACAGCATGTCAGGGAGTGGGGGGACCCGGCCATAAAGC AAGAGTTTTGGCTGAAGCAATGAGCCAAGTAACAAATCCA GCTACCATAATGATACAGAAAGGCAATTTTAGGAACCAAA GAAAGACTGTTAAGTGTTTCAATTGTGGCAAAGAAGGGCA CATAGCCAAAAATTGCAGGGCCCCTAGGAAAAAGGGCTGT TGGAAATGTGGAAAGGAAGGACACCAAATGAAAGATTGTA CTGAGAGACAGGCTAATTTTTTTAGGGAAGATCTGGCCTTCC CACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAG AGCCAACAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGA AGAGACAACAACTCCCTCTCAGAAGCAGGAGCCGATAGAC AAGGAACTGTATCCTTTAGCTTCCCTCAGATCACTCTTTGG CAGCGACCCCTCGTCACAATAA
22	HIV Pol	ATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGG GAATTGGAGGTTTTATCAAAGTAGGACAGTATGATCAGAT ACTCATAGAAATCTGCGGACATAAAGCTATAGGTACAGTA TTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAATCT GTTGACTCAGATTGGCTGCACTTTAAATTTTCCCATTAGTC CTATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGA TGGCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAA ATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGG AAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAA TACTCCAGTATTTGCCATAAAGAAAAAAGACAGTACTAAA TGGAGAAAATTAGTAGATTTCAGAGAACTTAATAAGAGAA CTCAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCT

		GCAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATG

		TGGGCGATGCATATTTTTCAGTTCCCTTAGATAAAGACTTC AGGAAGTATACTGCATTTACCATACCTAGTATAAACAATG AGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACA GGGATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATG ACAAAAATCTTAGAGCCTTTTAGAAAACAAAATCCAGACA TAGTCATCTATCAATACATGGATGATTTGTATGTAGGATCT GACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAA CTGAGACAACATCTGTTGAGGTGGGGATTTACCACACCAG ACAAAAAACATCAGAAAGAACCTCCATTCCTTTGGATGGG TTATGAACTCCATCCTGATAAATGGACAGTACAGCCTATAG TGCTGCCAGAAAAGGACAGCTGGACTGTCAATGACATACA GAAATTAGTGGGAAAATTGAATTGGGCAAGTCAGATTTAT GCAGGGATTAAAGTAAGGCAATTATGTAAACTTCTTAGGG GAACCAAAGCACTAACAGAAGTAGTACCACTAACAGAAGA AGCAGAGCTAGAACTGGCAGAAAACAGGGAGATTCTAAA AGAACCGGTACATGGAGTGTATTATGACCCATCAAAAGAC TTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGG ACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAA CAGGAAAATATGCAAGAATGAAGGGTGCCCACACTAATGA TGTGAAACAATTAACAGAGGCAGTACAAAAAATAGCCACA GAAAGCATAGTAATATGGGGAAAGACTCCTAAATTTAAAT TACCCATACAAAAGGAAACATGGGAAGCATGGTGGACAGA GTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGTTTGTCA ATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAA GAACCCATAATAGGAGCAGAAACTTTCTATGTAGATGGGG CAGCCAATAGGGAAACTAAATTAGGAAAAGCAGGATATGT AACTGACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGAC ACAACAAATCAGAAGACTGAGTTACAAGCAATTCATCTAG CTTTGCAGGATTCGGGATTAGAAGTAAACATAGTGACAGA CTCACAATATGCATTGGGAATCATTCAAGCACAACCAGAT AAGAGTGAATCAGAGTTAGTCAGTCAAATAATAGAGCAGT TAATAAAAAAGGAAAAAGTCTACCTGGCATGGGTACCAGC ACACAAAGGAATTGGAGGAAATGAACAAGTAGATGGGTTG GTCAGTGCTGGAATCAGGAAAGTACTA
23	HIV Int	TTTTTAGATGGAATAGATAAGGCCCAAGAAGAACATGAGA

25	HIV-1m	TTTTATCATCAATTATCATATAGCTGTCAACATCATTCATTGTC AATATCACAGTAATTGGAGAGCAATGGCTAGTGATTTTAA

		CCTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGT GATAAATGTCAGCTAAAAGGGGAAGCCATGCATGGACAAG TAGACTGTAGCCCAGGAATATGGCAGCTAGATTGTACACA TTTAGAAGGAAAAGTTATCTTGGTAGCAGTTCATGTAGCCA GTGGATATATAGAAGCAGAAGTAATTCCAGCAGAGACAGG GCAAGAAACAGCATACTTCCTCTTAAAATTAGCAGGAAGA TGGCCAGTAAAAACAGTACATACAGACAATGGCAGCAATT TCACCAGTACTACAGTTAAGGCCGCCTGTTGGTGGGCGGG GATCAAGCAGGAATTTGGCATTCCCTACAATCCCCAAAGTC AAGGAGTAATAGAATCTATGAATAAAGAATTAAAGAAAAT TATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACA GCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGAA AAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAG TAGACATAATAGCAACAGACATACAAACTAAAGAATTACA AAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACA GGGACAGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGCT CCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAAT AGTGACATAAAAGTAGTGCCAAGAAGAAAAGCAAAGATC ATCAGGGATTATGGAAAACAGATGGCAGGTGATGATTGTG TGGCAAGTAGACAGGATGAGGATTAA
24	HIV RRE	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGC ACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCA GACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTG CTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCA CAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGC TGTGGAAAGATACCTAAAGGATCAACAGCTCCT
25	HIV Rev	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAACTCCTC AAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCC ACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAAT AGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCAT TCGATTAGTGAACGGATCCTTAGCACTTATCTGGGACGATC TGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGAC TTACTCTTGATTGTAACGAGGATTGTGGAACTTCTGGGACG CAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTA CAATATTGGAGTCAGGAGCTAAAGAATAG

		CAATATTGGAGTCAGGAGCTAAAGAATAG
26	ウサギベータ	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGA

	グロビンポリA	AGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATC
27	CMVプロモーター	ACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
28	ベータグロビンイントロン	GTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTCGCTATTGTAAAATTCATGTTATATGGAGGGGGCAAAGTTTTCAGGGTGTTGTTTAGAATGGGAAGATGTCCCTTGTATCACCATGGACCCTCATGATAATTTTGTTTCTTTCACTTTCTACTCTGTTGACAACCATTGTCTCCTCTTATTTTCTTTTCATTTTCTGTAACTTTTTCGTTAAACTTTAGCTTGCATTTGTAACGAATTTTTAAATTCACTTTTGTTTATTTGTCAGATTGTAAGTACTTTCTCTAATCACTTTTTTTTCAAGGCAATCAGGGTATATTATATTGTACTTCAGCACAGTTTTAGAGAACAATTGTTATAATTAAATGATAAGGTAGAATATTTCTGCATATAAATTCTGGCTGGCGTGGAAA

		GCTAGAATATTTCTGCATATAAATTCTGGCTGGCGTGAAA
		TATTCTTATTGGTAGAAACAACTACACCCTGGTCATCATCC

		TGCCTTTCTCTTTATGGTTACAATGATATACACTGTTTGAGA TGAGGATAAAATACTCTGAGTCCAAACCGGGCCCCTCTGCT AACCATGTTCATGCCTTCTTCTCTTTCCTACAG
29	VSV-G/VSV-G を含有する DNA 断片	GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATT GGGGTGAATTGCAAGTTCACCATAGTTTTTCCACACAACCA AAAAGGAAACTGGAAAAATGTTCCTTCTAATTACCATTATT GCCCGTCAAGCTCAGATTTAAATTGGCATAATGACTTAATA GGCACAGCCTTACAAGTCAAAATGCCCAAGAGTCACAAGG CTATTCAAGCAGACGGTTGGATGTGTCATGCTTCCAAATGG GTCACTACTTGTGATTTCCGCTGGTATGGACCGAAGTATAT AACACATTCCATCCGATCCTTCACTCCATCTGTAGAACAAT GCAAGGAAAGCATTGAACAAACGAAACAAGGAACTTGGCT GAATCCAGGCTTCCCTCCTCAAAGTTGTGGATATGCAACTG TGACGGATGCCGAAGCAGTGATTGTCCAGGTGACTCCTCA CCATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGAT TCACAGTTCATCAACGGAAAATGCAGCAATTACATATGCC CCACTGTCCATAACTCTACAACCTGGCATTCTGACTATAAG GTCAAAGGGCTATGTGATTCTAACCTCATTTCCATGGACAT CACCTTCTTCTCAGAGGACGGAGAGCTATCATCCCTGGGAA AGGAGGGCACAGGGTTCAGAAGTAACTACTTTGCTTATGA AACTGGAGGCAAGGCCTGCAAAATGCAATACTGCAAGCAT TGGGGAGTCAGACTCCCATCAGGTGTCTGGTTCGAGATGG CTGATAAGGATCTCTTTGCTGCAGCCAGATTCCCTGAATGC CCAGAAGGGTCAAGTATCTCTGCTCCATCTCAGACCTCAGT GGATGTAAGTCTAATTCAGGACGTTGAGAGGATCTTGGATT ATTCCCTCTGCCAAGAAACCTGGAGCAAAATCAGAGCGGG TCTTCCAATCTCTCCAGTGGATCTCAGCTATCTTGCTCCTAA AAACCCAGGAACCGGTCCTGCTTTCACCATAATCAATGGTA CCCTAAAATACTTTGAGACCAGATACATCAGAGTCGATATT GCTGCTCCAATCCTCTCAAGAATGGTCGGAATGATCAGTGG AACTACCACAGAAAGGGAACTGTGGGATGACTGGGCACCA TATGAAGACGTGGAAATTGGACCCAATGGAGTTCTGAGGA CCAGTTCAGGATATAAGTTTCCTTTATACATGATTGGACAT GGTATGTTGGACTCCGATCTTCATCTTAGCTCAAAGGCTCA GGTGTTCGAACATCCTCACATTCAAGACGCTGCTTCGCAAC

		TTCCTGATGATGAGAGTTTATTTTTTGGTGATACTGGGCTA

		TCCAAAAATCCAATCGAGCTTGTAGAAGGTTGGTTCAGTA GTTGGAAAAGCTCTATTGCCTCTTTTTTCTTTATCATAGGGT TAATCATTGGACTATTCTTGGTTCTCCGAGTTGGTATCCATC TTTGCATTAAATTAAAGCACACCAAGAAAAGACAGATTTA TACAGACATAGAGATGAGAATTC
30	ウサギベータグロビンポリA	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGA AGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTT ATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCA CTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATC AGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCCAT ATGCTGGCTGCCATGAACAAAGGTTGGCTATAAAGAGGTC ATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTC CATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATT TTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCC TTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTA CTCCCAGTCATAGCTGTCCCTCTTCTCTTATGGAGATC
31	プライマー	TAAGCAGAATTCATGAATTTGCCAGGAAGAT
32	プライマー	CCATACAATGAATGGACACTAGGCGGCCGCACGAAT
33	Gag、Pol、インテグラーゼの断片	GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGA TAGGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGA TCAGATACTCATAGAAATCTGCGGACATAAAGCTATAGGT ACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAA GAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCCC ATTAGTCCTATTGAGACTGTACCAGTAAAATTAAAGCCAG GAATGGATGGCCCAAAAGTTAAACAATGGCCATTGACAGA AGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATG GAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATC CATACAATACTCCAGTATTTGCCATAAAGAAAAAAGACAG TACTAAATGGAGAAAATTAGTAGATTTCAGAGAACTTAAT AAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGGAATAC CACATCCTGCAGGGTTAAAACAGAAAAAATCAGTAACAGT ACTGGATGTGGGCGATGCATATTTTTCAGTTCCCTTAGATA AAGACTTCAGGAAGTATACTGCATTTACCATACCTAGTATA AACAATGAGACACCAGGGATTAGATATCAGTACAATGTGC

		TTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGTG TAGCATGACAAAAATCTTAGAGCCTTTTAGAAAACAAAAT

		CCAGACATAGTCATCTATCAATACATGGATGATTTGTATGT AGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATA GAGGAACTGAGACAACATCTGTTGAGGTGGGGATTTACCA CACCAGACAAAAAACATCAGAAAGAACCTCCATTCCTTTG GATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAG CCTATAGTGCTGCCAGAAAAGGACAGCTGGACTGTCAATG ACATACAGAAATTAGTGGGAAAATTGAATTGGGCAAGTCA GATTTATGCAGGGATTAAAGTAAGGCAATTATGTAAACTTC TTAGGGGAACCAAAGCACTAACAGAAGTAGTACCACTAAC AGAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGGAGAT TCTAAAAGAACCGGTACATGGAGTGTATTATGACCCATCA AAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGC CAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCT GAAAACAGGAAAGTATGCAAGAATGAAGGGTGCCCACACT AATGATGTGAAACAATTAACAGAGGCAGTACAAAAAATAG CCACAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATT TAAATTACCCATACAAAAGGAAACATGGGAAGCATGGTGG ACAGAGTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGT TTGTCAATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTA GAGAAAGAACCCATAATAGGAGCAGAAACTTTCTATGTAG ATGGGGCAGCCAATAGGGAAACTAAATTAGGAAAAGCAG GATATGTAACTGACAGAGGAAGACAAAAAGTTGTCCCCCT AACGGACACAACAAATCAGAAGACTGAGTTACAAGCAATT CATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAACATAGT GACAGACTCACAATATGCATTGGGAATCATTCAAGCACAA CCAGATAAGAGTGAATCAGAGTTAGTCAGTCAAATAATAG AGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGT ACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGA TAAATTGGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAG ATGGAATAGATAAGGCCCAAGAAGAACATGAGAAATATCA CAGTAATTGGAGAGCAATGGCTAGTGATTTTAACCTACCAC CTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATG TCAGCTAAAAGGGGAAGCCATGCATGGACAAGTAGACTGT AGCCCAGGAATATGGCAGCTAGATTGTACACATTTAGAAG GAAAAGTTATCTTGGTAGCAGTTCATGTAGCCAGTGGATAT

		ATAGAAGCAGAAGTAATTCCAGCAGAGACAGGGCAAGAA

		ACAGCATACTTCCTCTTAAAATTAGCAGGAAGATGGCCAG TAAAAACAGTACATACAGACAATGGCAGCAATTTCACCAG TACTACAGTTAAGGCCGCCTGTTGGTGGGCGGGGATCAAG CAGGAATTTGGCATTCCCTACAATCCCCAAAGTCAAGGAG TAATAGAATCTATGAATAAAGAATTAAAGAAAATTATAGG ACAGGTAAGAGATCAGGCTGAACATCTTAAGACAGCAGTA CAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGG GGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACAT AATAGCAACAGACATACAAACTAAAGAATTACAAAAACAA ATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGACAG CAGAGATCCAGTTTGGAAAGGACCAGCAAAGCTCCTCTGG AAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACA TAAAAGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGG ATTATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAG TAGACAGGATGAGGATTAA
34	Rev、RRE、およびウサギベータグロビンポリ A を含有する DNA 断片	TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAG CTCATCAGAACAGTCAGACTCATCAAGCTTCTCTATCAAAG CAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGA AGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAG ATCCATTCGATTAGTGAACGGATCCTTGGCACTTATCTGGG ACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTG AGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACTTCT GGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAAT CTCCTACAATATTGGAGTCAGGAGCTAAAGAATAGAGGAG CTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATG GGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAAT TATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGG GCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTG GGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAA AGATACCTAAAGGATCAACAGCTCCTAGATCTTTTTCCCTC TGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCAT CTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAAT AGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACAT ATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTT GGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCAT

		GAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAAC

		AGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTG ACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTATTTT TTTCTTTAACATCCCTAAAATTTTCCTTACATGTTTTACTAG CCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTG TCCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAG CTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATT GTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAG CATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAA CTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTC GGGAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTC AGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCC TAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA CTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGC CTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAG GCCTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCTT ATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCAC AAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTC CAAACTCATCAATGTATCTTATCAGCGGCCGCCCCGGG
35	CAG エンハンサー/プロモーター/イントロン配列を含有する DNA 断片	ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAG TTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACG GTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCA ATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACG GTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGC CAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTC CTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACC ATGGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCA TCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTT TTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGG GGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGG GCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGA GCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCG GCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGC GGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCG

		CCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTA

		CTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGG GCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTC TGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCC TTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTG TGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCG GCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGC GCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTG CCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGC GTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGG GCGCGGCGGTCGGGCTGTAACCCCCCCCCTGCACCCCCCTCC CCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTC CGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGG GGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGC CTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCC CCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGC CATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACT TCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGC GCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGC GGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGT GCGTCGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCG GGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGG GCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGGAA TTC
36	RSV プロモーターおよびHIV Rev	CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGG GACTAGGGTGTGTTTAGGCGAAAAGCGGGGCTTCGGTTGT ACGCGGTTAGGAGTCCCCTCAGGATATAGTAGTTTCGCTTT TGCATAGGGAGGGGGAAATGTAGTCTTATGCAATACACTT GTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCT TACAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGA AGTAAGGTGGTACGATCGTGCCTTATTAGGAAGGCAACAG ACAGGTCTGACATGGATTGGACGAACCACTGAATTCCGCA TTGCAGAGATAATTGTATTTAAGTGCCTAGCTCGATACAAT AAACGCCATTTGACCATTCACCACATTGGTGTGCACCTCCA AGCTCGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGA CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGA

		CCGATCCAGCCTCCCCTCGAAGCTAGCGATTAGGCATCTCC

		TATGGCAGGAAGAAGCGGAGACAGCGACGAAGAACTCCTC AAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCC ACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAAT AGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCAT TCGATTAGTGAACGGATCCTTAGCACTTATCTGGGACGATC TGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGAC TTACTCTTGATTGTAACGAGGATTGTGGAACTTCTGGGACG CAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTA CAATATTGGAGTCAGGAGCTAAAGAATAGTCTAGA
37	伸長因子-1 アルファ(EF1-アルファ)プロモーター	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAG TGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGG GAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTT TTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCG TGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGC CCTTGCGTGCCTTGAATTACTTCCACGCCCCTGGCTGCAGT ACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGG GAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCG TGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGC GTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTT CGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTG CGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGC CAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGG CGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCG AGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGG GGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCT CGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTG GCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGC TTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCG GCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGG AAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTC CACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCT CGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGG GTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGA CTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTG GAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAG

		CCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTG

		TCGTGA
38	プロモーター; PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTG CGCAGGGACGCGGCTGCTCTGGGCGTGGTTCCGGGAAACG CAGCGGCGCCGACCCTGGGTCTCGCACATTCTTCACGTCCG TTCGCAGCGTCACCCGGATCTTCGCCGCTACCCTTGTGGGC CCCCCGGCGACGCTTCCTGCTCCGCCCCTAAGTCGGGAAGG TTCCTTGCGGTTCGCGGCGTGCCGGACGTGACAAACGGAA GCCGCACGTCTCACTAGTACCCTCGCAGACGGACAGCGCC AGGGAGCAATGGCAGCGCGCCGACCGCGATGGGCTGTGGC CAATAGCGGCTGCTCAGCAGGGCGCGCCGAGAGCAGCGGC CGGGAAGGGGCGGTGCGGGAGGCGGGGTGTGGGGCGGTA GTGTGGGCCCTGTTCCTGCCCGCGCGGTGTTCCGCATTCTG CAAGCCTCCGGAGCGCACGTCGGCAGTCGGCTCCCTCGTTG ACCGAATCACCGACCTCTCTCCCCAG
39	プロモーター; UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCTCCTCA CGGCGAGCGCTGCCACGTCAGACGAAGGGCGCAGGAGCGT TCCTGATCCTTCCGCCCGGACGCTCAGGACAGCGGCCCGCT GCTCATAAGACTCGGCCTTAGAACCCCAGTATCAGCAGAA GGACATTTTAGGACGGGACTTGGGTGACTCTAGGGCACTG GTTTTCTTTCCAGAGAGCGGAACAGGCGAGGAAAAGTAGT CCCTTCTCGGCGATTCTGCGGAGGGATCTCCGTGGGGCGGT GAACGCCGATGATTATATAAGGACGCGCCGGGTGTGGCAC AGCTAGTTCCGTCGCAGCCGGGATTTGGGTCGCGGTTCTTG TTTGTGGATCGCTGTGATCGTCACTTGGTGAGTTGCGGGCT GCTGGGCTGGCCGGGGCTTTCGTGGCCGCCGGGCCGCTCG GTGGGACGGAAGCGTGTGGAGAGACCGCCAAGGGCTGTAG TCTGGGTCCGCGAGCAAGGTTGCCCTGAACTGGGGGTTGG GGGGAGCGCACAAAATGGCGGCTGTTCCCGAGTCTTGAAT GGAAGACGCTTGTAAGGCGGGCTGTGAGGTCGTTGAAACA AGGTGGGGGGCATGGTGGGCGGCAAGAACCCAAGGTCTTG AGGCCTTCGCTAATGCGGGAAAGCTCTTATTCGGGTGAGAT GGGCTGGGGCACCATCTGGGGACCCTGACGTGAAGTTTGT CACTGACTGGAGAACTCGGGTTTGTCGTCTGGTTGCGGGGG CGGCAGTTATGCGGTGCCGTTGGGCAGTGCACCCGTACCTT TGGGAGCGCGCGCCTCGTCGTGTCGTGACGTCACCCGTTCT

		GTTGGCTTATAATGCAGGGTGGGGCCACCTGCCGGTAGGT

		GTGCGGTAGGCTTTTCTCCGTCGCAGGACGCAGGGTTCGGG CCTAGGGTAGGCTCTCCTGAATCGACAGGCGCCGGACCTCT GGTGAGGGGAGGGATAAGTGAGGCGTCAGTTTCTTTGGTC GGTTTTATGTACCTATCTTCTTAAGTAGCTGAAGCTCCGGT TTTGAACTATGCGCTCGGGGTTGGCGAGTGTGTTTTGTGAA GTTTTTTAGGCACCTTTTGAAATGTAATCATTTGGGTCAAT ATGTAATTTTCAGTGTTAGACTAGTAAA
40	ポリ A; SV40	GTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCA TCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCT AGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCA
41	ポリ A; bGH	GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTC CCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTG TCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTG AGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGG ACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATG CTGGGGATGCGGTGGGCTCTATGG
42	エンベロープ; RD114	ATGAAACTCCCAACAGGAATGGTCATTTTATGTAGCCTAAT AATAGTTCGGGCAGGGTTTGACGACCCCCGCAAGGCTATC GCATTAGTACAAAAACAACATGGTAAACCATGCGAATGCA GCGGAGGGCAGGTATCCGAGGCCCCACCGAACTCCATCCA ACAGGTAACTTGCCCAGGCAAGACGGCCTACTTAATGACC AACCAAAAATGGAAATGCAGAGTCACTCCAAAAAATCTCA CCCCTAGCGGGGGAGAACTCCAGAACTGCCCCTGTAACAC TTTCCAGGACTCGATGCACAGTTCTTGTTATACTGAATACC GGCAATGCAGGGCGAATAATAAGACATACTACACGGCCAC CTTGCTTAAAATACGGTCTGGGAGCCTCAACGAGGTACAG ATATTACAAAACCCCAATCAGCTCCTACAGTCCCCTTGTAG GGGCTCTATAAATCAGCCCGTTTGCTGGAGTGCCACAGCCC CCATCCATATCTCCGATGGTGGAGGACCCCTCGATACTAAG AGAGTGTGGACAGTCCAAAAAAGGCTAGAACAAATTCATA AGGCTATGCATCCTGAACTTCAATACCACCCCTTAGCCCTG CCCAAAGTCAGAGATGACCTTAGCCTTGATGCACGGACTTT TGATATCCTGAATACCACTTTTAGGTTACTCCAGATGTCCA ATTTTAGCCTTGCCCAAGATTGTTGGCTCTGTTTAAAACTA GGTACCCCTACCCCTCTTGCGATACCCACTCCCTCTTTAAC

		CGTACCCCTACCCCTCTTGCGATACCCACTCCCTCTTTAAC
		CTACTCCCTAGCAGACTCCCTAGCGAATGCCTCCTGTCAGA

		TTATACCTCCCCTCTTGGTTCAACCGATGCAGTTCTCCAACT CGTCCTGTTTATCTTCCCCTTTCATTAACGATACGGAACAA ATAGACTTAGGTGCAGTCACCTTTACTAACTGCACCTCTGT AGCCAATGTCAGTAGTCCTTTATGTGCCCTAAACGGGTCAG TCTTCCTCTGTGGAAATAACATGGCATACACCTATTTACCC CAAAACTGGACAGGACTTTGCGTCCAAGCCTCCCTCCTCCC CGACATTGACATCATCCCGGGGGATGAGCCAGTCCCCATTC CTGCCATTGATCATTATATACATAGACCTAAACGAGCTGTA CAGTTCATCCCTTTACTAGCTGGACTGGGAATCACCGCAGC ATTCACCACCGGAGCTACAGGCCTAGGTGTCTCCGTCACCC AGTATACAAAATTATCCCATCAGTTAATATCTGATGTCCAA GTCTTATCCGGTACCATACAAGATTTACAAGACCAGGTAG ACTCGTTAGCTGAAGTAGTTCTCCAAAATAGGAGGGGACT GGACCTACTAACGGCAGAACAAGGAGGAATTTGTTTAGCC TTACAAGAAAAATGCTGTTTTTATGCTAACAAGTCAGGAAT TGTGAGAAACAAAATAAGAACCCTACAAGAAGAATTACAA AAACGCAGGGAAAGCCTGGCATCCAACCCTCTCTGGACCG GGCTGCAGGGCTTTCTTCCGTACCTCCTACCTCTCCTGGGA CCCCTACTCACCCTCCTACTCATACTAACCATTGGGCCATG CGTTTTCAATCGATTGGTCCAATTTGTTAAAGACAGGATCT CAGTGGTCCAGGCTCTGGTTTTGACTCAGCAATATCACCAG CTAAAACCCATAGAGTACGAGCCATGA
43	エンベロープ; GALV	ATGCTTCTCACCTCAAGCCCGCACCACCTTCGGCACCAGAT GAGTCCTGGGAGCTGGAAAAGACTGATCATCCTCTTAAGC TGCGTATTCGGAGACGGCAAAACGAGTCTGCAGAATAAGA ACCCCCACCAGCCTGTGACCCTCACCTGGCAGGTACTGTCC CAAACTGGGGACGTTGTCTGGGACAAAAAGGCAGTCCAGC CCCTTTGGACTTGGTGGCCCTCTCTTACACCTGATGTATGT GCCCTGGCGGCCGGTCTTGAGTCCTGGGATATCCCGGGATC CGATGTATCGTCCTCTAAAAGAGTTAGACCTCCTGATTCAG ACTATACTGCCGCTTATAAGCAAATCACCTGGGGAGCCAT AGGGTGCAGCTACCCTCGGGCTAGGACCAGGATGGCAAAT TCCCCCTTCTACGTGTGTCCCCGAGCTGGCCGAACCCATTC AGAAGCTAGGAGGTGTGGGGGGCTAGAATCCCTATACTGT AAAGAATGGAGTTGTGAGACCACGGGTACCGTTTATTGGC

		AACCCAAGTCCTCATGGGACCTCATAACTGTAAAATGGGA

		CCAAAATGTGAAATGGGAGCAAAAATTTCAAAAGTGTGAA CAAACCGGCTGGTGTAACCCCCTCAAGATAGACTTCACAG AAAAAGGAAAACTCTCCAGAGATTGGATAACGGAAAAAA CCTGGGAATTAAGGTTCTATGTATATGGACACCCAGGCATA CAGTTGACTATCCGCTTAGAGGTCACTAACATGCCGGTTGT GGCAGTGGGCCCAGACCCTGTCCTTGCGGAACAGGGACCT CCTAGCAAGCCCCTCACTCTCCCTCTCTCCCCACGGAAAGC GCCGCCCACCCCTCTACCCCCGGCGGCTAGTGAGCAAACC CCTGCGGTGCATGGAGAAACTGTTACCCTAAACTCTCCGCC TCCCACCAGTGGCGACCGACTCTTTGGCCTTGTGCAGGGGG CCTTCCTAACCTTGAATGCTACCAACCCAGGGGCCACTAAG TCTTGCTGGCTCTGTTTGGGCATGAGCCCCCCTTATTATGA AGGGATAGCCTCTTCAGGAGAGGTCGCTTATACCTCCAACC ATACCCGATGCCACTGGGGGGCCCAAGGAAAGCTTACCCT CACTGAGGTCTCCGGACTCGGGTCATGCATAGGGAAGGTG CCTCTTACCCATCAACATCTTTGCAACCAGACCTTACCCAT CAATTCCTCTAAAAACCATCAGTATCTGCTCCCCTCAAACC ATAGCTGGTGGGCCTGCAGCACTGGCCTCACCCCCTGCCTC TCCACCTCAGTTTTTAATCAGTCTAAAGACTTCTGTGTCCA GGTCCAGCTGATCCCCCGCATCTATTACCATTCTGAAGAAA CCTTGTTACAAGCCTATGACAAATCACCCCCCAGGTTTAAA AGAGAGCCTGCCTCACTTACCCTAGCTGTCTTCCTGGGGTT AGGGATTGCGGCAGGTATAGGTACTGGCTCAACCGCCCTA ATTAAAGGGCCCATAGACCTCCAGCAAGGCCTAACCAGCC TCCAAATCGCCATTGACGCTGACCTCCGGGCCCTTCAGGAC TCAATCAGCAAGCTAGAGGACTCACTGACTTCCCTATCTGA GGTAGTACTCCAAAATAGGAGAGGCCTTGACTTACTATTCC TTAAAGAAGGAGGCCTCTGCGCGGCCCTAAAAGAAGAGTG CTGTTTTTATGTAGACCACTCAGGTGCAGTACGAGACTCCA TGAAAAAACTTAAAGAAAGACTAGATAAAAGACAGTTAGA GCGCCAGAAAAACCAAAACTGGTATGAAGGGTGGTTCAAT AACTCCCCTTGGTTTACTACCCTACTATCAACCATCGCTGG GCCCCTATTGCTCCTCCTTTTGTTACTCACTCTTGGGCCCTG CATCATCAATAAATTAATCCAATTCATCAATGATAGGATAA GTGCAGTCAAAATTTTAGTCCTTAGACAGAAATATCAGACC

		CTAGATAACGAGGAAAACCTTTAA

44	エンベロープ; FUG	ATGGTTCCGCAGGTTCTTTTGTTTGTACTCCTTCTGGGTTTT TCGTTGTGTTTCGGGAAGTTCCCCATTTACACGATACCAGA CGAACTTGGTCCCTGGAGCCCTATTGACATACACCATCTCA GCTGTCCAAATAACCTGGTTGTGGAGGATGAAGGATGTAC CAACCTGTCCGAGTTCTCCTACATGGAACTCAAAGTGGGAT ACATCTCAGCCATCAAAGTGAACGGGTTCACTTGCACAGG TGTTGTGACAGAGGCAGAGACCTACACCAACTTTGTTGGTT ATGTCACAACCACATTCAAGAGAAAGCATTTCCGCCCCAC CCCAGACGCATGTAGAGCCGCGTATAACTGGAAGATGGCC GGTGACCCCAGATATGAAGAGTCCCTACACAATCCATACC CCGACTACCACTGGCTTCGAACTGTAAGAACCACCAAAGA GTCCCTCATTATCATATCCCCAAGTGTGACAGATTTGGACC CATATGACAAATCCCTTCACTCAAGGGTCTTCCCTGGCGGA AAGTGCTCAGGAATAACGGTGTCCTCTACCTACTGCTCAAC TAACCATGATTACACCATTTGGATGCCCGAGAATCCGAGA CCAAGGACACCTTGTGACATTTTTACCAATAGCAGAGGGA AGAGAGCATCCAACGGGAACAAGACTTGCGGCTTTGTGGA TGAAAGAGGCCTGTATAAGTCTCTAAAAGGAGCATGCAGG CTCAAGTTATGTGGAGTTCTTGGACTTAGACTTATGGATGG AACATGGGTCGCGATGCAAACATCAGATGAGACCAAATGG TGCCCTCCAGATCAGTTGGTGAATTTGCACGACTTTCGCTC AGACGAGATCGAGCATCTCGTTGTGGAGGAGTTAGTTAAG AAAAGAGAGGAATGTCTGGATGCATTAGAGTCCATCATGA CCACCAAGTCAGTAAGTTTCAGACGTCTCAGTCACCTGAGA AAACTTGTCCCAGGGTTTGGAAAAGCATATACCATATTCAA CAAAACCTTGATGGAGGCTGATGCTCACTACAAGTCAGTC CGGACCTGGAATGAGATCATCCCCTCAAAAGGGTGTTTGA AAGTTGGAGGAAGGTGCCATCCTCATGTGAACGGGGTGTT TTTCAATGGTATAATATTAGGGCCTGACGACCATGTCCTAA TCCCAGAGATGCAATCATCCCTCCTCCAGCAACATATGGAG TTGTTGGAATCTTCAGTTATCCCCCTGATGCACCCCCTGGC AGACCCTTCTACAGTTTTCAAAGAAGGTGATGAGGCTGAG GATTTTGTTGAAGTTCACCTCCCCGATGTGTACAAACAGAT CTCAGGGGTTGACCTGGGTCTCCCGAACTGGGGAAAGTAT GTATTGATGACTGCAGGGGCCATGATTGGCCTGGTGTTGAT

		ATTTTCCCTAATGACATGGTGCAGAGTTGGTATCCATCTTT

		GCATTAAATTAAAGCACACCAAGAAAAGACAGATTTATAC AGACATAGAGATGAACCGACTTGGAAAGTAA
45	エンベロープ; LCMV	ATGGGTCAGATTGTGACAATGTTTGAGGCTCTGCCTCACAT CATCGATGAGGTGATCAACATTGTCATTATTGTGCTTATCG TGATCACGGGTATCAAGGCTGTCTACAATTTTGCCACCTGT GGGATATTCGCATTGATCAGTTTCCTACTTCTGGCTGGCAG GTCCTGTGGCATGTACGGTCTTAAGGGACCCGACATTTACA AAGGAGTTTACCAATTTAAGTCAGTGGAGTTTGATATGTCA CATCTGAACCTGACCATGCCCAACGCATGTTCAGCCAACA ACTCCCACCATTACATCAGTATGGGGACTTCTGGACTAGAA TTGACCTTCACCAATGATTCCATCATCAGTCACAACTTTTG CAATCTGACCTCTGCCTTCAACAAAAAGACCTTTGACCACA CACTCATGAGTATAGTTTCGAGCCTACACCTCAGTATCAGA GGGAACTCCAACTATAAGGCAGTATCCTGCGACTTCAACA ATGGCATAACCATCCAATACAACTTGACATTCTCAGATCGA CAAAGTGCTCAGAGCCAGTGTAGAACCTTCAGAGGTAGAG TCCTAGATATGTTTAGAACTGCCTTCGGGGGGAAATACATG AGGAGTGGCTGGGGCTGGACAGGCTCAGATGGCAAGACCA CCTGGTGTAGCCAGACGAGTTACCAATACCTGATTATACAA AATAGAACCTGGGAAAACCACTGCACATATGCAGGTCCTT TTGGGATGTCCAGGATTCTCCTTTCCCAAGAGAAGACTAAG TTCTTCACTAGGAGACTAGCGGGCACATTCACCTGGACTTT GTCAGACTCTTCAGGGGTGGAGAATCCAGGTGGTTATTGCC TGACCAAATGGATGATTCTTGCTGCAGAGCTTAAGTGTTTC GGGAACACAGCAGTTGCGAAATGCAATGTAAATCATGATG CCGAATTCTGTGACATGCTGCGACTAATTGACTACAACAAG GCTGCTTTGAGTAAGTTCAAAGAGGACGTAGAATCTGCCTT GCACTTATTCAAAACAACAGTGAATTCTTTGATTTCAGATC AACTACTGATGAGGAACCACTTGAGAGATCTGATGGGGGT GCCATATTGCAATTACTCAAAGTTTTGGTACCTAGAACATG CAAAGACCGGCGAAACTAGTGTCCCCAAGTGCTGGCTTGT CACCAATGGTTCTTACTTAAATGAGACCCACTTCAGTGATC AAATCGAACAGGAAGCCGATAACATGATTACAGAGATGTT GAGGAAGGATTACATAAAGAGGCAGGGGAGTACCCCCCTA GCATTGATGGACCTTCTGATGTTTTCCACATCTGCATATCT

		AGTCAGCATCTTCCTGCACCTTGTCAAAATACCAACACACA

		GGCACATAAAAGGTGGCTCATGTCCAAAGCCACACCGATT AACCAACAAAGGAATTTGTAGTTGTGGTGCATTTAAGGTG CCTGGTGTAAAAACCGTCTGGAAAAGACGCTGA
46	エンベロープ; FPV	ATGAACACTCAAATCCTGGTTTTCGCCCTTGTGGCAGTCAT CCCCACAAATGCAGACAAAATTTGTCTTGGACATCATGCTG TATCAAATGGCACCAAAGTAAACACACTCACTGAGAGAGG AGTAGAAGTTGTCAATGCAACGGAAACAGTGGAGCGGACA AACATCCCCAAAATTTGCTCAAAAGGGAAAAGAACCACTG ATCTTGGCCAATGCGGACTGTTAGGGACCATTACCGGACC ACCTCAATGCGACCAATTTCTAGAATTTTCAGCTGATCTAA TAATCGAGAGACGAGAAGGAAATGATGTTTGTTACCCGGG GAAGTTTGTTAATGAAGAGGCATTGCGACAAATCCTCAGA GGATCAGGTGGGATTGACAAAGAAACAATGGGATTCACAT ATAGTGGAATAAGGACCAACGGAACAACTAGTGCATGTAG AAGATCAGGGTCTTCATTCTATGCAGAAATGGAGTGGCTCC TGTCAAATACAGACAATGCTGCTTTCCCACAAATGACAAA ATCATACAAAAACACAAGGAGAGAATCAGCTCTGATAGTC TGGGGAATCCACCATTCAGGATCAACCACCGAACAGACCA AACTATATGGGAGTGGAAATAAACTGATAACAGTCGGGAG TTCCAAATATCATCAATCTTTTGTGCCGAGTCCAGGAACAC GACCGCAGATAAATGGCCAGTCCGGACGGATTGATTTTCA TTGGTTGATCTTGGATCCCAATGATACAGTTACTTTTAGTTT CAATGGGGCTTTCATAGCTCCAAATCGTGCCAGCTTCTTGA GGGGAAAGTCCATGGGGATCCAGAGCGATGTGCAGGTTGA TGCCAATTGCGAAGGGGAATGCTACCACAGTGGAGGGACT ATAACAAGCAGATTGCCTTTTCAAAACATCAATAGCAGAG CAGTTGGCAAATGCCCAAGATATGTAAAACAGGAAAGTTT ATTATTGGCAACTGGGATGAAGAACGTTCCCGAACCTTCCA AAAAAAGGAAAAAAAGAGGCCTGTTTGGCGCTATAGCAGG GTTTATTGAAAATGGTTGGGAAGGTCTGGTCGACGGGTGG TACGGTTTCAGGCATCAGAATGCACAAGGAGAAGGAACTG CAGCAGACTACAAAAGCACCCAATCGGCAATTGATCAGAT AACCGGAAAGTTAAATAGACTCATTGAGAAAACCAACCAG CAATTTGAGCTAATAGATAATGAATTCACTGAGGTGGAAA AGCAGATTGGCAATTTAATTAACTGGACCAAAGACTCCAT

		CACAGAAGTATGGTCTTACAATGCTGAACTTCTTGTGGCAA

		TGGAAAACCAGCACACTATTGATTTGGCTGATTCAGAGAT GAACAAGCTGTATGAGCGAGTGAGGAAACAATTAAGGGA AAATGCTGAAGAGGATGGCACTGGTTGCTTTGAAATTTTTC ATAAATGTGACGATGATTGTATGGCTAGTATAAGGAACAA TACTTATGATCACAGCAAATACAGAGAAGAAGCGATGCAA AATAGAATACAAATTGACCCAGTCAAATTGAGTAGTGGCT ACAAAGATGTGATACTTTGGTTTAGCTTCGGGGCATCATGC TTTTTGCTTCTTGCCATTGCAATGGGCCTTGTTTTCATATGT GTGAAGAACGGAAACATGCGGTGCACTATTTGTATATAA
47	エンベロープ; RRV	AGTGTAACAGAGCACTTTAATGTGTATAAGGCTACTAGAC CATACCTAGCACATTGCGCCGATTGCGGGGACGGGTACTTC TGCTATAGCCCAGTTGCTATCGAGGAGATCCGAGATGAGG CGTCTGATGGCATGCTTAAGATCCAAGTCTCCGCCCAAATA GGTCTGGACAAGGCAGGCACCCACGCCCACACGAAGCTCC GATATATGGCTGGTCATGATGTTCAGGAATCTAAGAGAGA TTCCTTGAGGGTGTACACGTCCGCAGCGTGCTCCATACATG GGACGATGGGACACTTCATCGTCGCACACTGTCCACCAGG CGACTACCTCAAGGTTTCGTTCGAGGACGCAGATTCGCACG TGAAGGCATGTAAGGTCCAATACAAGCACAATCCATTGCC GGTGGGTAGAGAGAAGTTCGTGGTTAGACCACACTTTGGC GTAGAGCTGCCATGCACCTCATACCAGCTGACAACGGCTC CCACCGACGAGGAGATTGACATGCATACACCGCCAGATAT ACCGGATCGCACCCTGCTATCACAGACGGCGGGCAACGTC AAAATAACAGCAGGCGGCAGGACTATCAGGTACAACTGTA CCTGCGGCCGTGACAACGTAGGCACTACCAGTACTGACAA GACCATCAACACATGCAAGATTGACCAATGCCATGCTGCC GTCACCAGCCATGACAAATGGCAATTTACCTCTCCATTTGT TCCCAGGGCTGATCAGACAGCTAGGAAAGGCAAGGTACAC GTTCCGTTCCCTCTGACTAACGTCACCTGCCGAGTGCCGTT GGCTCGAGCGCCGGATGCCACCTATGGTAAGAAGGAGGTG ACCCTGAGATTACACCCAGATCATCCGACGCTCTTCTCCTA TAGGAGTTTAGGAGCCGAACCGCACCCGTACGAGGAATGG GTTGACAAGTTCTCTGAGCGCATCATCCCAGTGACGGAAG AAGGGATTGAGTACCAGTGGGGCAACAACCCGCCGGTCTG CCTGTGGGCGCAACTGACGACCGAGGGCAAACCCCATGGC

		TGGCCACATGAAATCATTCAGTACTATTATGGACTATACCC

		CGCCGCCACTATTGCCGCAGTATCCGGGGCGAGTCTGATG GCCCTCCTAACTCTGGCGGCCACATGCTGCATGCTGGCCAC CGCGAGGAGAAAGTGCCTAACACCGTACGCCCTGACGCCA GGAGCGGTGGTACCGTTGACACTGGGGCTGCTTTGCTGCGC ACCGAGGGCGAATGCA
48	エンベロープ; エボラ	ATGGGTGTTACAGGAATATTGCAGTTACCTCGTGATCGATT CAAGAGGACATCATTCTTTCTTTGGGTAATTATCCTTTTCCA AAGAACATTTTCCATCCCACTTGGAGTCATCCACAATAGCA CATTACAGGTTAGTGATGTCGACAAACTGGTTTGCCGTGAC AAACTGTCATCCACAAATCAATTGAGATCAGTTGGACTGA ATCTCGAAGGGAATGGAGTGGCAACTGACGTGCCATCTGC AACTAAAAGATGGGGCTTCAGGTCCGGTGTCCCACCAAAG GTGGTCAATTATGAAGCTGGTGAATGGGCTGAAAACTGCT ACAATCTTGAAATCAAAAAACCTGACGGGAGTGAGTGTCT ACCAGCAGCGCCAGACGGGATTCGGGGCTTCCCCCGGTGC CGGTATGTGCACAAAGTATCAGGAACGGGACCGTGTGCCG GAGACTTTGCCTTCCACAAAGAGGGTGCTTTCTTCCTGTAT GACCGACTTGCTTCCACAGTTATCTACCGAGGAACGACTTT CGCTGAAGGTGTCGTTGCATTTCTGATACTGCCCCAAGCTA AGAAGGACTTCTTCAGCTCACACCCCTTGAGAGAGCCGGT CAATGCAACGGAGGACCCGTCTAGTGGCTACTATTCTACCA CAATTAGATATCAAGCTACCGGTTTTGGAACCAATGAGAC AGAGTATTTGTTCGAGGTTGACAATTTGACCTACGTCCAAC TTGAATCAAGATTCACACCACAGTTTCTGCTCCAGCTGAAT GAGACAATATATACAAGTGGGAAAAGGAGCAATACCACG GGAAAACTAATTTGGAAGGTCAACCCCGAAATTGATACAA CAATCGGGGAGTGGGCCTTCTGGGAAACTAAAAAAACCTC ACTAGAAAAATTCGCAGTGAAGAGTTGTCTTTCACAGCTGT ATCAAACAGAGCCAAAAACATCAGTGGTCAGAGTCCGGCG CGAACTTCTTCCGACCCAGGGACCAACACAACAACTGAAG ACCACAAAATCATGGCTTCAGAAAATTCCTCTGCAATGGTT CAAGTGCACAGTCAAGGAAGGGAAGCTGCAGTGTCGCATC TGACAACCCTTGCCACAATCTCCACGAGTCCTCAACCCCCC ACAACCAAACCAGGTCCGGACAACAGCACCCACAATACAC CCGTGTATAAACTTGACATCTCTGAGGCAACTCAAGTTGAA

		CAACATCACCGCAGAACAGACAACGACAGCACAGCCTCCG

		ACACTCCCCCCGCCACGACCGCAGCCGGACCCCTAAAAGC AGAGAACACCAACACGAGCAAGGGTACCGACCTCCTGGAC CCCGCCACCACAACAAGTCCCCAAAACCACAGCGAGACCG CTGGCAACAACAACACTCATCACCAAGATACCGGAGAAGA GAGTGCCAGCAGCGGGAAGCTAGGCTTAATTACCAATACT ATTGCTGGAGTCGCAGGACTGATCACAGGCGGGAGGAGAG CTCGAAGAGAAGCAATTGTCAATGCTCAACCCAAATGCAA CCCTAATTTACATTACTGGACTACTCAGGATGAAGGTGCTG CAATCGGACTGGCCTGGATACCATATTTCGGGCCAGCAGC CGAGGGAATTTACATAGAGGGGCTGATGCACAATCAAGAT GGTTTAATCTGTGGGTTGAGACAGCTGGCCAACGAGACGA CTCAAGCTCTTCAACTGTTCCTGAGAGCCACAACCGAGCTA CGCACCTTTTCAATCCTCAACCGTAAGGCAATTGATTTCTT GCTGCAGCGATGGGGCGGCACATGCCACATTTTGGGACCG GACTGCTGTATCGAACCACATGATTGGACCAAGAACATAA CAGACAAAATTGATCAGATTATTCATGATTTTGTTGATAAA ACCCTTCCGGACCAGGGGGACAATGACAATTGGTGGACAG GATGGAGACAATGGATACCGGCAGGTATTGGAGTTACAGG CGTTATAATTGCAGTTATCGCTTTATTCTGTATATGCAAATT TGTCTTTTAG
49	FDPS 標的配列#1	GTCCTGGAGTACAATGCCATT
50	FDPS 標的配列#2	GCAGGATTTCGTTCAGCACTT
51	FDPS 標的配列#3	GCCATGTACATGGCAGGAATT
52	FDPS 標的配列#4	GCAGAAGGAGGCTGAGAAAGT
53	miR30 FDPS 配列#1	AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCC TCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAG AAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT
54	miR30 FDPS 配列#2	AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCC TCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAA

		AGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT

55	miR30 FDPS 配列#3	TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGT GAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTA CTGCCTCGGA
56	miR155 FDPS 配列#1	CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCC TCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGGCTGAGA AAGTCAGGACACAAGGCCTGTTACTAGCACTCA
57	miR21 FDPS 配列#1	CATCTCCATGGCTGTACCACCTTGTCGGGACTTTCTCAGCC TCCTTCTGCCTGTTGAATCTCATGGCAGAAGGAGGCGAGA AAGTCTGACATTTTGGTATCTTTCATCTGACCA
58	miR185 FDPS 配列#1	GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGC CTCCTTCTGCTGGTCCCCTCCCCGCAGAAGGAGGCTGAGAA AGTCCTTCCCTCCCAATGACCGCGTCTTCGTCG
59	フォワードプライマー	AGGAATTGATGGCGAGAAGG
60	リバースプライマー	CCCAAAGAGGTCAAGGTAATCA
61	フォワードプライマー	AGCGCGGCTACAGCTTCA
62	リバースプライマー	GGCGACGTAGCACAGCTTCT
63	フォワードプライマー	CACTGTCGTCATTCCATGCT
64	リバースプライマー	GCCTCTTGACATTCTCCTC
65	リバースプライマー	AAAGTCAGTGGGGACAGTGG
66	miR155 CD47 標的配列#2	CCTGGAGGCTTGCTGAAGGCTGTATGCTGTTAGCTCGATGA TCGTTTCACGTTTTGGCCACTGACTGACGTGAAACGCATCG AGCTAACAGGACACAAGGCCTGTTACTAGCACTCA
67	miR155 CD47 標的配列#3	CCTGGAGGCTTGCTGAAGGCTGTATGCTGAAGAATGGCTC CAACAATGACGTTTTGGCCACTGACTGACGTCATTGTGAGC CATTCTTCAGGACACAAGGCCTGTTACTAGCACTCA

68	miR155 CD47 標的配列#4	CCTGGAGGCTTGCTGAAGGCTGTATGCTGTATACACGCCGC AATACAGAGGTTTTGGCCACTGACTGACCTCTGTATCGGCG

		TGTATACAGGACACAAGGCCTGTTACTAGCACTCA
69	フォワードプライマー	GGACTATCCTGCTGCCAA
70	miR155 cMyc 配列	CCTGGAGGCTTGCTGAAGGCTGTATGCTGTGTTCGCCTCTT GACATTCTCTTTTGGCCACTGACTGAGAGAATGTAGAGGCG AACACAGGACACAAGGCCTGTTACTAGCACTCA
71	cMyc 標的配列	GAGAATGTCAAGAGGCGAACA
72	CMV プロモーター配列	ATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGG CAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGAT GCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTG ACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAAT GGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAA ATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGT AGGCGTGTACGGTGGGAGGTTTATATAAGCAGAGCTCGTT TAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTG TTTT
73	GFP T2A ルシフェラーゼ配列	ATGCCCGCCATGAAGATCGAGTGCCGCATCACCGGCACCC TGAACGGCGTGGAGTTCGAGCTGGTGGGCGGCGGAGAGGG CACCCCCGAGCAGGGCCGCATGACCAACAAGATGAAGAGC ACCAAAGGCGCCCTGACCTTCAGCCCCTACCTGCTGAGCCA CGTGATGGGCTACGGCTTCTACCACTTCGGCACCTACCCCA GCGGCTACGAGAACCCCTTCCTGCACGCCATCAACAACGG CGGCTACACCAACACCCGCATCGAGAAGTACGAGGACGGC GGCGTGCTGCACGTGAGCTTCAGCTACCGCTACGAGGCCG GCCGCGTGATCGGCGACTTCAAGGTGGTGGGCACCGGCTT CCCCGAGGACAGCGTGATCTTCACCGACAAGATCATCCGC AGCAACGCCACCGTGGAGCACCTGCACCCCATGGGCGATA ACGTGCTGGTGGGCAGCTTCGCCCGCACCTTCAGCCTGCGC GACGGCGGCTACTACAGCTTCGTGGTGGACAGCCACATGC ACTTCAAGAGCGCCATCCACCCCAGCATCCTGCAGAACGG GGGCCCCATGTTCGCCTTCCGCCGCGTGGAGGAGCTGCAC AGCAACACCGAGCTGGGCATCGTGGAGTACCAGCACGCCT TCAAGACCCCCATCGCCTTCGCCAGATCTCGAGATATCAGC

		CATGGCTTCCCGCCGGCGGTGGCGGCGCAGGATGATGGCA

		CGCTGCCCATGTCTTGTGCCCAGGAGAGCGGGATGGACCG TCACCCTGCAGCCTGTGCTTCTGCTAGGATCAATGTGACCG GTGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGA GGAGAATCCCGGCCCTTCCGGTATGGAAGACGCCAAAAAC ATAAAGAAAGGCCCGGCGCCATTCTATCCGCTAGAGGATG GAACCGCTGGAGAGCAACTGCATAAGGCTATGAAGAGATA CGCCCTGGTTCCTGGAACAATTGCTTTTACAGATGCACATA TCGAGGTGAACATCACGTACGCGGAATACTTCGAAATGTC CGTTCGGTTGGCAGAAGCTATGAAACGATATGGGCTGAAT ACAAATCACAGAATCGTCGTATGCAGTGAAAACTCTCTTCA ATTCTTTATGCCGGTGTTGGGCGCGTTATTTATCGGAGTTG CAGTTGCGCCCGCGAACGACATTTATAATGAACGTGAATT GCTCAACAGTATGAACATTTCGCAGCCTACCGTAGTGTTTG TTTCCAAAAAGGGGTTGCAAAAAATTTTGAACGTGCAAAA AAAATTACCAATAATCCAGAAAATTATTATCATGGATTCTA AAACGGATTACCAGGGATTTCAGTCGATGTACACGTTCGTC ACATCTCATCTACCTCCCGGTTTTAATGAATACGATTTTGT ACCAGAGTCCTTTGATCGTGACAAAACAATTGCACTGATA ATGAACTCCTCTGGATCTACTGGGTTACCTAAGGGTGTGGC CCTTCCGCATAGAACTGCCTGCGTCAGATTCTCGCATGCCA GAGATCCTATTTTTGGCAATCAAATCATTCCGGATACTGCG ATTTTAAGTGTTGTTCCATTCCATCACGGTTTTGGAATGTTT ACTACACTCGGATATTTGATATGTGGATTTCGAGTCGTCTT AATGTATAGATTTGAAGAAGAGCTGTTTTTACGATCCCTTC AGGATTACAAAATTCAAAGTGCGTTGCTAGTACCAACCCT ATTTTCATTCTTCGCCAAAAGCACTCTGATTGACAAATACG ATTTATCTAATTTACACGAAATTGCTTCTGGGGGCGCACCT CTTTCGAAAGAAGTCGGGGAAGCGGTTGCAAAACGCTTCC ATCTTCCAGGGATACGACAAGGATATGGGCTCACTGAGAC TACATCAGCTATTCTGATTACACCCGAGGGGGATGATAAA CCGGGCGCGGTCGGTAAAGTTGTTCCATTTTTTGAAGCGAA GGTTGTGGATCTGGATACCGGGAAAACGCTGGGCGTTAAT CAGAGAGGCGAATTATGTGTCAGAGGACCTATGATTATGT CCGGTTATGTAAACAATCCGGAAGCGACCAACGCCTTGAT TGACAAGGATGGATGGCTACATTCTGGAGACATAGCTTAC

		TGGGACGAAGACGAACACTTCTTCATAGTTGACCGCTTGA

		AGTCTTTAATTAAATACAAAGGATACCAGGTGGCCCCCGCT GAATTGGAGTCGATATTGTTACAACACCCCAACATCTTCGA CGCGGGCGTGGCAGGTCTTCCCGACGATGACGCCGGTGAA CTTCCCGCCGCCGTTGTTGTTTTGGAGCACGGAAAGACGAT GACGGAAAAAGAGATCGTGGATTACGTCGCCAGTCAAGTA ACAACCGCGAAAAAGTTGCGCGGAGGAGTTGTGTTTGTGG ACGAAGTACCGAAAGGTCTTACCGGAAAACTCGACGCAAG AAAAATCAGAGAGATCCTCATAAAGGCCAAGAAGGGCGG AAAGTCCAAATTGTAA
74	ラウス肉腫ウイルス(RSV)プロモーター	GTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTAA CGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAAGCA CCGTGCATGCCGATTGGTGGAAGTAAGGTGGTACGATCGT GCCTTATTAGGAAGGCAACAGACGGGTCTGACATGGATTG GACGAACCACTGAATTGCCGCATTGCAGAGATATTGTATTT AAGTGCCTAGCTCGATACAATAAACG
75	5'末端反復配列(LTR)	GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCT GGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCT TGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGT GACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGT GTGGAAAATCTCTAGCA
76	プサイパッケージングシグナル	TACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGA G
77	Rev 応答エレメント(RRE)	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGC ACTATGGGCGCAGCCTCAATGACGCTGACGGTACAGGCCA GACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTG CTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCA CAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGC TGTGGAAAGATACCTAAAGGATCAACAGCTCC
78	中央ポリプリントラクト(cPPT)	TTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGG GAAAGAATAGTAGACATAATAGCAACAGACATACAAACTA AAGAATTACAAAAACAAATTACAAAATTCAAAATTTTA
79	長鎖 WPRE 配列	AATCAACCTCTGATTACAAAATTTGTGAAAGATTGACTGGT ATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCT

		GCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCT

		TTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTT TATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGG TGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGC ATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTC CCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCT TGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGAC AATTCCGTGGTGTTGTCGGGGAAATCATCGTCCTTTCCTTG GCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGT CCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTT CCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCG TCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGG CCGCCTCCCCGCCT
80	3'デルタ LTR	TGGAAGGGCTAATTCACTCCCAACGAAGATAAGATCTGCT TTTTGCTTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAG CCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAG CCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGC CCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGAC CCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTAGTAGTTCA TGTCA
81	エンベロープ; MLV 10A1	ATGGAAGGTCCAGCGTTCTCAAAACCCCTTAAAGATAAGA TTAACCCGTGGAAGTCCTTAATGGTCATGGGGGTCTATTTA AGAGTAGGGATGGCAGAGAGCCCCCATCAGGTCTTTAATG TAACCTGGAGAGTCACCAACCTGATGACTGGGCGTACCGC CAATGCCACCTCCCTTTTAGGAACTGTACAAGATGCCTTCC CAAGATTATATTTTGATCTATGTGATCTGGTCGGAGAAGAG TGGGACCCTTCAGACCAGGAACCATATGTCGGGTATGGCT GCAAATACCCCGGAGGGAGAAAGCGGACCCGGACTTTTGA CTTTTACGTGTGCCCTGGGCATACCGTAAAATCGGGGTGTG GGGGGCCAAGAGAGGGCTACTGTGGTGAATGGGGTTGTGA AACCACCGGACAGGCTTACTGGAAGCCCACATCATCATGG GACCTAATCTCCCTTAAGCGCGGTAACACCCCCTGGGACAC GGGATGCTCCAAAATGGCTTGTGGCCCCTGCTACGACCTCT CCAAAGTATCCAATTCCTTCCAAGGGGCTACTCGAGGGGG CAGATGCAACCCTCTAGTCCTAGAATTCACTGATGCAGGA AAAAAGGCTAATTGGGACGGGCCCAAATCGTGGGGACTGA

		AAAAAGGCTAATTGGGACGGGCCCAATACGTGCGGACTGA
		GACTGTACCGGACAGGAACAGATCCTATTACCATGTTCTCC

		CTGACCCGCCAGGTCCTCAATATAGGGCCCCGCATCCCCAT TGGGCCTAATCCCGTGATCACTGGTCAACTACCCCCCTCCC GACCCGTGCAGATCAGGCTCCCCAGGCCTCCTCAGCCTCCT CCTACAGGCGCAGCCTCTATAGTCCCTGAGACTGCCCCACC TTCTCAACAACCTGGGACGGGAGACAGGCTGCTAAACCTG GTAGAAGGAGCCTATCAGGCGCTTAACCTCACCAATCCCG ACAAGACCCAAGAATGTTGGCTGTGCTTAGTGTCGGGACC TCCTTATTACGAAGGAGTAGCGGTCGTGGGCACTTATACCA ATCATTCTACCGCCCCGGCCAGCTGTACGGCCACTTCCCAA CATAAGCTTACCCTATCTGAAGTGACAGGACAGGGCCTAT GCATGGGAGCACTACCTAAAACTCACCAGGCCTTATGTAA CACCACCCAAAGTGCCGGCTCAGGATCCTACTACCTTGCAG CACCCGCTGGAACAATGTGGGCTTGTAGCACTGGATTGACT CCCTGCTTGTCCACCACGATGCTCAATCTAACCACAGACTA TTGTGTATTAGTTGAGCTCTGGCCCAGAATAATTTACCACT CCCCCGATTATATGTATGGTCAGCTTGAACAGCGTACCAAA TATAAGAGGGAGCCAGTATCGTTGACCCTGGCCCTTCTGCT AGGAGGATTAACCATGGGAGGGATTGCAGCTGGAATAGGG ACGGGGACCACTGCCCTAATCAAAACCCAGCAGTTTGAGC AGCTTCACGCCGCTATCCAGACAGACCTCAACGAAGTCGA AAAATCAATTACCAACCTAGAAAAGTCACTGACCTCGTTGT CTGAAGTAGTCCTACAGAACCGAAGAGGCCTAGATTTGCT CTTCCTAAAAGAGGGAGGTCTCTGCGCAGCCCTAAAAGAA GAATGTTGTTTTTATGCAGACCACACGGGACTAGTGAGAG ACAGCATGGCCAAACTAAGGGAAAGGCTTAATCAGAGACA AAAACTATTTGAGTCAGGCCAAGGTTGGTTCGAAGGGCAG TTTAATAGATCCCCCTGGTTTACCACCTTAATCTCCACCATC ATGGGACCTCTAATAGTACTCTTACTGATCTTACTCTTTGG ACCCTGCATTCTCAATCGATTGGTCCAATTTGTTAAAGACA GGATCTCAGTGGTCCAGGCTCTGGTTTTGACTCAACAATAT CACCAGCTAAAACCTATAGAGTACGAGCCATGA
82	miR155 CD47 標的配列#1	CCTGGAGGCTTGCTGAAGGCTGTATGCTGTTATCCATCTTC AAAGAGGCAGTTTTGGCCACTGACTGACTGCCTCTTAAGAT GGATAACAGGACACAAGGCCTGTTACTAGCACTCA
83	miR21 cMyc	CATCTCCATGGCTGTACCACCTTGTCGGGTGTTCGCCTCTT

65	miR21-cMyc 配列	GACATTCTCCTGTTGAATCTCATGGAGAATGTCAAGGGCGA
		ACACTGACATTTTGGTATCTTTCATCTGACCA

Sequences The following sequences are referred to herein:

配列番号	説明	配列
1	FDPS shRNA 配列#1	GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACT CCAGGACTTTTT
2	FDPS shRNA 配列#2	GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGA AATCCTGCTTTTT
3	FDPS shRNA 配列#3	GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGT ACATGGCTTTTT
4	FDPS shRNA 配列#4	GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCT CCTTCTGCTTTTT
5	CD47 shRNA 配列#1	GGTGAAACGATCATCGAGCCTCGAGGCTCGATGATCGTTTC ACCTTTTT
6	CD47 shRNA 配列#2	GCTACTGGCCTTGGTTTAACTCGAGTTAAACCAAGGCCAGT AGCTTTTT
7	CD47 shRNA 配列#3	CCTCCTTCGTCATTGCCATCTCGAGATGGCAATGACGAAGG AGGTTTTT
8	CD47 shRNA 配列#4	GCATGGCCCTCTTCTGATTCTCGAGAATCAGAAGAGGGCC ATGCTTTTT
9	CD47 shRNA 配列#5	GGTGAAACGATCATCGAGCTACTCGAGTAGCTCGATGATC GTTTCACCTTTTT
10	cMyc shRNA 配列#1	GCTTCACCAACAGGAACTATGCTCGAGCATAGTTCCTGTTG GTGAAGCTTTT
11	cMyc shRNA 配列#2	GCGAACACACAACGTCTTGGACTCGAGTCCAAGACGTTGT GTGTTCGCTTTT
12	cMyc shRNA 配列#3	GACATGGTGAACCAGAGTTTCCTCGAGGAAACTCTGGTTC ACCATGTCTTTTT
13	cMyc shRNA 配列#4	GAGAATGTCAAGAGGCGAACACTCGAGTGTTCGCCTCTTG ACATTCTCTTTTT
14	cMyc shRNA 配列#5	GCTCATTTCTGAAGAGGACTTCTCGAGAAGTCCTCTTCAGA AATGAGCTTTTT
15	H1 プロモーター	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGC CCAGTGTCACTAGGCGGGAACACCCAGCGCGCGTGCGCCC TGGCAGGAAGATGGCTGTGAGGGACAGGGGAGTGGCGCCC

		TGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCA TAAACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGTTC TGTATGAGACCACTT
16	U6プロモーター	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACG ATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACT GTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAA AGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGT TTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTA TTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAA ACACC
17	7SKプロモーター	CTGCAGTATTTAGCATGCCCCACCCATCTGCAAGGCATTCT GGATAGTGTCAAAACAGCCGGAAATCAAGTCCGTTTATCT CAAACTTTAGCATTTTGGGAATAAATGATATTTGCTATGCT GGTTAAATTAGATTTTAGTTAAATTTCCTGCTGAAGCTCTA GTACGATAAGCAACTTGACCTAAGTGTAAAGTTGAGATTTC CTTCAGGTTTATATAGCTTGTGCGCCGCCTGGCTACCTC
18	CAGエンハンサー	TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAAT GGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGA CGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGG GACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAA CTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTG GCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTT GGCAGTACATCTACGTATTAGTCATC
19	CAGプロモーター	GCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCA CTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATT TATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGG GGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGA GGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGC CAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGA GGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGC GGCGGGCG
20	ニワトリベー	GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGC

	ダアクチンイントロン	CGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTAC TCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGG

		CTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCT GTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCT TTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGT GTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGG CGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCG CTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGC CCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGC GTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGG GCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCC CCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTC CGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGG GGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGC CTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCC CCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGC CATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACT TCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGC GCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGC GGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGT GCGTCGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCG GGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGG GCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGG
21	HIV gag	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAG ATCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAGA AAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGA GCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACAT CAGAAGGCTGTAGACAAATACTGGGACAGCTACAACCATC CCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAAT ACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGA TAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAG AGCAAAACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAG CTGACACAGGACACAGCAATCAGGTCAGCCAAAATTACCC TATAGTGCAGAACATCCAGGGGCAAATGGTACATCAGGCC ATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAG AAGAGAAGGCTTTCAGCCCAGAAGTGATACCCATGTTTTC AGCATTATCAGAAGGAGCCACCCCACAAGATTTAAACACC

		ATGCTAAACACAGTGGGGGGACATCAAGCAGCCATGCAAA

		TGTTAAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGA TAGAGTGCATCCAGTGCATGCAGGGCCTATTGCACCAGGC CAGATGAGAGAACCAAGGGGAAGTGACATAGCAGGAACT ACTAGTACCCTTCAGGAACAAATAGGATGGATGACACATA ATCCACCTATCCCAGTAGGAGAAATCTATAAAAGATGGAT AATCCTGGGATTAAATAAAATAGTAAGAATGTATAGCCCT ACCAGCATTCTGGACATAAGACAAGGACCAAAGGAACCCT TTAGAGACTATGTAGACCGATTCTATAAAACTCTAAGAGCC GAGCAAGCTTCACAAGAGGTAAAAAATTGGATGACAGAAA CCTTGTTGGTCCAAAATGCGAACCCAGATTGTAAGACTATT TTAAAAGCATTGGGACCAGGAGCGACACTAGAAGAAATGA TGACAGCATGTCAGGGAGTGGGGGGACCCGGCCATAAAGC AAGAGTTTTGGCTGAAGCAATGAGCCAAGTAACAAATCCA GCTACCATAATGATACAGAAAGGCAATTTTAGGAACCAAA GAAAGACTGTTAAGTGTTTCAATTGTGGCAAAGAAGGGCA CATAGCCAAAAATTGCAGGGCCCCTAGGAAAAAGGGCTGT TGGAAATGTGGAAAGGAAGGACACCAAATGAAAGATTGTA CTGAGAGACAGGCTAATTTTTTTAGGGAAGATCTGGCCTTCC CACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAG AGCCAACAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGA AGAGACAACAACTCCCTCTCAGAAGCAGGAGCCGATAGAC AAGGAACTGTATCCTTTAGCTTCCCTCAGATCACTCTTTGG CAGCGACCCCTCGTCACAATAA
22	HIV Pol	ATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGG GAATTGGAGGTTTTATCAAAGTAGGACAGTATGATCAGAT ACTCATAGAAATCTGCGGACATAAAGCTATAGGTACAGTA TTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAATCT GTTGACTCAGATTGGCTGCACTTTAAATTTTCCCATTAGTC CTATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGA TGGCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAA ATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGG AAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAA TACTCCAGTATTTGCCATAAAGAAAAAAGACAGTACTAAA TGGAGAAAATTAGTAGATTTCAGAGAACTTAATAAGAGAA CTCAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCT

		GCAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATG

		TGGGCGATGCATATTTTTCAGTTCCCTTAGATAAAGACTTC AGGAAGTATACTGCATTTACCATACCTAGTATAAACAATG AGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACA GGGATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATG ACAAAAATCTTAGAGCCTTTTAGAAAACAAAATCCAGACA TAGTCATCTATCAATACATGGATGATTTGTATGTAGGATCT GACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAA CTGAGACAACATCTGTTGAGGTGGGGATTTACCACACCAG ACAAAAAACATCAGAAAGAACCTCCATTCCTTTGGATGGG TTATGAACTCCATCCTGATAAATGGACAGTACAGCCTATAG TGCTGCCAGAAAAGGACAGCTGGACTGTCAATGACATACA GAAATTAGTGGGAAAATTGAATTGGGCAAGTCAGATTTAT GCAGGGATTAAAGTAAGGCAATTATGTAAACTTCTTAGGG GAACCAAAGCACTAACAGAAGTAGTACCACTAACAGAAGA AGCAGAGCTAGAACTGGCAGAAAACAGGGAGATTCTAAA AGAACCGGTACATGGAGTGTATTATGACCCATCAAAAGAC TTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGG ACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAA CAGGAAAATATGCAAGAATGAAGGGTGCCCACACTAATGA TGTGAAACAATTAACAGAGGCAGTACAAAAAATAGCCACA GAAAGCATAGTAATATGGGGAAAGACTCCTAAATTTAAAT TACCCATACAAAAGGAAACATGGGAAGCATGGTGGACAGA GTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGTTTGTCA ATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAA GAACCCATAATAGGAGCAGAAACTTTCTATGTAGATGGGG CAGCCAATAGGGAAACTAAATTAGGAAAAGCAGGATATGT AACTGACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGAC ACAACAAATCAGAAGACTGAGTTACAAGCAATTCATCTAG CTTTGCAGGATTCGGGATTAGAAGTAAACATAGTGACAGA CTCACAATATGCATTGGGAATCATTCAAGCACAACCAGAT AAGAGTGAATCAGAGTTAGTCAGTCAAATAATAGAGCAGT TAATAAAAAAGGAAAAAGTCTACCTGGCATGGGTACCAGC ACACAAAGGAATTGGAGGAAATGAACAAGTAGATGGGTTG GTCAGTGCTGGAATCAGGAAAGTACTA
23	HIV Int	TTTTTAGATGGAATAGATAAGGCCCAAGAAGAACATGAGA

25	HIV-1m	TTTTATCATCAATTATCATATAGCTGTCAACATCATTCATTGTC AATATCACAGTAATTGGAGAGCAATGGCTAGTGATTTTAA

		CCTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGT GATAAATGTCAGCTAAAAGGGGAAGCCATGCATGGACAAG TAGACTGTAGCCCAGGAATATGGCAGCTAGATTGTACACA TTTAGAAGGAAAAGTTATCTTGGTAGCAGTTCATGTAGCCA GTGGATATATAGAAGCAGAAGTAATTCCAGCAGAGACAGG GCAAGAAACAGCATACTTCCTCTTAAAATTAGCAGGAAGA TGGCCAGTAAAAACAGTACATACAGACAATGGCAGCAATT TCACCAGTACTACAGTTAAGGCCGCCTGTTGGTGGGCGGG GATCAAGCAGGAATTTGGCATTCCCTACAATCCCCAAAGTC AAGGAGTAATAGAATCTATGAATAAAGAATTAAAGAAAAT TATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACA GCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGAA AAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAG TAGACATAATAGCAACAGACATACAAACTAAAGAATTACA AAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACA GGGACAGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGCT CCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAAT AGTGACATAAAAGTAGTGCCAAGAAGAAAAGCAAAGATC ATCAGGGATTATGGAAAACAGATGGCAGGTGATGATTGTG TGGCAAGTAGACAGGATGAGGATTAA
24	HIV RRE	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGC ACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCA GACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTG CTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCA CAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGC TGTGGAAAGATACCTAAAGGATCAACAGCTCCT
25	HIV Rev	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAACTCCTC AAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCC ACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAAT AGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCAT TCGATTAGTGAACGGATCCTTAGCACTTATCTGGGACGATC TGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGAC TTACTCTTGATTGTAACGAGGATTGTGGAACTTCTGGGACG CAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTA CAATATTGGAGTCAGGAGCTAAAGAATAG

		CAATATTGGAGTCAGGAGCTAAAGAATAG
26	ウサギベータ	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGA

	グロビンポリA	AGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATC
27	CMVプロモーター	ACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
28	ベータグロビンイントロン	GTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTCGCTATTGTAAAATTCATGTTATATGGAGGGGGCAAAGTTTTCAGGGTGTTGTTTAGAATGGGAAGATGTCCCTTGTATCACCATGGACCCTCATGATAATTTTGTTTCTTTCACTTTCTACTCTGTTGACAACCATTGTCTCCTCTTATTTTCTTTTCATTTTCTGTAACTTTTTCGTTAAACTTTAGCTTGCATTTGTAACGAATTTTTAAATTCACTTTTGTTTATTTGTCAGATTGTAAGTACTTTCTCTAATCACTTTTTTTTCAAGGCAATCAGGGTATATTATATTGTACTTCAGCACAGTTTTAGAGAACAATTGTTATAATTAAATGATAAGGTAGAATATTTCTGCATATAAATTCTGGCTGGCGTGGAAA

		GCTAGAATATTTCTGCATATAAATTCTGGCTGGCGTGAAA
		TATTCTTATTGGTAGAAACAACTACACCCTGGTCATCATCC

		TGCCTTTCTCTTTATGGTTACAATGATATACACTGTTTGAGA TGAGGATAAAATACTCTGAGTCCAAACCGGGCCCCTCTGCT AACCATGTTCATGCCTTCTTCTCTTTCCTACAG
29	VSV-G/VSV-G を含有する DNA 断片	GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATT GGGGTGAATTGCAAGTTCACCATAGTTTTTCCACACAACCA AAAAGGAAACTGGAAAAATGTTCCTTCTAATTACCATTATT GCCCGTCAAGCTCAGATTTAAATTGGCATAATGACTTAATA GGCACAGCCTTACAAGTCAAAATGCCCAAGAGTCACAAGG CTATTCAAGCAGACGGTTGGATGTGTCATGCTTCCAAATGG GTCACTACTTGTGATTTCCGCTGGTATGGACCGAAGTATAT AACACATTCCATCCGATCCTTCACTCCATCTGTAGAACAAT GCAAGGAAAGCATTGAACAAACGAAACAAGGAACTTGGCT GAATCCAGGCTTCCCTCCTCAAAGTTGTGGATATGCAACTG TGACGGATGCCGAAGCAGTGATTGTCCAGGTGACTCCTCA CCATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGAT TCACAGTTCATCAACGGAAAATGCAGCAATTACATATGCC CCACTGTCCATAACTCTACAACCTGGCATTCTGACTATAAG GTCAAAGGGCTATGTGATTCTAACCTCATTTCCATGGACAT CACCTTCTTCTCAGAGGACGGAGAGCTATCATCCCTGGGAA AGGAGGGCACAGGGTTCAGAAGTAACTACTTTGCTTATGA AACTGGAGGCAAGGCCTGCAAAATGCAATACTGCAAGCAT TGGGGAGTCAGACTCCCATCAGGTGTCTGGTTCGAGATGG CTGATAAGGATCTCTTTGCTGCAGCCAGATTCCCTGAATGC CCAGAAGGGTCAAGTATCTCTGCTCCATCTCAGACCTCAGT GGATGTAAGTCTAATTCAGGACGTTGAGAGGATCTTGGATT ATTCCCTCTGCCAAGAAACCTGGAGCAAAATCAGAGCGGG TCTTCCAATCTCTCCAGTGGATCTCAGCTATCTTGCTCCTAA AAACCCAGGAACCGGTCCTGCTTTCACCATAATCAATGGTA CCCTAAAATACTTTGAGACCAGATACATCAGAGTCGATATT GCTGCTCCAATCCTCTCAAGAATGGTCGGAATGATCAGTGG AACTACCACAGAAAGGGAACTGTGGGATGACTGGGCACCA TATGAAGACGTGGAAATTGGACCCAATGGAGTTCTGAGGA CCAGTTCAGGATATAAGTTTCCTTTATACATGATTGGACAT GGTATGTTGGACTCCGATCTTCATCTTAGCTCAAAGGCTCA GGTGTTCGAACATCCTCACATTCAAGACGCTGCTTCGCAAC

		TTCCTGATGATGAGAGTTTATTTTTTGGTGATACTGGGCTA

		TCCAAAAATCCAATCGAGCTTGTAGAAGGTTGGTTCAGTA GTTGGAAAAGCTCTATTGCCTCTTTTTTCTTTATCATAGGGT TAATCATTGGACTATTCTTGGTTCTCCGAGTTGGTATCCATC TTTGCATTAAATTAAAGCACACCAAGAAAAGACAGATTTA TACAGACATAGAGATGAGAATTC
30	ウサギベータグロビンポリA	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGA AGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTT ATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCA CTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATC AGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCCAT ATGCTGGCTGCCATGAACAAAGGTTGGCTATAAAGAGGTC ATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTC CATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATT TTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCC TTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTA CTCCCAGTCATAGCTGTCCCTCTTCTCTTATGGAGATC
31	プライマー	TAAGCAGAATTCATGAATTTGCCAGGAAGAT
32	プライマー	CCATACAATGAATGGACACTAGGCGGCCGCACGAAT
33	Gag、Pol、インテグラーゼの断片	GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGA TAGGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGA TCAGATACTCATAGAAATCTGCGGACATAAAGCTATAGGT ACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAA GAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCCC ATTAGTCCTATTGAGACTGTACCAGTAAAATTAAAGCCAG GAATGGATGGCCCAAAAGTTAAACAATGGCCATTGACAGA AGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATG GAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATC CATACAATACTCCAGTATTTGCCATAAAGAAAAAAGACAG TACTAAATGGAGAAAATTAGTAGATTTCAGAGAACTTAAT AAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGGAATAC CACATCCTGCAGGGTTAAAACAGAAAAAATCAGTAACAGT ACTGGATGTGGGCGATGCATATTTTTCAGTTCCCTTAGATA AAGACTTCAGGAAGTATACTGCATTTACCATACCTAGTATA AACAATGAGACACCAGGGATTAGATATCAGTACAATGTGC

		TTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGTG TAGCATGACAAAAATCTTAGAGCCTTTTAGAAAACAAAAT

		CCAGACATAGTCATCTATCAATACATGGATGATTTGTATGT AGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATA GAGGAACTGAGACAACATCTGTTGAGGTGGGGATTTACCA CACCAGACAAAAAACATCAGAAAGAACCTCCATTCCTTTG GATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAG CCTATAGTGCTGCCAGAAAAGGACAGCTGGACTGTCAATG ACATACAGAAATTAGTGGGAAAATTGAATTGGGCAAGTCA GATTTATGCAGGGATTAAAGTAAGGCAATTATGTAAACTTC TTAGGGGAACCAAAGCACTAACAGAAGTAGTACCACTAAC AGAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGGAGAT TCTAAAAGAACCGGTACATGGAGTGTATTATGACCCATCA AAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGC CAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCT GAAAACAGGAAAGTATGCAAGAATGAAGGGTGCCCACACT AATGATGTGAAACAATTAACAGAGGCAGTACAAAAAATAG CCACAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATT TAAATTACCCATACAAAAGGAAACATGGGAAGCATGGTGG ACAGAGTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGT TTGTCAATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTA GAGAAAGAACCCATAATAGGAGCAGAAACTTTCTATGTAG ATGGGGCAGCCAATAGGGAAACTAAATTAGGAAAAGCAG GATATGTAACTGACAGAGGAAGACAAAAAGTTGTCCCCCT AACGGACACAACAAATCAGAAGACTGAGTTACAAGCAATT CATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAACATAGT GACAGACTCACAATATGCATTGGGAATCATTCAAGCACAA CCAGATAAGAGTGAATCAGAGTTAGTCAGTCAAATAATAG AGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGT ACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGA TAAATTGGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAG ATGGAATAGATAAGGCCCAAGAAGAACATGAGAAATATCA CAGTAATTGGAGAGCAATGGCTAGTGATTTTAACCTACCAC CTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATG TCAGCTAAAAGGGGAAGCCATGCATGGACAAGTAGACTGT AGCCCAGGAATATGGCAGCTAGATTGTACACATTTAGAAG GAAAAGTTATCTTGGTAGCAGTTCATGTAGCCAGTGGATAT

		ATAGAAGCAGAAGTAATTCCAGCAGAGACAGGGCAAGAA

		ACAGCATACTTCCTCTTAAAATTAGCAGGAAGATGGCCAG TAAAAACAGTACATACAGACAATGGCAGCAATTTCACCAG TACTACAGTTAAGGCCGCCTGTTGGTGGGCGGGGATCAAG CAGGAATTTGGCATTCCCTACAATCCCCAAAGTCAAGGAG TAATAGAATCTATGAATAAAGAATTAAAGAAAATTATAGG ACAGGTAAGAGATCAGGCTGAACATCTTAAGACAGCAGTA CAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGG GGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACAT AATAGCAACAGACATACAAACTAAAGAATTACAAAAACAA ATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGACAG CAGAGATCCAGTTTGGAAAGGACCAGCAAAGCTCCTCTGG AAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACA TAAAAGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGG ATTATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAG TAGACAGGATGAGGATTAA
34	Rev、RRE、およびウサギベータグロビンポリ A を含有する DNA 断片	TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAG CTCATCAGAACAGTCAGACTCATCAAGCTTCTCTATCAAAG CAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGA AGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAG ATCCATTCGATTAGTGAACGGATCCTTGGCACTTATCTGGG ACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTG AGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACTTCT GGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAAT CTCCTACAATATTGGAGTCAGGAGCTAAAGAATAGAGGAG CTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATG GGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAAT TATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGG GCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTG GGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAA AGATACCTAAAGGATCAACAGCTCCTAGATCTTTTTCCCTC TGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCAT CTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAAT AGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACAT ATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTT GGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCAT

		GAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAAC

		AGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTG ACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTATTTT TTTCTTTAACATCCCTAAAATTTTCCTTACATGTTTTACTAG CCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTG TCCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAG CTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATT GTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAG CATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAA CTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTC GGGAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTC AGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCC TAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA CTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGC CTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAG GCCTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCTT ATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCAC AAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTC CAAACTCATCAATGTATCTTATCAGCGGCCGCCCCGGG
35	CAG エンハンサー/プロモーター/イントロン配列を含有する DNA 断片	ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAG TTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACG GTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCA ATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACG GTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGC CAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTC CTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACC ATGGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCA TCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTT TTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGG GGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGG GCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGA GCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCG GCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGC GGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCG

		CCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTA

		CTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGG GCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTC TGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCC TTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTG TGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCG GCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGC GCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTG CCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGC GTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGG GCGCGGCGGTCGGGCTGTAACCCCCCCCCTGCACCCCCCTCC CCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTC CGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGG GGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGC CTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCC CCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGC CATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACT TCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGC GCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGC GGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGT GCGTCGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCG GGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGG GCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGGAA TTC
36	RSV プロモーターおよびHIV Rev	CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGG GACTAGGGTGTGTTTAGGCGAAAAGCGGGGCTTCGGTTGT ACGCGGTTAGGAGTCCCCTCAGGATATAGTAGTTTCGCTTT TGCATAGGGAGGGGGAAATGTAGTCTTATGCAATACACTT GTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCT TACAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGA AGTAAGGTGGTACGATCGTGCCTTATTAGGAAGGCAACAG ACAGGTCTGACATGGATTGGACGAACCACTGAATTCCGCA TTGCAGAGATAATTGTATTTAAGTGCCTAGCTCGATACAAT AAACGCCATTTGACCATTCACCACATTGGTGTGCACCTCCA AGCTCGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGA CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGA

		CCGATCCAGCCTCCCCTCGAAGCTAGCGATTAGGCATCTCC

		TATGGCAGGAAGAAGCGGAGACAGCGACGAAGAACTCCTC AAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCC ACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAAT AGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCAT TCGATTAGTGAACGGATCCTTAGCACTTATCTGGGACGATC TGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGAC TTACTCTTGATTGTAACGAGGATTGTGGAACTTCTGGGACG CAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTA CAATATTGGAGTCAGGAGCTAAAGAATAGTCTAGA
37	伸長因子-1 アルファ(EF1-アルファ)プロモーター	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAG TGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGG GAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTT TTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCG TGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGC CCTTGCGTGCCTTGAATTACTTCCACGCCCCTGGCTGCAGT ACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGG GAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCG TGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGC GTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTT CGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTG CGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGC CAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGG CGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCG AGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGG GGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCT CGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTG GCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGC TTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCG GCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGG AAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTC CACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCT CGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGG GTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGA CTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTG GAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAG

		CCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTG

		TCGTGA
38	プロモーター; PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTG CGCAGGGACGCGGCTGCTCTGGGCGTGGTTCCGGGAAACG CAGCGGCGCCGACCCTGGGTCTCGCACATTCTTCACGTCCG TTCGCAGCGTCACCCGGATCTTCGCCGCTACCCTTGTGGGC CCCCCGGCGACGCTTCCTGCTCCGCCCCTAAGTCGGGAAGG TTCCTTGCGGTTCGCGGCGTGCCGGACGTGACAAACGGAA GCCGCACGTCTCACTAGTACCCTCGCAGACGGACAGCGCC AGGGAGCAATGGCAGCGCGCCGACCGCGATGGGCTGTGGC CAATAGCGGCTGCTCAGCAGGGCGCGCCGAGAGCAGCGGC CGGGAAGGGGCGGTGCGGGAGGCGGGGTGTGGGGCGGTA GTGTGGGCCCTGTTCCTGCCCGCGCGGTGTTCCGCATTCTG CAAGCCTCCGGAGCGCACGTCGGCAGTCGGCTCCCTCGTTG ACCGAATCACCGACCTCTCTCCCCAG
39	プロモーター; UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCTCCTCA CGGCGAGCGCTGCCACGTCAGACGAAGGGCGCAGGAGCGT TCCTGATCCTTCCGCCCGGACGCTCAGGACAGCGGCCCGCT GCTCATAAGACTCGGCCTTAGAACCCCAGTATCAGCAGAA GGACATTTTAGGACGGGACTTGGGTGACTCTAGGGCACTG GTTTTCTTTCCAGAGAGCGGAACAGGCGAGGAAAAGTAGT CCCTTCTCGGCGATTCTGCGGAGGGATCTCCGTGGGGCGGT GAACGCCGATGATTATATAAGGACGCGCCGGGTGTGGCAC AGCTAGTTCCGTCGCAGCCGGGATTTGGGTCGCGGTTCTTG TTTGTGGATCGCTGTGATCGTCACTTGGTGAGTTGCGGGCT GCTGGGCTGGCCGGGGCTTTCGTGGCCGCCGGGCCGCTCG GTGGGACGGAAGCGTGTGGAGAGACCGCCAAGGGCTGTAG TCTGGGTCCGCGAGCAAGGTTGCCCTGAACTGGGGGTTGG GGGGAGCGCACAAAATGGCGGCTGTTCCCGAGTCTTGAAT GGAAGACGCTTGTAAGGCGGGCTGTGAGGTCGTTGAAACA AGGTGGGGGGCATGGTGGGCGGCAAGAACCCAAGGTCTTG AGGCCTTCGCTAATGCGGGAAAGCTCTTATTCGGGTGAGAT GGGCTGGGGCACCATCTGGGGACCCTGACGTGAAGTTTGT CACTGACTGGAGAACTCGGGTTTGTCGTCTGGTTGCGGGGG CGGCAGTTATGCGGTGCCGTTGGGCAGTGCACCCGTACCTT TGGGAGCGCGCGCCTCGTCGTGTCGTGACGTCACCCGTTCT

		GTTGGCTTATAATGCAGGGTGGGGCCACCTGCCGGTAGGT

		GTGCGGTAGGCTTTTCTCCGTCGCAGGACGCAGGGTTCGGG CCTAGGGTAGGCTCTCCTGAATCGACAGGCGCCGGACCTCT GGTGAGGGGAGGGATAAGTGAGGCGTCAGTTTCTTTGGTC GGTTTTATGTACCTATCTTCTTAAGTAGCTGAAGCTCCGGT TTTGAACTATGCGCTCGGGGTTGGCGAGTGTGTTTTGTGAA GTTTTTTAGGCACCTTTTGAAATGTAATCATTTGGGTCAAT ATGTAATTTTCAGTGTTAGACTAGTAAA
40	ポリ A; SV40	GTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCA TCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCT AGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCA
41	ポリ A; bGH	GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTC CCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTG TCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTG AGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGG ACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATG CTGGGGATGCGGTGGGCTCTATGG
42	エンベロープ; RD114	ATGAAACTCCCAACAGGAATGGTCATTTTATGTAGCCTAAT AATAGTTCGGGCAGGGTTTGACGACCCCCGCAAGGCTATC GCATTAGTACAAAAACAACATGGTAAACCATGCGAATGCA GCGGAGGGCAGGTATCCGAGGCCCCACCGAACTCCATCCA ACAGGTAACTTGCCCAGGCAAGACGGCCTACTTAATGACC AACCAAAAATGGAAATGCAGAGTCACTCCAAAAAATCTCA CCCCTAGCGGGGGAGAACTCCAGAACTGCCCCTGTAACAC TTTCCAGGACTCGATGCACAGTTCTTGTTATACTGAATACC GGCAATGCAGGGCGAATAATAAGACATACTACACGGCCAC CTTGCTTAAAATACGGTCTGGGAGCCTCAACGAGGTACAG ATATTACAAAACCCCAATCAGCTCCTACAGTCCCCTTGTAG GGGCTCTATAAATCAGCCCGTTTGCTGGAGTGCCACAGCCC CCATCCATATCTCCGATGGTGGAGGACCCCTCGATACTAAG AGAGTGTGGACAGTCCAAAAAAGGCTAGAACAAATTCATA AGGCTATGCATCCTGAACTTCAATACCACCCCTTAGCCCTG CCCAAAGTCAGAGATGACCTTAGCCTTGATGCACGGACTTT TGATATCCTGAATACCACTTTTAGGTTACTCCAGATGTCCA ATTTTAGCCTTGCCCAAGATTGTTGGCTCTGTTTAAAACTA GGTACCCCTACCCCTCTTGCGATACCCACTCCCTCTTTAAC

		CGTACCCCTACCCCTCTTGCGATACCCACTCCCTCTTTAAC
		CTACTCCCTAGCAGACTCCCTAGCGAATGCCTCCTGTCAGA

		TTATACCTCCCCTCTTGGTTCAACCGATGCAGTTCTCCAACT CGTCCTGTTTATCTTCCCCTTTCATTAACGATACGGAACAA ATAGACTTAGGTGCAGTCACCTTTACTAACTGCACCTCTGT AGCCAATGTCAGTAGTCCTTTATGTGCCCTAAACGGGTCAG TCTTCCTCTGTGGAAATAACATGGCATACACCTATTTACCC CAAAACTGGACAGGACTTTGCGTCCAAGCCTCCCTCCTCCC CGACATTGACATCATCCCGGGGGATGAGCCAGTCCCCATTC CTGCCATTGATCATTATATACATAGACCTAAACGAGCTGTA CAGTTCATCCCTTTACTAGCTGGACTGGGAATCACCGCAGC ATTCACCACCGGAGCTACAGGCCTAGGTGTCTCCGTCACCC AGTATACAAAATTATCCCATCAGTTAATATCTGATGTCCAA GTCTTATCCGGTACCATACAAGATTTACAAGACCAGGTAG ACTCGTTAGCTGAAGTAGTTCTCCAAAATAGGAGGGGACT GGACCTACTAACGGCAGAACAAGGAGGAATTTGTTTAGCC TTACAAGAAAAATGCTGTTTTTATGCTAACAAGTCAGGAAT TGTGAGAAACAAAATAAGAACCCTACAAGAAGAATTACAA AAACGCAGGGAAAGCCTGGCATCCAACCCTCTCTGGACCG GGCTGCAGGGCTTTCTTCCGTACCTCCTACCTCTCCTGGGA CCCCTACTCACCCTCCTACTCATACTAACCATTGGGCCATG CGTTTTCAATCGATTGGTCCAATTTGTTAAAGACAGGATCT CAGTGGTCCAGGCTCTGGTTTTGACTCAGCAATATCACCAG CTAAAACCCATAGAGTACGAGCCATGA
43	エンベロープ; GALV	ATGCTTCTCACCTCAAGCCCGCACCACCTTCGGCACCAGAT GAGTCCTGGGAGCTGGAAAAGACTGATCATCCTCTTAAGC TGCGTATTCGGAGACGGCAAAACGAGTCTGCAGAATAAGA ACCCCCACCAGCCTGTGACCCTCACCTGGCAGGTACTGTCC CAAACTGGGGACGTTGTCTGGGACAAAAAGGCAGTCCAGC CCCTTTGGACTTGGTGGCCCTCTCTTACACCTGATGTATGT GCCCTGGCGGCCGGTCTTGAGTCCTGGGATATCCCGGGATC CGATGTATCGTCCTCTAAAAGAGTTAGACCTCCTGATTCAG ACTATACTGCCGCTTATAAGCAAATCACCTGGGGAGCCAT AGGGTGCAGCTACCCTCGGGCTAGGACCAGGATGGCAAAT TCCCCCTTCTACGTGTGTCCCCGAGCTGGCCGAACCCATTC AGAAGCTAGGAGGTGTGGGGGGCTAGAATCCCTATACTGT AAAGAATGGAGTTGTGAGACCACGGGTACCGTTTATTGGC

		AACCCAAGTCCTCATGGGACCTCATAACTGTAAAATGGGA

		CCAAAATGTGAAATGGGAGCAAAAATTTCAAAAGTGTGAA CAAACCGGCTGGTGTAACCCCCTCAAGATAGACTTCACAG AAAAAGGAAAACTCTCCAGAGATTGGATAACGGAAAAAA CCTGGGAATTAAGGTTCTATGTATATGGACACCCAGGCATA CAGTTGACTATCCGCTTAGAGGTCACTAACATGCCGGTTGT GGCAGTGGGCCCAGACCCTGTCCTTGCGGAACAGGGACCT CCTAGCAAGCCCCTCACTCTCCCTCTCTCCCCACGGAAAGC GCCGCCCACCCCTCTACCCCCGGCGGCTAGTGAGCAAACC CCTGCGGTGCATGGAGAAACTGTTACCCTAAACTCTCCGCC TCCCACCAGTGGCGACCGACTCTTTGGCCTTGTGCAGGGGG CCTTCCTAACCTTGAATGCTACCAACCCAGGGGCCACTAAG TCTTGCTGGCTCTGTTTGGGCATGAGCCCCCCTTATTATGA AGGGATAGCCTCTTCAGGAGAGGTCGCTTATACCTCCAACC ATACCCGATGCCACTGGGGGGCCCAAGGAAAGCTTACCCT CACTGAGGTCTCCGGACTCGGGTCATGCATAGGGAAGGTG CCTCTTACCCATCAACATCTTTGCAACCAGACCTTACCCAT CAATTCCTCTAAAAACCATCAGTATCTGCTCCCCTCAAACC ATAGCTGGTGGGCCTGCAGCACTGGCCTCACCCCCTGCCTC TCCACCTCAGTTTTTAATCAGTCTAAAGACTTCTGTGTCCA GGTCCAGCTGATCCCCCGCATCTATTACCATTCTGAAGAAA CCTTGTTACAAGCCTATGACAAATCACCCCCCAGGTTTAAA AGAGAGCCTGCCTCACTTACCCTAGCTGTCTTCCTGGGGTT AGGGATTGCGGCAGGTATAGGTACTGGCTCAACCGCCCTA ATTAAAGGGCCCATAGACCTCCAGCAAGGCCTAACCAGCC TCCAAATCGCCATTGACGCTGACCTCCGGGCCCTTCAGGAC TCAATCAGCAAGCTAGAGGACTCACTGACTTCCCTATCTGA GGTAGTACTCCAAAATAGGAGAGGCCTTGACTTACTATTCC TTAAAGAAGGAGGCCTCTGCGCGGCCCTAAAAGAAGAGTG CTGTTTTTATGTAGACCACTCAGGTGCAGTACGAGACTCCA TGAAAAAACTTAAAGAAAGACTAGATAAAAGACAGTTAGA GCGCCAGAAAAACCAAAACTGGTATGAAGGGTGGTTCAAT AACTCCCCTTGGTTTACTACCCTACTATCAACCATCGCTGG GCCCCTATTGCTCCTCCTTTTGTTACTCACTCTTGGGCCCTG CATCATCAATAAATTAATCCAATTCATCAATGATAGGATAA GTGCAGTCAAAATTTTAGTCCTTAGACAGAAATATCAGACC

		CTAGATAACGAGGAAAACCTTTAA

44	エンベロープ; FUG	ATGGTTCCGCAGGTTCTTTTGTTTGTACTCCTTCTGGGTTTT TCGTTGTGTTTCGGGAAGTTCCCCATTTACACGATACCAGA CGAACTTGGTCCCTGGAGCCCTATTGACATACACCATCTCA GCTGTCCAAATAACCTGGTTGTGGAGGATGAAGGATGTAC CAACCTGTCCGAGTTCTCCTACATGGAACTCAAAGTGGGAT ACATCTCAGCCATCAAAGTGAACGGGTTCACTTGCACAGG TGTTGTGACAGAGGCAGAGACCTACACCAACTTTGTTGGTT ATGTCACAACCACATTCAAGAGAAAGCATTTCCGCCCCAC CCCAGACGCATGTAGAGCCGCGTATAACTGGAAGATGGCC GGTGACCCCAGATATGAAGAGTCCCTACACAATCCATACC CCGACTACCACTGGCTTCGAACTGTAAGAACCACCAAAGA GTCCCTCATTATCATATCCCCAAGTGTGACAGATTTGGACC CATATGACAAATCCCTTCACTCAAGGGTCTTCCCTGGCGGA AAGTGCTCAGGAATAACGGTGTCCTCTACCTACTGCTCAAC TAACCATGATTACACCATTTGGATGCCCGAGAATCCGAGA CCAAGGACACCTTGTGACATTTTTACCAATAGCAGAGGGA AGAGAGCATCCAACGGGAACAAGACTTGCGGCTTTGTGGA TGAAAGAGGCCTGTATAAGTCTCTAAAAGGAGCATGCAGG CTCAAGTTATGTGGAGTTCTTGGACTTAGACTTATGGATGG AACATGGGTCGCGATGCAAACATCAGATGAGACCAAATGG TGCCCTCCAGATCAGTTGGTGAATTTGCACGACTTTCGCTC AGACGAGATCGAGCATCTCGTTGTGGAGGAGTTAGTTAAG AAAAGAGAGGAATGTCTGGATGCATTAGAGTCCATCATGA CCACCAAGTCAGTAAGTTTCAGACGTCTCAGTCACCTGAGA AAACTTGTCCCAGGGTTTGGAAAAGCATATACCATATTCAA CAAAACCTTGATGGAGGCTGATGCTCACTACAAGTCAGTC CGGACCTGGAATGAGATCATCCCCTCAAAAGGGTGTTTGA AAGTTGGAGGAAGGTGCCATCCTCATGTGAACGGGGTGTT TTTCAATGGTATAATATTAGGGCCTGACGACCATGTCCTAA TCCCAGAGATGCAATCATCCCTCCTCCAGCAACATATGGAG TTGTTGGAATCTTCAGTTATCCCCCTGATGCACCCCCTGGC AGACCCTTCTACAGTTTTCAAAGAAGGTGATGAGGCTGAG GATTTTGTTGAAGTTCACCTCCCCGATGTGTACAAACAGAT CTCAGGGGTTGACCTGGGTCTCCCGAACTGGGGAAAGTAT GTATTGATGACTGCAGGGGCCATGATTGGCCTGGTGTTGAT

		ATTTTCCCTAATGACATGGTGCAGAGTTGGTATCCATCTTT

		GCATTAAATTAAAGCACACCAAGAAAAGACAGATTTATAC AGACATAGAGATGAACCGACTTGGAAAGTAA
45	エンベロープ; LCMV	ATGGGTCAGATTGTGACAATGTTTGAGGCTCTGCCTCACAT CATCGATGAGGTGATCAACATTGTCATTATTGTGCTTATCG TGATCACGGGTATCAAGGCTGTCTACAATTTTGCCACCTGT GGGATATTCGCATTGATCAGTTTCCTACTTCTGGCTGGCAG GTCCTGTGGCATGTACGGTCTTAAGGGACCCGACATTTACA AAGGAGTTTACCAATTTAAGTCAGTGGAGTTTGATATGTCA CATCTGAACCTGACCATGCCCAACGCATGTTCAGCCAACA ACTCCCACCATTACATCAGTATGGGGACTTCTGGACTAGAA TTGACCTTCACCAATGATTCCATCATCAGTCACAACTTTTG CAATCTGACCTCTGCCTTCAACAAAAAGACCTTTGACCACA CACTCATGAGTATAGTTTCGAGCCTACACCTCAGTATCAGA GGGAACTCCAACTATAAGGCAGTATCCTGCGACTTCAACA ATGGCATAACCATCCAATACAACTTGACATTCTCAGATCGA CAAAGTGCTCAGAGCCAGTGTAGAACCTTCAGAGGTAGAG TCCTAGATATGTTTAGAACTGCCTTCGGGGGGAAATACATG AGGAGTGGCTGGGGCTGGACAGGCTCAGATGGCAAGACCA CCTGGTGTAGCCAGACGAGTTACCAATACCTGATTATACAA AATAGAACCTGGGAAAACCACTGCACATATGCAGGTCCTT TTGGGATGTCCAGGATTCTCCTTTCCCAAGAGAAGACTAAG TTCTTCACTAGGAGACTAGCGGGCACATTCACCTGGACTTT GTCAGACTCTTCAGGGGTGGAGAATCCAGGTGGTTATTGCC TGACCAAATGGATGATTCTTGCTGCAGAGCTTAAGTGTTTC GGGAACACAGCAGTTGCGAAATGCAATGTAAATCATGATG CCGAATTCTGTGACATGCTGCGACTAATTGACTACAACAAG GCTGCTTTGAGTAAGTTCAAAGAGGACGTAGAATCTGCCTT GCACTTATTCAAAACAACAGTGAATTCTTTGATTTCAGATC AACTACTGATGAGGAACCACTTGAGAGATCTGATGGGGGT GCCATATTGCAATTACTCAAAGTTTTGGTACCTAGAACATG CAAAGACCGGCGAAACTAGTGTCCCCAAGTGCTGGCTTGT CACCAATGGTTCTTACTTAAATGAGACCCACTTCAGTGATC AAATCGAACAGGAAGCCGATAACATGATTACAGAGATGTT GAGGAAGGATTACATAAAGAGGCAGGGGAGTACCCCCCTA GCATTGATGGACCTTCTGATGTTTTCCACATCTGCATATCT

		AGTCAGCATCTTCCTGCACCTTGTCAAAATACCAACACACA

		GGCACATAAAAGGTGGCTCATGTCCAAAGCCACACCGATT AACCAACAAAGGAATTTGTAGTTGTGGTGCATTTAAGGTG CCTGGTGTAAAAACCGTCTGGAAAAGACGCTGA
46	エンベロープ; FPV	ATGAACACTCAAATCCTGGTTTTCGCCCTTGTGGCAGTCAT CCCCACAAATGCAGACAAAATTTGTCTTGGACATCATGCTG TATCAAATGGCACCAAAGTAAACACACTCACTGAGAGAGG AGTAGAAGTTGTCAATGCAACGGAAACAGTGGAGCGGACA AACATCCCCAAAATTTGCTCAAAAGGGAAAAGAACCACTG ATCTTGGCCAATGCGGACTGTTAGGGACCATTACCGGACC ACCTCAATGCGACCAATTTCTAGAATTTTCAGCTGATCTAA TAATCGAGAGACGAGAAGGAAATGATGTTTGTTACCCGGG GAAGTTTGTTAATGAAGAGGCATTGCGACAAATCCTCAGA GGATCAGGTGGGATTGACAAAGAAACAATGGGATTCACAT ATAGTGGAATAAGGACCAACGGAACAACTAGTGCATGTAG AAGATCAGGGTCTTCATTCTATGCAGAAATGGAGTGGCTCC TGTCAAATACAGACAATGCTGCTTTCCCACAAATGACAAA ATCATACAAAAACACAAGGAGAGAATCAGCTCTGATAGTC TGGGGAATCCACCATTCAGGATCAACCACCGAACAGACCA AACTATATGGGAGTGGAAATAAACTGATAACAGTCGGGAG TTCCAAATATCATCAATCTTTTGTGCCGAGTCCAGGAACAC GACCGCAGATAAATGGCCAGTCCGGACGGATTGATTTTCA TTGGTTGATCTTGGATCCCAATGATACAGTTACTTTTAGTTT CAATGGGGCTTTCATAGCTCCAAATCGTGCCAGCTTCTTGA GGGGAAAGTCCATGGGGATCCAGAGCGATGTGCAGGTTGA TGCCAATTGCGAAGGGGAATGCTACCACAGTGGAGGGACT ATAACAAGCAGATTGCCTTTTCAAAACATCAATAGCAGAG CAGTTGGCAAATGCCCAAGATATGTAAAACAGGAAAGTTT ATTATTGGCAACTGGGATGAAGAACGTTCCCGAACCTTCCA AAAAAAGGAAAAAAAGAGGCCTGTTTGGCGCTATAGCAGG GTTTATTGAAAATGGTTGGGAAGGTCTGGTCGACGGGTGG TACGGTTTCAGGCATCAGAATGCACAAGGAGAAGGAACTG CAGCAGACTACAAAAGCACCCAATCGGCAATTGATCAGAT AACCGGAAAGTTAAATAGACTCATTGAGAAAACCAACCAG CAATTTGAGCTAATAGATAATGAATTCACTGAGGTGGAAA AGCAGATTGGCAATTTAATTAACTGGACCAAAGACTCCAT

		CACAGAAGTATGGTCTTACAATGCTGAACTTCTTGTGGCAA

		TGGAAAACCAGCACACTATTGATTTGGCTGATTCAGAGAT GAACAAGCTGTATGAGCGAGTGAGGAAACAATTAAGGGA AAATGCTGAAGAGGATGGCACTGGTTGCTTTGAAATTTTTC ATAAATGTGACGATGATTGTATGGCTAGTATAAGGAACAA TACTTATGATCACAGCAAATACAGAGAAGAAGCGATGCAA AATAGAATACAAATTGACCCAGTCAAATTGAGTAGTGGCT ACAAAGATGTGATACTTTGGTTTAGCTTCGGGGCATCATGC TTTTTGCTTCTTGCCATTGCAATGGGCCTTGTTTTCATATGT GTGAAGAACGGAAACATGCGGTGCACTATTTGTATATAA
47	エンベロープ; RRV	AGTGTAACAGAGCACTTTAATGTGTATAAGGCTACTAGAC CATACCTAGCACATTGCGCCGATTGCGGGGACGGGTACTTC TGCTATAGCCCAGTTGCTATCGAGGAGATCCGAGATGAGG CGTCTGATGGCATGCTTAAGATCCAAGTCTCCGCCCAAATA GGTCTGGACAAGGCAGGCACCCACGCCCACACGAAGCTCC GATATATGGCTGGTCATGATGTTCAGGAATCTAAGAGAGA TTCCTTGAGGGTGTACACGTCCGCAGCGTGCTCCATACATG GGACGATGGGACACTTCATCGTCGCACACTGTCCACCAGG CGACTACCTCAAGGTTTCGTTCGAGGACGCAGATTCGCACG TGAAGGCATGTAAGGTCCAATACAAGCACAATCCATTGCC GGTGGGTAGAGAGAAGTTCGTGGTTAGACCACACTTTGGC GTAGAGCTGCCATGCACCTCATACCAGCTGACAACGGCTC CCACCGACGAGGAGATTGACATGCATACACCGCCAGATAT ACCGGATCGCACCCTGCTATCACAGACGGCGGGCAACGTC AAAATAACAGCAGGCGGCAGGACTATCAGGTACAACTGTA CCTGCGGCCGTGACAACGTAGGCACTACCAGTACTGACAA GACCATCAACACATGCAAGATTGACCAATGCCATGCTGCC GTCACCAGCCATGACAAATGGCAATTTACCTCTCCATTTGT TCCCAGGGCTGATCAGACAGCTAGGAAAGGCAAGGTACAC GTTCCGTTCCCTCTGACTAACGTCACCTGCCGAGTGCCGTT GGCTCGAGCGCCGGATGCCACCTATGGTAAGAAGGAGGTG ACCCTGAGATTACACCCAGATCATCCGACGCTCTTCTCCTA TAGGAGTTTAGGAGCCGAACCGCACCCGTACGAGGAATGG GTTGACAAGTTCTCTGAGCGCATCATCCCAGTGACGGAAG AAGGGATTGAGTACCAGTGGGGCAACAACCCGCCGGTCTG CCTGTGGGCGCAACTGACGACCGAGGGCAAACCCCATGGC

		TGGCCACATGAAATCATTCAGTACTATTATGGACTATACCC

		CGCCGCCACTATTGCCGCAGTATCCGGGGCGAGTCTGATG GCCCTCCTAACTCTGGCGGCCACATGCTGCATGCTGGCCAC CGCGAGGAGAAAGTGCCTAACACCGTACGCCCTGACGCCA GGAGCGGTGGTACCGTTGACACTGGGGCTGCTTTGCTGCGC ACCGAGGGCGAATGCA
48	エンベロープ; エボラ	ATGGGTGTTACAGGAATATTGCAGTTACCTCGTGATCGATT CAAGAGGACATCATTCTTTCTTTGGGTAATTATCCTTTTCCA AAGAACATTTTCCATCCCACTTGGAGTCATCCACAATAGCA CATTACAGGTTAGTGATGTCGACAAACTGGTTTGCCGTGAC AAACTGTCATCCACAAATCAATTGAGATCAGTTGGACTGA ATCTCGAAGGGAATGGAGTGGCAACTGACGTGCCATCTGC AACTAAAAGATGGGGCTTCAGGTCCGGTGTCCCACCAAAG GTGGTCAATTATGAAGCTGGTGAATGGGCTGAAAACTGCT ACAATCTTGAAATCAAAAAACCTGACGGGAGTGAGTGTCT ACCAGCAGCGCCAGACGGGATTCGGGGCTTCCCCCGGTGC CGGTATGTGCACAAAGTATCAGGAACGGGACCGTGTGCCG GAGACTTTGCCTTCCACAAAGAGGGTGCTTTCTTCCTGTAT GACCGACTTGCTTCCACAGTTATCTACCGAGGAACGACTTT CGCTGAAGGTGTCGTTGCATTTCTGATACTGCCCCAAGCTA AGAAGGACTTCTTCAGCTCACACCCCTTGAGAGAGCCGGT CAATGCAACGGAGGACCCGTCTAGTGGCTACTATTCTACCA CAATTAGATATCAAGCTACCGGTTTTGGAACCAATGAGAC AGAGTATTTGTTCGAGGTTGACAATTTGACCTACGTCCAAC TTGAATCAAGATTCACACCACAGTTTCTGCTCCAGCTGAAT GAGACAATATATACAAGTGGGAAAAGGAGCAATACCACG GGAAAACTAATTTGGAAGGTCAACCCCGAAATTGATACAA CAATCGGGGAGTGGGCCTTCTGGGAAACTAAAAAAACCTC ACTAGAAAAATTCGCAGTGAAGAGTTGTCTTTCACAGCTGT ATCAAACAGAGCCAAAAACATCAGTGGTCAGAGTCCGGCG CGAACTTCTTCCGACCCAGGGACCAACACAACAACTGAAG ACCACAAAATCATGGCTTCAGAAAATTCCTCTGCAATGGTT CAAGTGCACAGTCAAGGAAGGGAAGCTGCAGTGTCGCATC TGACAACCCTTGCCACAATCTCCACGAGTCCTCAACCCCCC ACAACCAAACCAGGTCCGGACAACAGCACCCACAATACAC CCGTGTATAAACTTGACATCTCTGAGGCAACTCAAGTTGAA

		CAACATCACCGCAGAACAGACAACGACAGCACAGCCTCCG

		ACACTCCCCCCGCCACGACCGCAGCCGGACCCCTAAAAGC AGAGAACACCAACACGAGCAAGGGTACCGACCTCCTGGAC CCCGCCACCACAACAAGTCCCCAAAACCACAGCGAGACCG CTGGCAACAACAACACTCATCACCAAGATACCGGAGAAGA GAGTGCCAGCAGCGGGAAGCTAGGCTTAATTACCAATACT ATTGCTGGAGTCGCAGGACTGATCACAGGCGGGAGGAGAG CTCGAAGAGAAGCAATTGTCAATGCTCAACCCAAATGCAA CCCTAATTTACATTACTGGACTACTCAGGATGAAGGTGCTG CAATCGGACTGGCCTGGATACCATATTTCGGGCCAGCAGC CGAGGGAATTTACATAGAGGGGCTGATGCACAATCAAGAT GGTTTAATCTGTGGGTTGAGACAGCTGGCCAACGAGACGA CTCAAGCTCTTCAACTGTTCCTGAGAGCCACAACCGAGCTA CGCACCTTTTCAATCCTCAACCGTAAGGCAATTGATTTCTT GCTGCAGCGATGGGGCGGCACATGCCACATTTTGGGACCG GACTGCTGTATCGAACCACATGATTGGACCAAGAACATAA CAGACAAAATTGATCAGATTATTCATGATTTTGTTGATAAA ACCCTTCCGGACCAGGGGGACAATGACAATTGGTGGACAG GATGGAGACAATGGATACCGGCAGGTATTGGAGTTACAGG CGTTATAATTGCAGTTATCGCTTTATTCTGTATATGCAAATT TGTCTTTTAG
49	FDPS 標的配列#1	GTCCTGGAGTACAATGCCATT
50	FDPS 標的配列#2	GCAGGATTTCGTTCAGCACTT
51	FDPS 標的配列#3	GCCATGTACATGGCAGGAATT
52	FDPS 標的配列#4	GCAGAAGGAGGCTGAGAAAGT
53	miR30 FDPS 配列#1	AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCC TCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAG AAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT
54	miR30 FDPS 配列#2	AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCC TCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAA

		AGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT

55	miR30 FDPS 配列#3	TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGT GAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTA CTGCCTCGGA
56	miR155 FDPS 配列#1	CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCC TCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGGCTGAGA AAGTCAGGACACAAGGCCTGTTACTAGCACTCA
57	miR21 FDPS 配列#1	CATCTCCATGGCTGTACCACCTTGTCGGGACTTTCTCAGCC TCCTTCTGCCTGTTGAATCTCATGGCAGAAGGAGGCGAGA AAGTCTGACATTTTGGTATCTTTCATCTGACCA
58	miR185 FDPS 配列#1	GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGC CTCCTTCTGCTGGTCCCCTCCCCGCAGAAGGAGGCTGAGAA AGTCCTTCCCTCCCAATGACCGCGTCTTCGTCG
59	フォワードプライマー	AGGAATTGATGGCGAGAAGG
60	リバースプライマー	CCCAAAGAGGTCAAGGTAATCA
61	フォワードプライマー	AGCGCGGCTACAGCTTCA
62	リバースプライマー	GGCGACGTAGCACAGCTTCT
63	フォワードプライマー	CACTGTCGTCATTCCATGCT
64	リバースプライマー	GCCTCTTGACATTCTCCTC
65	リバースプライマー	AAAGTCAGTGGGGACAGTGG
66	miR155 CD47 標的配列#2	CCTGGAGGCTTGCTGAAGGCTGTATGCTGTTAGCTCGATGA TCGTTTCACGTTTTGGCCACTGACTGACGTGAAACGCATCG AGCTAACAGGACACAAGGCCTGTTACTAGCACTCA
67	miR155 CD47 標的配列#3	CCTGGAGGCTTGCTGAAGGCTGTATGCTGAAGAATGGCTC CAACAATGACGTTTTGGCCACTGACTGACGTCATTGTGAGC CATTCTTCAGGACACAAGGCCTGTTACTAGCACTCA

68	miR155 CD47 標的配列#4	CCTGGAGGCTTGCTGAAGGCTGTATGCTGTATACACGCCGC AATACAGAGGTTTTGGCCACTGACTGACCTCTGTATCGGCG

		TGTATACAGGACACAAGGCCTGTTACTAGCACTCA
69	フォワードプライマー	GGACTATCCTGCTGCCAA
70	miR155 cMyc 配列	CCTGGAGGCTTGCTGAAGGCTGTATGCTGTGTTCGCCTCTT GACATTCTCTTTTGGCCACTGACTGAGAGAATGTAGAGGCG AACACAGGACACAAGGCCTGTTACTAGCACTCA
71	cMyc 標的配列	GAGAATGTCAAGAGGCGAACA
72	CMV プロモーター配列	ATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGG CAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGAT GCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTG ACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAAT GGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAA ATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGT AGGCGTGTACGGTGGGAGGTTTATATAAGCAGAGCTCGTT TAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTG TTTT
73	GFP T2A ルシフェラーゼ配列	ATGCCCGCCATGAAGATCGAGTGCCGCATCACCGGCACCC TGAACGGCGTGGAGTTCGAGCTGGTGGGCGGCGGAGAGGG CACCCCCGAGCAGGGCCGCATGACCAACAAGATGAAGAGC ACCAAAGGCGCCCTGACCTTCAGCCCCTACCTGCTGAGCCA CGTGATGGGCTACGGCTTCTACCACTTCGGCACCTACCCCA GCGGCTACGAGAACCCCTTCCTGCACGCCATCAACAACGG CGGCTACACCAACACCCGCATCGAGAAGTACGAGGACGGC GGCGTGCTGCACGTGAGCTTCAGCTACCGCTACGAGGCCG GCCGCGTGATCGGCGACTTCAAGGTGGTGGGCACCGGCTT CCCCGAGGACAGCGTGATCTTCACCGACAAGATCATCCGC AGCAACGCCACCGTGGAGCACCTGCACCCCATGGGCGATA ACGTGCTGGTGGGCAGCTTCGCCCGCACCTTCAGCCTGCGC GACGGCGGCTACTACAGCTTCGTGGTGGACAGCCACATGC ACTTCAAGAGCGCCATCCACCCCAGCATCCTGCAGAACGG GGGCCCCATGTTCGCCTTCCGCCGCGTGGAGGAGCTGCAC AGCAACACCGAGCTGGGCATCGTGGAGTACCAGCACGCCT TCAAGACCCCCATCGCCTTCGCCAGATCTCGAGATATCAGC

		CATGGCTTCCCGCCGGCGGTGGCGGCGCAGGATGATGGCA

		CGCTGCCCATGTCTTGTGCCCAGGAGAGCGGGATGGACCG TCACCCTGCAGCCTGTGCTTCTGCTAGGATCAATGTGACCG GTGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGA GGAGAATCCCGGCCCTTCCGGTATGGAAGACGCCAAAAAC ATAAAGAAAGGCCCGGCGCCATTCTATCCGCTAGAGGATG GAACCGCTGGAGAGCAACTGCATAAGGCTATGAAGAGATA CGCCCTGGTTCCTGGAACAATTGCTTTTACAGATGCACATA TCGAGGTGAACATCACGTACGCGGAATACTTCGAAATGTC CGTTCGGTTGGCAGAAGCTATGAAACGATATGGGCTGAAT ACAAATCACAGAATCGTCGTATGCAGTGAAAACTCTCTTCA ATTCTTTATGCCGGTGTTGGGCGCGTTATTTATCGGAGTTG CAGTTGCGCCCGCGAACGACATTTATAATGAACGTGAATT GCTCAACAGTATGAACATTTCGCAGCCTACCGTAGTGTTTG TTTCCAAAAAGGGGTTGCAAAAAATTTTGAACGTGCAAAA AAAATTACCAATAATCCAGAAAATTATTATCATGGATTCTA AAACGGATTACCAGGGATTTCAGTCGATGTACACGTTCGTC ACATCTCATCTACCTCCCGGTTTTAATGAATACGATTTTGT ACCAGAGTCCTTTGATCGTGACAAAACAATTGCACTGATA ATGAACTCCTCTGGATCTACTGGGTTACCTAAGGGTGTGGC CCTTCCGCATAGAACTGCCTGCGTCAGATTCTCGCATGCCA GAGATCCTATTTTTGGCAATCAAATCATTCCGGATACTGCG ATTTTAAGTGTTGTTCCATTCCATCACGGTTTTGGAATGTTT ACTACACTCGGATATTTGATATGTGGATTTCGAGTCGTCTT AATGTATAGATTTGAAGAAGAGCTGTTTTTACGATCCCTTC AGGATTACAAAATTCAAAGTGCGTTGCTAGTACCAACCCT ATTTTCATTCTTCGCCAAAAGCACTCTGATTGACAAATACG ATTTATCTAATTTACACGAAATTGCTTCTGGGGGCGCACCT CTTTCGAAAGAAGTCGGGGAAGCGGTTGCAAAACGCTTCC ATCTTCCAGGGATACGACAAGGATATGGGCTCACTGAGAC TACATCAGCTATTCTGATTACACCCGAGGGGGATGATAAA CCGGGCGCGGTCGGTAAAGTTGTTCCATTTTTTGAAGCGAA GGTTGTGGATCTGGATACCGGGAAAACGCTGGGCGTTAAT CAGAGAGGCGAATTATGTGTCAGAGGACCTATGATTATGT CCGGTTATGTAAACAATCCGGAAGCGACCAACGCCTTGAT TGACAAGGATGGATGGCTACATTCTGGAGACATAGCTTAC

		TGGGACGAAGACGAACACTTCTTCATAGTTGACCGCTTGA

		AGTCTTTAATTAAATACAAAGGATACCAGGTGGCCCCCGCT GAATTGGAGTCGATATTGTTACAACACCCCAACATCTTCGA CGCGGGCGTGGCAGGTCTTCCCGACGATGACGCCGGTGAA CTTCCCGCCGCCGTTGTTGTTTTGGAGCACGGAAAGACGAT GACGGAAAAAGAGATCGTGGATTACGTCGCCAGTCAAGTA ACAACCGCGAAAAAGTTGCGCGGAGGAGTTGTGTTTGTGG ACGAAGTACCGAAAGGTCTTACCGGAAAACTCGACGCAAG AAAAATCAGAGAGATCCTCATAAAGGCCAAGAAGGGCGG AAAGTCCAAATTGTAA
74	ラウス肉腫ウイルス(RSV)プロモーター	GTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTAA CGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAAGCA CCGTGCATGCCGATTGGTGGAAGTAAGGTGGTACGATCGT GCCTTATTAGGAAGGCAACAGACGGGTCTGACATGGATTG GACGAACCACTGAATTGCCGCATTGCAGAGATATTGTATTT AAGTGCCTAGCTCGATACAATAAACG
75	5'末端反復配列(LTR)	GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCT GGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCT TGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGT GACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGT GTGGAAAATCTCTAGCA
76	プサイパッケージングシグナル	TACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGA G
77	Rev 応答エレメント(RRE)	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGC ACTATGGGCGCAGCCTCAATGACGCTGACGGTACAGGCCA GACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTG CTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCA CAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGC TGTGGAAAGATACCTAAAGGATCAACAGCTCC
78	中央ポリプリントラクト(cPPT)	TTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGG GAAAGAATAGTAGACATAATAGCAACAGACATACAAACTA AAGAATTACAAAAACAAATTACAAAATTCAAAATTTTA
79	長鎖 WPRE 配列	AATCAACCTCTGATTACAAAATTTGTGAAAGATTGACTGGT ATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCT

		GCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCT

		TTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTT TATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGG TGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGC ATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTC CCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCT TGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGAC AATTCCGTGGTGTTGTCGGGGAAATCATCGTCCTTTCCTTG GCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGT CCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTT CCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCG TCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGG CCGCCTCCCCGCCT
80	3'デルタ LTR	TGGAAGGGCTAATTCACTCCCAACGAAGATAAGATCTGCT TTTTGCTTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAG CCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAG CCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGC CCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGAC CCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTAGTAGTTCA TGTCA
81	エンベロープ; MLV 10A1	ATGGAAGGTCCAGCGTTCTCAAAACCCCTTAAAGATAAGA TTAACCCGTGGAAGTCCTTAATGGTCATGGGGGTCTATTTA AGAGTAGGGATGGCAGAGAGCCCCCATCAGGTCTTTAATG TAACCTGGAGAGTCACCAACCTGATGACTGGGCGTACCGC CAATGCCACCTCCCTTTTAGGAACTGTACAAGATGCCTTCC CAAGATTATATTTTGATCTATGTGATCTGGTCGGAGAAGAG TGGGACCCTTCAGACCAGGAACCATATGTCGGGTATGGCT GCAAATACCCCGGAGGGAGAAAGCGGACCCGGACTTTTGA CTTTTACGTGTGCCCTGGGCATACCGTAAAATCGGGGTGTG GGGGGCCAAGAGAGGGCTACTGTGGTGAATGGGGTTGTGA AACCACCGGACAGGCTTACTGGAAGCCCACATCATCATGG GACCTAATCTCCCTTAAGCGCGGTAACACCCCCTGGGACAC GGGATGCTCCAAAATGGCTTGTGGCCCCTGCTACGACCTCT CCAAAGTATCCAATTCCTTCCAAGGGGCTACTCGAGGGGG CAGATGCAACCCTCTAGTCCTAGAATTCACTGATGCAGGA AAAAAGGCTAATTGGGACGGGCCCAAATCGTGGGGACTGA

		AAAAAGGCTAATTGGGACGGGCCCAATACGTGCGGACTGA
		GACTGTACCGGACAGGAACAGATCCTATTACCATGTTCTCC

		CTGACCCGCCAGGTCCTCAATATAGGGCCCCGCATCCCCAT TGGGCCTAATCCCGTGATCACTGGTCAACTACCCCCCTCCC GACCCGTGCAGATCAGGCTCCCCAGGCCTCCTCAGCCTCCT CCTACAGGCGCAGCCTCTATAGTCCCTGAGACTGCCCCACC TTCTCAACAACCTGGGACGGGAGACAGGCTGCTAAACCTG GTAGAAGGAGCCTATCAGGCGCTTAACCTCACCAATCCCG ACAAGACCCAAGAATGTTGGCTGTGCTTAGTGTCGGGACC TCCTTATTACGAAGGAGTAGCGGTCGTGGGCACTTATACCA ATCATTCTACCGCCCCGGCCAGCTGTACGGCCACTTCCCAA CATAAGCTTACCCTATCTGAAGTGACAGGACAGGGCCTAT GCATGGGAGCACTACCTAAAACTCACCAGGCCTTATGTAA CACCACCCAAAGTGCCGGCTCAGGATCCTACTACCTTGCAG CACCCGCTGGAACAATGTGGGCTTGTAGCACTGGATTGACT CCCTGCTTGTCCACCACGATGCTCAATCTAACCACAGACTA TTGTGTATTAGTTGAGCTCTGGCCCAGAATAATTTACCACT CCCCCGATTATATGTATGGTCAGCTTGAACAGCGTACCAAA TATAAGAGGGAGCCAGTATCGTTGACCCTGGCCCTTCTGCT AGGAGGATTAACCATGGGAGGGATTGCAGCTGGAATAGGG ACGGGGACCACTGCCCTAATCAAAACCCAGCAGTTTGAGC AGCTTCACGCCGCTATCCAGACAGACCTCAACGAAGTCGA AAAATCAATTACCAACCTAGAAAAGTCACTGACCTCGTTGT CTGAAGTAGTCCTACAGAACCGAAGAGGCCTAGATTTGCT CTTCCTAAAAGAGGGAGGTCTCTGCGCAGCCCTAAAAGAA GAATGTTGTTTTTATGCAGACCACACGGGACTAGTGAGAG ACAGCATGGCCAAACTAAGGGAAAGGCTTAATCAGAGACA AAAACTATTTGAGTCAGGCCAAGGTTGGTTCGAAGGGCAG TTTAATAGATCCCCCTGGTTTACCACCTTAATCTCCACCATC ATGGGACCTCTAATAGTACTCTTACTGATCTTACTCTTTGG ACCCTGCATTCTCAATCGATTGGTCCAATTTGTTAAAGACA GGATCTCAGTGGTCCAGGCTCTGGTTTTGACTCAACAATAT CACCAGCTAAAACCTATAGAGTACGAGCCATGA
82	miR155 CD47 標的配列#1	CCTGGAGGCTTGCTGAAGGCTGTATGCTGTTATCCATCTTC AAAGAGGCAGTTTTGGCCACTGACTGACTGCCTCTTAAGAT GGATAACAGGACACAAGGCCTGTTACTAGCACTCA
83	miR21 cMyc	CATCTCCATGGCTGTACCACCTTGTCGGGTGTTCGCCTCTT

	配列	GACATTCTCCTGTTGAATCTCATGGAGAATGTCAAGGGCGA

		ACACTGACATTTTGGTATCTTTCATCTGACCA

Although certain specific portions of preferred embodiments of the present invention have been described above and specifically exemplified, the present invention is not intended to be limited to such embodiments. Various modifications can be made to it without departing from the scope and spirit of the invention.

Examples of embodiments of the present invention include the following items.

(Item 1)

A viral vector comprising a therapeutic cargo moiety, wherein the therapeutic cargo moiety comprises at least one small RNA sequence capable of binding to at least one predetermined complementary mRNA sequence, said at least one complementary mRNA. A viral vector in which the sequence comprises the FDPS mRNA sequence.

(Item 2)

The therapeutic cargo moiety further comprises a second small RNA sequence capable of binding to a second predetermined complementary mRNA sequence, wherein the second predetermined complementary mRNA sequence is the mRNA of CD47. The viral vector according to item 1, which comprises a sequence or an mRNA sequence of cMyc.

(Item 3)

The viral vector according to item 2, wherein the at least one small RNA is under the control of a first promoter and the second small RNA sequence is under the control of a second promoter.

(Item 4)

The therapeutic cargo moiety further comprises a third small RNA sequence capable of binding to a third predetermined complementary mRNA sequence, wherein the third predetermined complementary mRNA sequence is the mRNA of CD47. The viral vector according to item 2, which comprises a sequence or an mRNA sequence of cMyc.

(Item 5)

Item 4. The viral vector according to item 4, wherein the third small RNA sequence is under the control of a third promoter.

(Item 6)

The viral vector according to item 4, wherein the small RNA sequence is under the control of a single promoter.

(Item 7)

The viral vector according to item 1, wherein the small RNA sequence comprises miRNA or shRNA.

(Item 8)

The small RNA sequence

GTCCTGGAGTACAATGCCATTCTCCGAGAATGGCATTGTACTCAGGACTTTTT (SEQ ID NO: 1);

GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTCTGCTTTTT (SEQ ID NO: 2);

GCCATGTACATGGCAGGGAATTCTCCGAATTCCCTGCCATGTCATGGGTTTTT (SEQ ID NO: 3); or

GCAGAAGGAGGCTGAGAAAGTTCGAGACTTTCTCAGCCTCCCTTCTGCTTTTT (SEQ ID NO: 4)

The viral vector according to item 1, which comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% identity percent with a small RNA sequence of FDPS comprising.

(Item 9)

Item 8. The viral vector according to item 8, wherein the small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, or 4.

(Item 10)

The second small RNA sequence is

GGTGAAACGATCATCGAGCCTCGAGGCTCGATGATCGTTTCACTTTTT (SEQ ID NO: 5);

GCTACTGGCCTTGGTTTAACTCGAGTTAAAACCAAGGCCAGTAGCTTTTT (SEQ ID NO: 6);

CCTCCTTCGTCATTGCCATTCGAGAGGCAATGAAGGAGGGTTTTT (SEQ ID NO: 7);

GCATGGCCCTTTCTTGATTCTCCGAGAATCAGAAGAGGGCCATTGCTTTTT (SEQ ID NO: 8); or

Small RNA sequence of CD47 containing GGTGAAAACCATCATCGAGCTACTCCGAGTAGCTCGATGATCGTTTCCACTTTTT (SEQ ID NO: 9), or
GCTTCACCAACAGGAACTATTGCTCGAGCATAGTTTCCTGTTGGTGAAGCTTTT (SEQ ID NO: 10);
GCGAACACACAAACGTCTTGGACTCGAGTCCAAAGACGTTGTGTGTGTTCGCTTTT (SEQ ID NO: 11);
GACATGGGTGAACCAGAGTTTCTCCGAGGAAACTCTGGGTTCACCATGTCTTTTT (SEQ ID NO: 12);
GAGAATGTCAAGAGGCGAACACTCGAGTGTTCGCCTTTGACATTTCTTTTT (SEQ ID NO: 13); or
GCTCATTTCTGAAGGACTTCTCGAGAAGTCCTTTCAGAAATTGAGCTTTTT (SEQ ID NO: 14)
2. The viral vector according to item 2, comprising a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% identity percent with a small RNA sequence of cMyc comprising.
(Item 11)
10. The viral vector according to item 10, wherein the second small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.
(Item 12)
The third small RNA sequence is at least a small RNA sequence of CD47 comprising SEQ ID NO: 5, 6, 7, 8 or 9 or a small RNA sequence of cMyc comprising SEQ ID NO: 10, 11, 12, 13 or 14. 4. The viral vector according to item 4, comprising a sequence having 80%, or at least 85%, or at least 90%, or at least 95% identity percent.
(Item 13)
12. The viral vector according to item 12, wherein the third small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.
(Item 14)
The virus vector according to any one of items 1 to 13, which is a lentiviral vector.
(Item 15)
Lentiviral particles that can infect target cells
a. Envelope proteins optimized to infect the target cells, and
b. The viral vector according to any one of items 1 to 14.
Including lentivirus particles.
(Item 16)
a. The lentiviral particles of item 15, and
b. Aminobisphosphonate drug
Composition containing.
(Item 17)
The composition according to item 16, wherein the aminobisphosphonate drug is zoledronic acid.
(Item 18)
A method for treating cancer in a subject, comprising administering to the subject the composition according to item 16 or 17 of a therapeutically effective amount.
(Item 19)
a. The lentiviral particles according to item 15 of the therapeutically effective amount, and
b. A therapeutically effective amount of aminobisphosphonate drug
A method of treating cancer in a subject, comprising administering to the subject.
(Item 20)
a. The lentiviral particles according to item 15 of the effective amount, and
b. Effective amount of aminobisphosphonate drug
A method of preventing cancer in a subject, comprising administering to the subject.
(Item 21)
19. The method of item 19 or 20, wherein the aminobisphosphonate drug is zoledronic acid.
(Item 22)
19. The method of item 19 or 20, wherein step (a) and step (b) are performed simultaneously.
(Item 23)
19. The method of item 19 or 20, wherein a period of defined length elapses between step (a) and step (b).
(Item 24)
19. The method of item 19 or 20, wherein the therapeutically effective amount of the lentiviral particles comprises a plurality of single doses of the lentiviral particles.

(Item 25)

19. The method of item 19 or 20, wherein the therapeutically effective amount of the aminobisphosphonate drug comprises a single dose of the aminobisphosphonate drug.

Patent Citations (143)

Publication number	Priority date	Publication date	Assignee	Title
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JP2015518838A	2012-05-23	2015-07-06	ガニメド ファーマシューティカルズ アーゲー	Combination therapy with antibodies to claudin 18.2 to treat cancer
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CA2124350A1	1992-09-30	1994-04-14	Robert S. Abrams	Method and apparatus for attaching a spout to a carton
AU6014094A	1992-12-02	1994-06-22	Baylor College Of Medicine	Episomal vectors for gene therapy
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ES2639568T3	2007-01-23	2017-10-27	Janssen Pharmaceutica Nv	Method to design a drug regimen for HIV-infected patients
CA2682694A1	2007-04-12	2008-10-23	The Board Of Trustees Of The University Of Illinois	Bisphosphonate compounds and methods with enhanced potency for multiple targets including fpps, ggpps, and dpps
EP2008656A1	2007-06-28	2008-12-31	Bergen Teknologioverforing AS	Compositions for the treatment of hyperphenylalaninemia
US8673477B2	2008-06-16	2014-03-18	Polyplus Battery Company	High energy density aqueous lithium/air-battery cells
WO2009026328A2	2007-08-21	2009-02-26	Immune Disease Institute, Inc.	Methods of delivery of agents to leukocytes and endothelial cells
EP2090659A1	2008-02-14	2009-08-19	Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V.	Infectious particle, process for its preparation and use thereof

GB0810209D0 *	2008-06-04	2008-07-09	Cambridge Entpr Ltd	Pluripotency associated epigenetic factor
US8629334B2	2008-07-16	2014-01-14	University Of Florida Research Foundation, Inc.	Viral-based transient-expression vector system for trees
WO2010022195A2	2008-08-20	2010-02-25	Virxsys Corporation	Non-integrating lenti/adeno-associated virus hybrid vector system
EP2342321B1	2008-09-17	2018-04-11	Isogenis, Inc.	Construction of fully-deleted adenovirus-based gene delivery vectors and uses thereof
WO2010045659A1	2008-10-17	2010-04-22	American Gene Technologies International Inc.	Safe lentiviral vectors for targeted delivery of multiple therapeutic molecules
US8734795B2	2008-10-31	2014-05-27	Biogen Idec Ma Inc.	Light targeting molecules and uses thereof
WO2010051521A1	2008-10-31	2010-05-06	Lentigen Corporation	Cell therapy product for the treatment of hiv infection
WO2011071476A2	2008-11-14	2011-06-16	Life Technologies Corporation	Compositions and methods for engineering cells
EP2191834A1	2008-11-26	2010-06-02	Centre National De La Recherche Scientifique (Cnrs)	Compositions and methods for treating retrovirus infections
WO2010117974A2	2009-04-09	2010-10-14	Stemcyte Inc.	Hiv-resistant stem cells and uses thereof
EP2419113B1	2009-04-13	2017-05-10	Apceth GmbH & Co. KG	Engineered mesenchymal stem cells and method of using same to treat tumors
EP2425001A4	2009-04-30	2012-11-14	Univ California	Combination anti-hiv vectors, targeting vectors, and methods of use
EP3329772B1	2009-07-15	2019-10-16	Calimmune, Inc.	Dual vector for inhibition of human immunodeficiency virus
US20120027725A1	2009-11-30	2012-02-02	Galvin Jeffrey A	Safe lentiviral vectors for targeted delivery of multiple therapeutic molecules to treat liver cancer
CN101805750B *	2009-12-29	2011-11-30	浙江大学	Construction and application of farnesyl pyrophosphoric acid synthetase RNA (Ribonucleic Acid) interference recombinant lentivirus vector
CN102782136A	2010-02-18	2012-11-14	爱默蕾大学	Vectors expressing HIV antigens and GM-CSF and related methods for generating an immune response
WO2011119942A1	2010-03-25	2011-09-29	Vistagen Therapeutics, Inc.	Induction of ips cells using transient episomal vectors
WO2011133687A2	2010-04-20	2011-10-27	President And Fellows Of Harvard College	Methods and compositions for inhibition of beta2-adrenergic receptor degradation
LT2561078T	2010-04-23	2019-01-10	Cold Spring Harbor Laboratory	NOVEL STRUCTURALLY DESIGNED shRNAs
US20110293571A1	2010-05-28	2011-12-01	Oxford Biomedica (Uk) Ltd.	Method for vector delivery

WO2012020757A1	2010-08-10	2012-02-16	タカラバイオ株式会社	Production method for cell populations
US20130281493A1	2010-10-07	2013-10-24	The Trustees Of The University Of Columbia In The City Of New York	Method for Treating Cancer Harboring a p53 Mutation
WO2012061075A2	2010-10-25	2012-05-10	The Regents Of The University Of California	Hiv resistant and functional hematopoietic stem/progenitor cells and macrophages from induced pluripotent stem cells
WO2012115980A1	2011-02-22	2012-08-30	California Institute Of Technology	Delivery of proteins using adeno-associated virus (aav) vectors
US9226976B2	2011-04-21	2016-01-05	University Of Massachusetts	RAAV-based compositions and methods for treating alpha-1 anti-trypsin deficiencies
EP2782596A4	2011-11-22	2015-07-29	Philadelphia Children Hospital	Virus vectors for highly efficient transgene delivery
US9745631B2	2011-12-20	2017-08-29	Dana-Farber Cancer Institute, Inc.	Methods for diagnosing and treating oncogenic kras-associated cancer
BR112014019431A8	2012-02-07	2017-07-11	Global Bio Therapeutics Usa Inc	COMPARTMENTALIZED METHOD OF DELIVERY OF NUCLEIC ACID AND COMPOSITIONS AND USES THEREOF
AU2013273483A1	2012-06-06	2014-12-11	Bionor Immuno As	Vaccine
WO2014016817A2	2012-07-17	2014-01-30	Universite De Geneve	Nucleic acids for down-regulation of gene expression
CA2922005A1	2012-09-27	2014-04-03	Population Diagnostics, Inc.	Methods and compositions for screening and treating developmental disorders
CA2892448A1	2012-12-05	2014-06-12	Sangamo Biosciences, Inc.	Methods and compositions for regulation of metabolic disorders
US9642921B2	2012-12-20	2017-05-09	Tocagen Inc.	Cancer combination therapy and recombinant vectors
WO2014117050A2	2013-01-26	2014-07-31	Mirimus, Inc.	Modified mirna as a scaffold for shrna
CN103184224A	2013-04-03	2013-07-03	衡阳师范学院	Triple minRNA for resisting virus infection of aids and construction method thereof
WO2014187881A1 *	2013-05-21	2014-11-27	Max-Planck Gesellschaft zur Förderung der Wissenschaften e.V.	Isoforms of gata6 and nkx2-1 as markers for diagnosis and therapy of cancer and as targets for anti-cancer therapy
WO2015042308A2	2013-09-18	2015-03-26	City Of Hope	Rna-based hiv inhibitors
AU2014340083B2	2013-10-22	2019-08-15	Translate Bio, Inc.	mRNA therapy for phenylketonuria
EP2878674A1	2013-11-28	2015-06-03	Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC)	Stable episomes based on non-integrative lentiviral vectors

GB201322091D0	2013-12-13	2014-01-29	Cambridge Entpr Ltd	Modified serpins for the treatment of bleeding disorders
CA2946312A1	2014-04-23	2015-10-29	Juno Therapeutics, Inc.	Methods for isolating, culturing, and genetically engineering immune cell populations for adoptive therapy
DK3851537T3	2014-04-25	2024-03-18	Genethon	TREATMENT OF HYPERBILIRUBINAMIA
PL3689899T3	2014-04-25	2022-01-31	2Seventy Bio, Inc.	Mnd promoter chimeric antigen receptors
CA2955254A1 *	2014-08-29	2016-03-03	Immunomedics, Inc.	Identification of cancer genes by in-vivo fusion of human cancer cells and animal cells
SI3197472T1	2014-09-22	2022-01-31	Tanea Medical Ab	Recombinant phe-free proteins for use in the treatment of phenylketonuria
AU2015329696A1 *	2014-10-10	2017-04-27	The United States Of America, As Represented By The Secretary, Department Of Health And Human Services	Methods to eliminate cancer stem cells by targeting CD47
CN107405357B	2014-10-14	2021-12-31	德克萨斯科技大学系统	Multiple shRNAs and application thereof
WO2016069716A1	2014-10-30	2016-05-06	The Scripps Research Institute	Compositions and methods comprising tyrosyl-trna synthetases and resveratrol compounds
GB201509202D0	2015-05-28	2015-07-15	Ge Healthcare Bio Sciences Ab	Semi-static cell culture
JP6924487B2	2015-06-10	2021-08-25	アメリカン ジーン テクノロジーズ インターナショナル インコーポレイテッド	Non-embedded virus delivery system and how to use it
WO2017007994A1	2015-07-08	2017-01-12	American Gene Technologies International Inc.	Hiv pre-immunization and immunotherapy
JP6780870B2	2015-08-13	2020-11-04	北昊干細胞与再生医学研究院有限公司Ｂｅｉｈａｏ ＳｔｅｍＣｅｌｌ Ａｎｄ ＲｅｇｅｎｅｒａｔｉｖｅＭｅｄｉｃｉｎｅ Ｒｅｓｅａｒｃｈ Ｉｎｓｔｉｔｕｔｅ Ｃｏ．， Ｌｔｄ．	Induced expanded pluripotent stem cells, how to make and use
CN105112370B	2015-08-25	2019-02-05	杭州优善生物科技有限公司	A kind of method and its application of stimulated in vitro peripheral blood gamma delta T cells high efficiently multiplying
JP7059179B2	2015-10-20	2022-04-25	アンスティチュ ナショナル ドゥ ラ サンテ エ ドゥ ラ ルシェルシュ メディカル	Methods and products for genetic engineering
US11389546B2	2015-12-09	2022-07-19	Modernatx, Inc.	Heterologous UTR sequences for enhanced mRNA expression
US10137144B2 *	2016-01-15	2018-11-27	American Gene Technologies International Inc.	Methods and compositions for the activation of gamma-delta T-cells

EP4310500A3	2016-01-15	2024-04-03	American Gene Technologies International Inc.	Methods and compositons for the activation of gamma-delta t-cells
EP3413926A4	2016-02-08	2019-10-09	American Gene Technologies International, Inc.	Hiv vaccination and immunotherapy
WO2017156311A2	2016-03-09	2017-09-14	American Gene Technologies International Inc.	Combination vectors and methods for treating cancer
WO2017173453A1	2016-04-01	2017-10-05	The Brigham And Women's Hospital, Inc.	Stimuli-responsive nanoparticles for biomedical applications
JP7173548B2	2016-06-08	2022-11-16	アメリカン ジーン テクノロジーズ インターナショナル インコーポレイテッド	Non-Integrating Viral Delivery Systems and Related Methods
AU2017292582C1	2016-07-08	2021-11-11	American Gene Technologies International Inc.	HIV pre-immunization and immunotherapy
EP3487507A4	2016-07-21	2020-04-08	American Gene Technologies International, Inc.	Viral vectors for treating parkinson's disease
KR20190100318A	2016-12-30	2019-08-28	더 트러스티스 오브 더 유니버시티 오브 펜실바니아	Gene therapy to treat phenylketonuria
EP3565564A4	2017-01-09	2020-09-23	American Gene Technologies International Inc.	Hiv immunotherapy with no pre-immunization step
CN110621322A	2017-02-08	2019-12-27	达纳-法伯癌症研究所有限公司	Modulatable endogenous protein degradation with heterobifunctional compounds
US11820999B2	2017-04-03	2023-11-21	American Gene Technologies International Inc.	Compositions and methods for treating phenylketonuria
US20200181645A1	2017-06-16	2020-06-11	American Gene Technologies International Inc.	Methods and compositions for the activation of tumor cytotoxicity via human gamma-delta t-cells
CN111433368A	2017-10-02	2020-07-17	美国基因技术国际有限公司	Vector with promoter and enhancer combination for treating phenylketonuria
WO2020011247A1	2018-07-13	2020-01-16	Nanjing Legend Biotech Co., Ltd.	Co-receptor systems for treating infectious diseases
US11352646B2	2018-11-05	2022-06-07	American Gene Technologies International Inc.	Vector system for expressing regulatory RNA
KR20220068954A	2019-05-31	2022-05-26	아메리칸 진 테크놀로지스 인터내셔널 인코포레이티드	Optimized phenylalanine hydroxylase expression
IL296096A	2020-03-03	2022-11-01	American Gene Tech Int Inc	On demand expression of exogenous factors in lymphocytes to treat hiv

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Family To Family Citations				
WO2010045659A1	2008-10-17	2010-04-22	American Gene Technologies International Inc.	Safe lentiviral vectors for targeted delivery of multiple therapeutic molecules
US10137144B2	2016-01-15	2018-11-27	American Gene Technologies International Inc.	Methods and compositions for the activation of gamma-delta T-cells
EP4310500A3	2016-01-15	2024-04-03	American Gene Technologies International Inc.	Methods and compositons for the activation of gamma-delta t-cells
EP3413926A4	2016-02-08	2019-10-09	American Gene Technologies International, Inc.	Hiv vaccination and immunotherapy
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EP3487507A4	2016-07-21	2020-04-08	American Gene Technologies International, Inc.	Viral vectors for treating parkinson's disease
US11820999B2	2017-04-03	2023-11-21	American Gene Technologies International Inc.	Compositions and methods for treating phenylketonuria
GB201807945D0 *	2018-05-16	2018-06-27	Ospedale San Raffaele Srl	Vector production
US11352646B2	2018-11-05	2022-06-07	American Gene Technologies International Inc.	Vector system for expressing regulatory RNA
CN110964727A *	2019-12-31	2020-04-07	深圳市疾病预防控制中心（深圳市卫生检验中心、深圳市预防医学研究所）	shRNA lentiviral expression vector construction method for specifically inhibiting c-myc gene expression and application thereof
WO2023225569A1 *	2022-05-17	2023-11-23	Umoja Biopharma, Inc.	Manufacturing viral particles

* Cited by examiner, † Cited by third party, ‡ Family to family citation

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JP7017247B2	2022-02-08	Combination Vectors and Methods for Treating Cancer
US11519006B2	2022-12-06	Methods and compositions for the activation of gamma-delta T-cells
JP2023133396A	2023-09-22	Methods and compositions for activating tumor cell cytotoxicity via human gamma-delta t cells

Priority And Related Applications

Child Applications (1)

Application	Priority date	Filing date	Relation	Title
JP2022006999A	2016-03-09	2022-01-20	Division	Combination Vectors and Methods for Treating Cancer

Priority Applications (1)

Application	Priority date	Filing date	Title
JP2022006999A	2016-03-09	2022-01-20	Combination Vectors and Methods for Treating Cancer

Applications Claiming Priority (3)

Application	Filing date	Title
US201662305944P	2016-03-09	
US62/305,944	2016-03-09	
PCT/US2017/021639	2017-03-09	Combination vectors and methods for treating cancer

Legal Events

Date	Code	Title	Description
2020-03-06	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20200306
2020-03-06	A621	Written request for application examination	**Free format text**: JAPANESE INTERMEDIATE CODE: A621

			Effective date: 20200306
2021-02-04	A977	Report on retrieval	**Free format text**: JAPANESE INTERMEDIATE CODE: A971007 **Effective date**: 20210204
2021-02-16	A131	Notification of reasons for refusal	**Free format text**: JAPANESE INTERMEDIATE CODE: A131 **Effective date**: 20210216
2021-05-06	A601	Written request for extension of time	**Free format text**: JAPANESE INTERMEDIATE CODE: A601 **Effective date**: 20210506
2021-07-15	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20210715
2021-12-17	TRDD	Decision of grant or rejection written	
2021-12-21	A01	Written decision to grant a patent or to grant a registration (utility model)	**Free format text**: JAPANESE INTERMEDIATE CODE: A01 **Effective date**: 20211221
2022-01-24	A61	First payment of annual fees (during grant procedure)	**Free format text**: JAPANESE INTERMEDIATE CODE: A61 **Effective date**: 20220120
2022-01-31	R150	Certificate of patent or registration of utility model	**Ref document number**: 7017247 **Country of ref document**: JP **Free format text**: JAPANESE INTERMEDIATE CODE: R150

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