



(86) Date de dépôt PCT/PCT Filing Date: 2001/06/29
(87) Date publication PCT/PCT Publication Date: 2002/01/10
(45) Date de délivrance/Issue Date: 2012/11/27
(85) Entrée phase nationale/National Entry: 2003/01/02
(86) N° demande PCT/PCT Application No.: US 2001/020710
(87) N° publication PCT/PCT Publication No.: 2002/002738
(30) Priorité/Priority: 2000/07/03 (US60/215,925)

(51) Cl.Int./Int.Cl. *C12N 15/85* (2006.01),
C07K 14/02 (2006.01), *C07K 16/12* (2006.01),
C07K 16/30 (2006.01), *C12N 15/82* (2006.01),
C12N 15/86 (2006.01), *C12N 15/867* (2006.01),
C12N 5/10 (2006.01), *C12N 7/04* (2006.01),
G01N 33/50 (2006.01)

(72) Inventeurs/Inventors:
BREMEL, ROBERT D., US;
MILLER, LINDA U., US;
BLECK, GREGORY T., US

(73) Propriétaire/Owner:
CATALANT PHARMA SOLUTIONS, LLC, US

(74) Agent: SMART & BIGGAR

(54) Titre : CELLULES HOTES CONTENANT DE MULTIPLES VECTEURS D'INTEGRATION
(54) Title: HOST CELLS CONTAINING MULTIPLE INTEGRATING VECTORS

(57) **Abrégé/Abstract:**

The present invention relates to the production of proteins in host cells, and more particularly to host cells containing multiple integrated copies of an integrating vector. Suitable integrating vectors for use in the present invention include retrovirus vectors, lentivirus vectors, transposon vectors, and adeno-associated virus vectors. Methods are provided in which the host cells are prepared by using the integrating vectors at a high multiplicity of infection. The host cells are useful for producing pharmaceutical proteins, variants of proteins for use in screening assays, and for direct use in high throughput screening.

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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
10 January 2002 (10.01.2002)

PCT

(10) International Publication Number
WO 02/02738 A3

(51) International Patent Classification⁷: **C12N 15/85**,
15/86, 5/10, G01N 33/50

(21) International Application Number: PCT/US01/20710

(22) International Filing Date: 29 June 2001 (29.06.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/215,925 3 July 2000 (03.07.2000) US

(71) Applicant (for all designated States except US): **GALA DESIGN, INC.** [US/US]; 280 Washington Street, Prarie du Sac, WI 53578 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BREMEL, Robert, D.** [US/US]; E3352 Chapel Road, Hillpoint, WI 53937 (US). **MILLER, Linda, U.** [US/US]; N1301 O'Conner Road, Lodi, WI 53555 (US). **BLECK, Gregory, T.** [US/US]; 1111 Silver Drive, Baraboo, WI 53913 (US). **YORK, Donna** [US/US]; E9923 Trout Road, Dells, WI 53965 (US).

(74) Agents: **JONES, J., Mitchell** et al.; Medlen & Carroll, LLP, Suite 350, 101 Howard Street, San Francisco, CA 94105 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
30 May 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: HOST CELLS CONTAINING MULTIPLE INTEGRATING VECTORS

(57) Abstract: The present invention relates to the production of proteins in host cells, and more particularly to host cells containing multiple integrated copies of an integrating vector. Suitable integrating vectors for use in the present invention include retrovirus vectors, lentivirus vectors, transposon vectors, and adeno-associated virus vectors. Methods are provided in which the host cells are prepared by using the integrating vectors at a high multiplicity of infection. The host cells are useful for producing pharmaceutical proteins, variants of proteins for use in screening assays, and for direct use in high throughput screening.



WO 02/02738 A3

74667-216

HOST CELLS CONTAINING MULTIPLE INTEGRATING VECTORS

FIELD OF THE INVENTION

The present invention relates to the production of proteins in host cells, and more particularly to host cells containing multiple integrated copies of an integrating vector.

BACKGROUND OF THE INVENTION

The pharmaceutical biotechnology industry is based on the production of recombinant proteins in mammalian cells. These proteins are essential to the therapeutic treatment of many diseases and conditions. In many cases, the market for these proteins exceeds a billion dollars a year. Examples of proteins produced recombinantly in mammalian cells include erythropoietin, factor VIII, factor IX, and insulin. For many of these proteins, expression in mammalian cells is preferred over expression in prokaryotic cells because of the need for correct post-translational modification (*e.g.*, glycosylation or sialation; *see, e.g.*, U.S. Pat. No. 5,721,121).

Several methods are known for creating host cells that express recombinant proteins. In the most basic methods, a nucleic acid construct containing a gene encoding a heterologous protein and appropriate regulatory regions is introduced into the host cell and allowed to integrate. Methods of introduction include calcium phosphate precipitation, microinjection, lipofection, and electroporation. In other methods, a selection scheme is used to amplify the introduced nucleic acid construct. In these methods, the cells are co-transfected with a gene encoding an amplifiable selection marker and a gene encoding a heterologous protein (*See, e.g.*, Schroder and Friedl, *Biotech. Bioeng.* 53(6):547-59 [1997]). After selection of the initial transformants, the transfected genes are amplified by the stepwise increase of the selective agent (*e.g.*, dihydrofolate reductase) in the culture medium. In some cases, the exogenous gene may be amplified several hundred-fold by these procedures. Other methods of recombinant protein expression in mammalian cells utilize transfection with episomal vectors (*e.g.*, plasmids).

Current methods for creating mammalian cell lines for expression of recombinant proteins suffer from several drawbacks. (*See, e.g.*, Mielke *et al.*, *Biochem.* 35:2239-52

[1996]). Episomal systems allow for high expression levels of the recombinant protein, but are frequently only stable for a short time period (*See, e.g.*, Klehr and Bode, Mol. Genet. (Life Sci. Adv.) 7:47-52 [1988]). Mammalian cell lines containing integrated exogenous genes are somewhat more stable, but there is increasing evidence that stability depends on the presence of only a few copies or even a single copy of the exogenous gene.

Standard transfection techniques favor the introduction of multiple copies of the transgene into the genome of the host cell. Multiple integration of the transgene has, in many cases, proven to be intrinsically unstable. This intrinsic instability may be due to the characteristic head-to-tail mode of integration which promotes the loss of coding sequences by homologous recombination (*See, e.g.*, Weidle *et al.*, Gene 66:193-203 [1988]) especially when the transgenes are transcribed (*See, e.g.*, McBurney *et al.*, Somatic Cell Molec. Genet. 20:529-40 [1994]). Host cells also have epigenetic defense mechanisms directed against multiple copy integration events. In plants, this mechanism has been termed "cosuppression." (*See, e.g.*, Allen *et al.*, Plant Cell 5:603-13 [1993]). Indeed, it is not uncommon that the level of expression is inversely related to copy number. These observations are consistent with findings that multiple copies of exogenous genes become inactivated by methylation (*See, e.g.*, Mehtali *et al.*, Gene 91:179-84 [1990]) and subsequent mutagenesis (*See, e.g.*, Kricker *et al.*, Proc. Natl. Acad. Sci. 89:1075-79 [1992]) or silenced by heterochromatin formation (*See, e.g.*, Dorer and Henikoff, Cell 77:993-1002 [1994]).

Accordingly, what is needed in the art are improved methods for making host cells that express recombinant proteins. Preferably, the host cells will be stable over extended periods of time and express the protein encoded by a transgene at high levels.

SUMMARY OF THE INVENTION

The present invention relates to the production of proteins in host cells, and more particularly to host cells containing multiple integrated copies of an integrating vector. The present invention is not limited to host cells transfected with a particular number of integrating vectors. Indeed, host cells containing a wide range of integrating vectors are contemplated. In some embodiments, the present invention provides a host cell comprising a genome containing preferably at least about two integrated integrating vectors. In still further embodiments, the genome preferably comprises at least 3

integrated integrating vectors and most preferably at least 4 integrated integrating vectors, 5 integrated integrating vectors, 6 integrated integrating vectors, 7 integrated integrating vectors, 10 integrated integrating vectors, 15 integrated integrating vectors, 20 integrated integrating vectors, or 50 integrated integrating vectors.

The present invention is not limited to host cells containing vectors encoding a single protein of interest (*i.e.*, exogenous protein). Indeed, it is contemplated that the host cells are transfected with vectors encoding multiple proteins of interest. In some embodiments, the integrating vector comprises at least two exogenous genes. In some preferred embodiments, the at least two exogenous genes are arranged in a polycistronic sequence. In some particularly preferred embodiments, the at least two exogenous genes are separated by an internal ribosome entry site. In other preferred embodiments, the at least two exogenous genes are arranged in a polycistronic sequence. In still further embodiments, the two exogenous genes comprise a heavy chain of an immunoglobulin molecule and a light chain of an immunoglobulin molecule. In other embodiments, one of the at least two exogenous genes is a selectable marker. In still other embodiments, the host cells comprise at least 2 integrated copies of a first integrating vector comprising a first exogenous gene, and at least 1 integrated copy of a second integrating vector or other vector comprising a second exogenous gene. In still further embodiments, the host cells comprise at least 10 integrated copies of a first integrating vector comprising a first exogenous gene, and at least 1 integrated copy of a second integrating vector or other vector comprising a second exogenous gene.

In some preferred embodiments, the integrating vectors comprise at least one exogenous gene operably linked to a promoter. The present invention is not limited to vectors containing a particular promoter. Indeed, a variety of promoters are contemplated. In some embodiments of the present invention, the promoter is selected from the group consisting of the alpha-lactalbumin promoter, cytomegalovirus promoter and the long terminal repeat of Moloney murine leukemia virus. In other preferred embodiments, the integrating vectors further comprise a secretion signal operably linked to the exogenous gene. In still other embodiments, the integrating vectors further comprise an RNA export element operably linked to the exogenous gene.

The present invention is not limited to a particular integrating vector. Indeed, a variety of integrating vectors are contemplated. In some embodiments of the present invention, the integrating vector is selected from the group consisting of a retroviral

vector, a lentiviral vector, and a transposon vector. In some preferred embodiments, the retroviral vector is a pseudotyped retroviral vector. In other preferred embodiments, the pseudotyped retroviral vector comprises a G glycoprotein. The retroviral vectors of the present invention are not limited to a particular G glycoprotein. Indeed, a variety of G glycoproteins are contemplated. In some particularly preferred embodiments, the G glycoprotein is selected from the group consisting of vesicular stomatitis virus, Piry virus, Chandipura virus, Spring viremia of carp virus and Mokola virus G glycoproteins. In still further embodiments, the retroviral vector comprises long terminal repeats. The retroviral vectors of the present invention are not limited to a particular LTR. Indeed, a variety of LTRs are contemplated, including, but not limited to MoMLV, MoMuSV, MMTV long terminal repeats.

In other embodiments, the retroviral vector is a lentiviral vector. In some preferred embodiments, the lentiviral vector is pseudotyped. In some particularly preferred embodiments, the lentiviral vector comprises a G glycoprotein. In still further embodiments, the G glycoprotein is selected from the group consisting of vesicular stomatitis virus, Piry virus, Chandipura virus, Spring viremia of carp virus and Mokola virus G glycoproteins. In still other embodiments, the lentiviral vector comprises long terminal repeats selected from the group consisting of HIV and equine infectious anemia long terminal repeats.

In still further embodiments of the present invention, the integrating vector is a transposon vector. In some preferred embodiments, the transposon vector is selected from Tn5, Tn7, and Tn10 transposon vectors.

The present invention is not limited to a particular host cell. Indeed, a variety of host cells are contemplated. In some embodiments of the present invention, the host cell is cultured *in vitro*. In still further embodiments of the present invention, the host cell is selected from chinese hamster ovary cells, baby hamster kidney cells, and bovine mammary epithelial cells. In some preferred embodiments, the host cells are clonally derived. In other embodiments, the host cells are non-clonally derived. In some embodiments, the genome of the host cell is stable for greater than 10 passages. In other embodiments, the genome is stable for greater than 50 passages, while in still other embodiments, the genome is stable for greater than 100 passages. In still other embodiments, the host cells can be an embryonic stem cell, oocyte, or embryo. In some embodiments, the integrated vector is stable in the absence of selection.

The present invention is not limited to vectors encoding a particular protein of interest. Indeed, vectors encoding a variety of proteins of interest encoded by exogenous genes are contemplated. In some embodiments, the protein of interest is selected from hepatitis B surface antigen, MN14 antibody, LL2 antibody, *botulinum* toxin antibody and cc49IL2. In some embodiments, the genes encoding the protein of interest are intronless, while in other embodiments, the genes encoding the protein of interest include at least one intron.

The present invention also provides a method for transfecting or transducing host cells comprising: 1) providing: a) a host cell comprising a genome, and b) a plurality of integrating vectors; and 2) contacting the host cell with the plurality of integrating vectors under conditions such that at least two integrating vectors integrate into the genome of the host cell. In some embodiments, the conditions comprise contacting the host cells at a multiplicity of infection of greater than 10. In other embodiments, the conditions comprise contacting the host cells at a multiplicity of infection of from about 10 to 1,000,000. In still further embodiments, the conditions comprise contacting the host cells at a multiplicity of infection of from about 100 to 10,000. In still further embodiments, the conditions comprise contacting the host cells at a multiplicity of infection of from about 100 to 1,000. In still other embodiments of the present invention, the method further comprises transfecting said host cells with at least two integrating vectors, each of said two integrating comprising a different exogenous gene. In still other embodiments, the conditions comprise serial transfection or transduction of host cells wherein the host cells are transfected or transduced in at least a first transfection or transduction with a vector encoding a protein of interest and then re-transfected or re-transduced in a separate transfection or transduction step.

The present invention further provides a method of producing a protein of interest comprising: 1) providing a host cell comprising a genome, the genome comprising at least two integrated copies of at least one integrating vector comprising an exogenous gene operably linked to a promotor, wherein the exogenous gene encodes a protein of interest, and 2) culturing the host cells under conditions such that the protein of interest is produced. In some preferred embodiments, the integrating vector further comprises a secretion signal sequence operably linked to said exogenous gene. In other embodiments, the methods further comprise step 3) isolating the protein of interest. The present invention is not limited to any particular culture system. Indeed, a variety of culture

systems are contemplated, including, but not limited to roller bottle cultures, perfusion cultures, batch fed cultures, and petri dish cultures. In some embodiments, the cell line is clonally selected, while in other embodiments, the cells are non-clonally selected.

The methods of the present invention are not limited to host cells containing any particular number of integrated integrating vectors. Indeed, in some embodiments, the genome of the host cell comprises greater than 3 integrated copies of the integrating vector; in other embodiments, genome of the host cell comprises greater than 4 integrated copies of the integrating vector.; in still other embodiments, the genome of the host cell comprises greater than 5 integrated copies of the integrating vector; in further embodiments, the genome of the host cell comprises greater than 7 integrated copies of the integrating vector; while in still further embodiments, the genome of the host cell comprises greater than 10 integrated copies of the integrating vector. In other embodiments, the genome of the host cell comprises between about 2 and 20 integrated copies of the integrating vector. In some embodiments, the genome of the host cell comprises between about 3 and 10 integrated copies of the integrating vector.

The methods of the present invention are not limited to any particular integrating vector. Indeed, the use of a variety of integrating vectors is contemplated. In some embodiments, the integrating vector is a retroviral vector. In some preferred embodiments, the retroviral vector is a pseudotyped retroviral vector. In other embodiments, the retroviral vector is a lentiviral vector.

The methods of the present invention are not limited to the use of any particular host cell. Indeed, the use of a variety of host cells is contemplated, including, but not limited to, Chinese hamster ovary cells, baby hamster kidney cells, bovine mammary epithelial cells, oocytes, embryos, stem cells, and embryonic stem cells.

The methods of the present invention are not limited to the production of any particular amount of exogenous protein (*i.e.*, protein of interest) from the host cells. Indeed, it is contemplated that a variety of expression levels are acceptable from the methods of the present invention. In some embodiments, the host cells synthesize greater than about 1 picogram per cell per day of the protein of interest. In other embodiments, the host cells synthesize greater than about 10 picograms per cell per day of the protein of interest. In still further embodiments, the host cells synthesize greater than about 50 picograms per cell per day of the protein of interest.

In other embodiments, the present invention provides a method for screening compounds comprising: 1) providing a) a host cell comprising a genome, the genome comprising at least two integrated copies of at least one integrating vector comprising an exogenous gene operably linked to a promoter, wherein the exogenous gene encodes a protein of interest; and b) one or more test compounds; 2) culturing the host cells under conditions such that the protein of interest is expressed; 3) treating the host cells with one or more test compounds; and 4) assaying for the presence or absence of a response in the host cells to the test compound. In some embodiments of the present invention, the exogenous gene encodes a protein selected from the group consisting of reporter proteins, membrane receptor proteins, nucleic acid binding proteins, cytoplasmic receptor proteins, ion channel proteins, signal transduction proteins, protein kinases, protein phosphatases, and proteins encoded by oncogenes.

In still further embodiments, the host cell further comprises a reporter gene. In some particularly preferred embodiments, the reporter gene is selected from the group consisting of green fluorescent protein, luciferase, beta-galactosidase, and beta-lactamase. In some embodiments, the assaying step further comprises detecting a signal from the reporter gene. In other embodiments, the genome of the host cell comprises at least two integrating vectors, each comprising a different exogenous gene.

In still other embodiments, the present invention provides methods for comparing protein activity comprising: 1) providing a) a first host cell comprising a first integrating vector comprising a promoter operably linked to a first exogenous gene, wherein the first exogenous gene encodes a first protein of interest, and b) at least a second host cell comprising a second integrating vector comprising a promoter operably linked to a second exogenous gene, wherein the second exogenous gene encodes a second exogenous gene that is a variant of the first protein of interest; 2) culturing the host cells under conditions such that the first and second proteins of interest are produced; and 3) comparing the activities of the first and second proteins of interest.

In some embodiments, the exogenous gene encodes a protein selected from the group consisting of membrane receptor proteins, nucleic acid binding proteins, cytoplasmic receptor proteins, ion channel proteins, signal transduction proteins, protein kinases, protein phosphatases, cell cycle proteins, and proteins encoded by oncogenes. In other embodiments, the first and second proteins of interest differ by a single amino acid. In still further embodiments, the first and second proteins of interest are greater than 95%

14667-216

identical, preferably greater than 90% identical, and most preferably greater than 80% identical.

In other embodiments, the present invention provides methods comprising: 1) providing: a) a host cell comprising a genome comprising at least one integrated exogenous gene; and b) a plurality of integrating vectors; and 2) contacting the host cell with the plurality of integrating vectors under conditions such that at least two of the integrating vectors integrate into the genome of the host cell. In some embodiments, the integrated exogenous gene comprises an integrating vector. In other embodiments, the host cell is clonally selected. In alternative embodiments, the host cell is non-clonally selected.

In still further embodiments, the present invention provides methods of indirectly detecting the expression of a protein of interest comprising providing a host cell transfected with a vector encoding a polycistronic sequence, wherein the polycistronic sequence comprises a signal protein and a protein of interest operably linked by an IRES, and culturing the host cells under conditions such that the signal protein and protein of interest are produced, wherein the presence of the signal protein indicates the presence of the protein of interest. The methods of the present invention are not limited to the expression of any particular protein of interest. Indeed, the expression of a variety of proteins of interest is contemplated, including, but not limited to, G-protein coupled receptors. The present invention is not limited to the use of any particular signal protein. Indeed, the use of variety of signal proteins is contemplated, including, but not limited to, immunoglobulin heavy and light chains, beta-galactosidase, beta-lactamase, green fluorescent protein, and luciferase. In particularly preferred embodiments, expression of the signal protein and protein of interest is driven by the same promoter and the signal protein and protein of interest are transcribed as a single transcriptional unit.

53116-13

In another aspect, the invention provides a clonally selected host cell comprising a genome, said genome comprising from 20 to 100 copies of a first integrated retroviral vector, wherein said retroviral vector comprises at least one exogenous gene operably linked to a promoter, said gene encoding a secreted
5 protein, wherein said host cell does not produce infectious retroviral particles.

In another aspect, the invention provides a method for transfecting host cells comprising: 1) providing: a) a host cell comprising a genome, and b) a plurality of retroviral vectors encoding a first exogenous gene operably linked to a promoter; and 2) serially transducing said host cell with said plurality of retroviral vectors to
10 provide said host cell with from 20 to 100 copies of the retroviral vector integrated into said genome of said host cell, said first exogenous gene encoding a secreted protein, wherein said host cell does not produce infectious retroviral particles; and 3) clonally selecting a transfected host cell.

In another aspect, the invention provides a method of producing a
15 protein of interest comprising: 1) providing a clonally selected host cell comprising a genome, said genome comprising from 20 to 100 integrated copies of a first retroviral integrating vector comprising an exogenous gene operably linked to a promoter, and a secretion signal sequence operably linked to said exogenous gene wherein said exogenous gene encodes a secretable protein of interest, and 2) culturing said host
20 cell under conditions such that said protein of interest is produced.

In another aspect, the invention provides a method for transfecting host cells comprising: 1) providing: a) a clonally selected host cell comprising a genome comprising at least one integrated exogenous gene; and b) a plurality of retroviral vectors comprising an exogenous gene identical to said exogenous gene in said
25 clonally selected host cell, said genes encoding a secreted protein; and 2) serially transducing said host cell with said plurality of retroviral vectors to provide said host cell with from 20 to 100 copies of said retroviral vector integrated into said genome of said host cell, wherein said host cell does not produce infectious retroviral particles.

53116-13

DESCRIPTION OF THE FIGURES

Figure 1 is a western blot of a 15% SDS-PAGE gel run under denaturing conditions and probed with anti-human IgG (Fc) and anti-human IgG (Kappa).

5 Figure 2 is a graph of MN14 expression over time.

Figure 3 is a Western blot of a 15% PAGE run under non-denaturing conditions and probed with anti-human IgG (Fc) and anti-human IgG (Kappa).

Figure 4 provides the sequence for the hybrid human-bovine alpha-lactalbumin promoter (SEQ ID NO:1).

Figure 5 provides the sequence for the mutated PPE sequence (SEQ ID NO:2).

Figure 6 provides the sequence for the IRES-Signal peptide sequence (SEQ ID NO:3).

Figures 7a and 7b provide the sequence for CMV MN14 vector (SEQ ID NO:4).

Figures 8a and 8b provide the sequence for the CMV LL2 vector (SEQ ID NO:5).

Figures 9a-c provide the sequence for the MMTV MN14 vector (SEQ ID NO:6).

Figures 10a-d provide the sequence for the alpha-lactalbumin MN14 Vector (SEQ ID NO:7).

Figures 11a-c provide the sequence for the alpha-lactalbumin Bot vector (SEQ ID NO:8).

Figures 12a-b provide the sequence for the LSRNL vector (SEQ ID NO:9).

Figures 13a-b provide the sequence for the alpha-lactalbumin cc49IL2 vector (SEQ ID NO:10).

Figures 14a-c provides the sequence for the alpha-lactalbumin YP vector (SEQ ID NO:11).

Figure 15 provides the sequence for the IRES-Casein signal peptide sequence (SEQ ID NO:12).

Figures 16a-c provide the sequence for the LNBOTDC vector (SEQ ID NO:13).

Figure 17 provides a graph depicting the INVADER Assay gene ratio in CMV promoter cell lines.

Figure 18 provides a graph depicting the INVADER Assay gene ratio in α -lactalbumin promoter cell lines.

Figures 19a-d provide the sequence of a retroviral vector that expresses a G-Protein coupled receptor and antibody light chain.

DEFINITIONS

To facilitate understanding of the invention, a number of terms are defined below.

As used herein, the term "host cell" refers to any eukaryotic cell (*e.g.*, mammalian cells, avian cells, amphibian cells, plant cells, fish cells, and insect cells), whether located *in vitro* or *in vivo*.

As used herein, the term "cell culture" refers to any *in vitro* culture of cells. Included within this term are continuous cell lines (*e.g.*, with an immortal phenotype), primary cell cultures, finite cell lines (*e.g.*, non-transformed cells), and any other cell population maintained *in vitro*, including oocytes and embryos.

As used herein, the term "vector" refers to any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc., which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

As used herein, the term "integrating vector" refers to a vector whose integration or insertion into a nucleic acid (*e.g.*, a chromosome) is accomplished via an integrase. Examples of "integrating vectors" include, but are not limited to, retroviral vectors, transposons, and adeno associated virus vectors.

As used herein, the term "integrated" refers to a vector that is stably inserted into the genome (*i.e.*, into a chromosome) of a host cell.

As used herein, the term "multiplicity of infection" or "MOI" refers to the ratio of integrating vectors:host cells used during transfection or transduction of host cells. For example, if 1,000,000 vectors are used to transduce 100,000 host cells, the multiplicity of infection is 10. The use of this term is not limited to events involving transduction, but instead encompasses introduction of a vector into a host by methods such as lipofection, microinjection, calcium phosphate precipitation, and electroporation.

As used herein, the term "genome" refers to the genetic material (*e.g.*, chromosomes) of an organism.

The term "nucleotide sequence of interest" refers to any nucleotide sequence (*e.g.*, RNA or DNA), the manipulation of which may be deemed desirable for any reason (*e.g.*, treat disease, confer improved qualities, expression of a protein of interest in a host cell, expression of a ribozyme, *etc.*), by one of ordinary skill in the art. Such nucleotide sequences include, but are not limited to, coding sequences of structural genes (*e.g.*, reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, *etc.*), and non-coding regulatory sequences which do not encode an mRNA or protein product (*e.g.*, promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, *etc.*).

As used herein, the term "protein of interest" refers to a protein encoded by a nucleic acid of interest.

As used herein, the term "signal protein" refers to a protein that is co-expressed with a protein of interest and which, when detected by a suitable assay, provides indirect evidence of expression of the protein of interest. Examples of signal protein useful in the present invention include, but are not limited to, immunoglobulin heavy and light chains, beta-galactosidase, beta-lactamase, green fluorescent protein, and luciferase.

As used herein, the term "exogenous gene" refers to a gene that is not naturally present in a host organism or cell, or is artificially introduced into a host organism or cell.

The term "gene" refers to a nucleic acid (*e.g.*, DNA or RNA) sequence that comprises coding sequences necessary for the production of a polypeptide or precursor (*e.g.*, proinsulin). The polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or functional properties (*e.g.*, enzymatic activity, ligand binding, signal transduction, etc.) of the full-length or fragment are retained. The term also encompasses the coding region of a structural gene and includes sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 1 kb or more on either end such that the gene corresponds to the length of the full-length mRNA. The sequences that are located 5' of the coding region and which are present on the mRNA are referred to as 5' untranslated sequences. The sequences that are located 3' or downstream of the coding region and which are present on the mRNA are referred to as 3' untranslated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." Introns are segments of a gene which are transcribed into nuclear RNA (hnRNA); introns may contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA (mRNA) transcript. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

As used herein, the term "gene expression" refers to the process of converting genetic information encoded in a gene into RNA (*e.g.*, mRNA, rRNA, tRNA, or snRNA) through "transcription" of the gene (*i.e.*, via the enzymatic action of an RNA

polymerase), and for protein encoding genes, into protein through "translation" of mRNA. Gene expression can be regulated at many stages in the process. "Up-regulation" or "activation" refers to regulation that increases the production of gene expression products (*i.e.*, RNA or protein), while "down-regulation" or "repression" refers to regulation that decrease production. Molecules (*e.g.*, transcription factors) that are involved in up-regulation or down-regulation are often called "activators" and "repressors," respectively.

Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms, such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

As used herein, the terms "nucleic acid molecule encoding," "DNA sequence encoding," "DNA encoding," "RNA sequence encoding," and "RNA encoding" refer to the order or sequence of deoxyribonucleotides or ribonucleotides along a strand of deoxyribonucleic acid or ribonucleic acid. The order of these deoxyribonucleotides or ribonucleotides determines the order of amino acids along the polypeptide (protein) chain. The DNA or RNA sequence thus codes for the amino acid sequence.

As used herein, the term "variant," when used in reference to a protein, refers to proteins encoded by partially homologous nucleic acids so that the amino acid sequence of the proteins varies. As used herein, the term "variant" encompasses proteins encoded by homologous genes having both conservative and nonconservative amino acid substitutions that do not result in a change in protein function, as well as proteins encoded by homologous genes having amino acid substitutions that cause decreased (*e.g.*, null mutations) protein function or increased protein function.

As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (*i.e.*, a sequence of nucleotides) related by the base-pairing rules. For example, for the sequence "A-G-T," is complementary to the sequence "T-C-A." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

The terms "homology" and "percent identity" when used in relation to nucleic acids refers to a degree of complementarity. There may be partial homology (*i.e.*, partial identity) or complete homology (*i.e.*, complete identity). A partially complementary sequence is one that at least partially inhibits a completely complementary sequence from hybridizing to a target nucleic acid sequence and is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe (*i.e.*, an oligonucleotide which is capable of hybridizing to another oligonucleotide of interest) will compete for and inhibit the binding (*i.e.*, the hybridization) of a completely homologous sequence to a target sequence under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (*i.e.*, selective) interaction. The absence of non-specific binding may be tested by the use of a second target which lacks even a partial degree of complementarity (*e.g.*, less than about 30% identity); in the absence of non-specific binding the probe will not hybridize to the second non-complementary target.

The art knows well that numerous equivalent conditions may be employed to comprise low stringency conditions; factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, etc.) and the concentration of the salts and other components (*e.g.*, the presence or absence of formamide, dextran sulfate, polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of low stringency hybridization different from, but equivalent to, the above listed conditions. In addition, the art knows conditions that promote hybridization under conditions of high stringency (*e.g.*, increasing the temperature of the hybridization and/or wash steps, the use of formamide in the hybridization solution, etc.).

When used in reference to a double-stranded nucleic acid sequence such as a cDNA or genomic clone, the term "substantially homologous" refers to any probe that can hybridize to either or both strands of the double-stranded nucleic acid sequence under conditions of low stringency as described above.

When used in reference to a single-stranded nucleic acid sequence, the term "substantially homologous" refers to any probe that can hybridize (*i.e.*, it is the complement of) the single-stranded nucleic acid sequence under conditions of low stringency as described above.

As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (*i.e.*, the strength of the association between the nucleic acids) is impacted by such factors as the degree of complementarity between the nucleic acids, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acids. A single molecule that contains pairing of complementary nucleic acids within its structure is said to be "self-hybridized."

As used herein, the term " T_m " is used in reference to the "melting temperature" of a nucleic acid. The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. The equation for calculating the T_m of nucleic acids is well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m = 81.5 + 0.41(\% G + C)$, when a nucleic acid is in aqueous solution at 1 M NaCl (See *e.g.*, Anderson and Young, Quantitative Filter Hybridization, in *Nucleic Acid Hybridization* [1985]). Other references include more sophisticated computations that take structural as well as sequence characteristics into account for the calculation of T_m .

As used herein the term "stringency" is used in reference to the conditions of temperature, ionic strength, and the presence of other compounds such as organic solvents, under which nucleic acid hybridizations are conducted. With "high stringency" conditions, nucleic acid base pairing will occur only between nucleic acid fragments that have a high frequency of complementary base sequences. Thus, conditions of "weak" or "low" stringency are often required with nucleic acids that are derived from organisms that are genetically diverse, as the frequency of complementary sequences is usually less.

"High stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1X SSPE, 1.0% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

"Medium stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH₂PO₄•H₂O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 1.0X SSPE, 1.0% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

"Low stringency conditions" comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH₂PO₄•H₂O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.1% SDS, 5X Denhardt's reagent [50X Denhardt's contains per 500 ml: 5 g Ficoll (Type 400, Pharmacia), 5 g BSA (Fraction V; Sigma)] and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 5X SSPE, 0.1% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

A gene may produce multiple RNA species that are generated by differential splicing of the primary RNA transcript. cDNAs that are splice variants of the same gene will contain regions of sequence identity or complete homology (representing the presence of the same exon or portion of the same exon on both cDNAs) and regions of complete non-identity (for example, representing the presence of exon "A" on cDNA 1 wherein cDNA 2 contains exon "B" instead). Because the two cDNAs contain regions of sequence identity they will both hybridize to a probe derived from the entire gene or portions of the gene containing sequences found on both cDNAs; the two splice variants are therefore substantially homologous to such a probe and to each other.

The terms "in operable combination," "in operable order," and "operably linked" as used herein refer to the linkage of nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced. The term also refers to the linkage of amino acid sequences in such a manner so that a functional protein is produced.

As used herein, the term "selectable marker" refers to a gene that encodes an enzymatic activity that confers the ability to grow in medium lacking what would otherwise be an essential nutrient (e.g. the *HIS3* gene in yeast cells); in addition, a selectable marker may confer resistance to an antibiotic or drug upon the cell in which the selectable marker is expressed. Selectable markers may be "dominant"; a dominant selectable marker encodes an enzymatic activity that can be detected in any eukaryotic

cell line. Examples of dominant selectable markers include the bacterial aminoglycoside 3' phosphotransferase gene (also referred to as the *neo* gene) that confers resistance to the drug G418 in mammalian cells, the bacterial hygromycin G phosphotransferase (*hyg*) gene that confers resistance to the antibiotic hygromycin and the bacterial xanthine-guanine phosphoribosyl transferase gene (also referred to as the *gpt* gene) that confers the ability to grow in the presence of mycophenolic acid. Other selectable markers are not dominant in that their use must be in conjunction with a cell line that lacks the relevant enzyme activity. Examples of non-dominant selectable markers include the thymidine kinase (*tk*) gene that is used in conjunction with *tk*⁻ cell lines, the CAD gene which is used in conjunction with CAD-deficient cells and the mammalian hypoxanthine-guanine phosphoribosyl transferase (*hprt*) gene which is used in conjunction with *hprt*⁻ cell lines. A review of the use of selectable markers in mammalian cell lines is provided in Sambrook, J. *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, New York (1989) pp.16.9-16.15.

As used herein, the term "regulatory element" refers to a genetic element which controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element that facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements are splicing signals, polyadenylation signals, termination signals, RNA export elements, internal ribosome entry sites, etc. (defined *infra*).

Transcriptional control signals in eukaryotes comprise "promoter" and "enhancer" elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription (Maniatis *et al.*, *Science* 236:1237 [1987]). Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in yeast, insect and mammalian cells, and viruses (analogous control elements, *i.e.*, promoters, are also found in prokaryotes). The selection of a particular promoter and enhancer depends on what cell type is to be used to express the protein of interest. Some eukaryotic promoters and enhancers have a broad host range while others are functional in a limited subset of cell types (for review see, Voss *et al.*, *Trends Biochem. Sci.*, 11:287 [1986]; and Maniatis *et al.*, *supra*). For example, the SV40 early gene enhancer is very active in a wide variety of cell types from many mammalian species and has been widely used for the expression of proteins in mammalian cells (Dijkema *et al.*, *EMBO J.* 4:761 [1985]). Two other examples of

promoter/enhancer elements active in a broad range of mammalian cell types are those from the human elongation factor 1 α gene (Uetsuki *et al.*, J. Biol. Chem., 264:5791 [1989]; Kim *et al.*, Gene 91:217 [1990]; and Mizushima and Nagata, Nuc. Acids. Res., 18:5322 [1990]) and the long terminal repeats of the Rous sarcoma virus (Gorman *et al.*, Proc. Natl. Acad. Sci. USA 79:6777 [1982]) and the human cytomegalovirus (Boshart *et al.*, Cell 41:521 [1985]).

As used herein, the term "promoter/enhancer" denotes a segment of DNA which contains sequences capable of providing both promoter and enhancer functions (*i.e.*, the functions provided by a promoter element and an enhancer element, see above for a discussion of these functions). For example, the long terminal repeats of retroviruses contain both promoter and enhancer functions. The enhancer/promoter may be "endogenous" or "exogenous" or "heterologous." An "endogenous" enhancer/promoter is one which is naturally linked with a given gene in the genome. An "exogenous" or "heterologous" enhancer/promoter is one which is placed in juxtaposition to a gene by means of genetic manipulation (*i.e.*, molecular biological techniques such as cloning and recombination) such that transcription of that gene is directed by the linked enhancer/promoter.

Regulatory elements may be tissue specific or cell specific. The term "tissue specific" as it applies to a regulatory element refers to a regulatory element that is capable of directing selective expression of a nucleotide sequence of interest to a specific type of tissue (*e.g.*, liver) in the relative absence of expression of the same nucleotide sequence of interest in a different type of tissue (*e.g.*, lung).

Tissue specificity of a regulatory element may be evaluated by, for example, operably linking a reporter gene to a promoter sequence (which is not tissue-specific) and to the regulatory element to generate a reporter construct, introducing the reporter construct into the genome of an animal such that the reporter construct is integrated into every tissue of the resulting transgenic animal, and detecting the expression of the reporter gene (*e.g.*, detecting mRNA, protein, or the activity of a protein encoded by the reporter gene) in different tissues of the transgenic animal. The detection of a greater level of expression of the reporter gene in one or more tissues relative to the level of expression of the reporter gene in other tissues shows that the regulatory element is "specific" for the tissues in which greater levels of expression are detected. Thus, the term "tissue-specific" (*e.g.*, liver-specific) as used herein is a relative term that does not

require absolute specificity of expression. In other words, the term "tissue-specific" does not require that one tissue have extremely high levels of expression and another tissue have no expression. It is sufficient that expression is greater in one tissue than another. By contrast, "strict" or "absolute" tissue-specific expression is meant to indicate expression in a single tissue type (*e.g.*, liver) with no detectable expression in other tissues.

The term "cell type specific" as applied to a regulatory element refers to a regulatory element which is capable of directing selective expression of a nucleotide sequence of interest in a specific type of cell in the relative absence of expression of the same nucleotide sequence of interest in a different type of cell within the same tissue. The term "cell type specific" when applied to a regulatory element also means a regulatory element capable of promoting selective expression of a nucleotide sequence of interest in a region within a single tissue.

Cell type specificity of a regulatory element may be assessed using methods well known in the art (*e.g.*, immunohistochemical staining and/or Northern blot analysis). Briefly, for immunohistochemical staining, tissue sections are embedded in paraffin, and paraffin sections are reacted with a primary antibody specific for the polypeptide product encoded by the nucleotide sequence of interest whose expression is regulated by the regulatory element. A labeled (*e.g.*, peroxidase conjugated) secondary antibody specific for the primary antibody is allowed to bind to the sectioned tissue and specific binding detected (*e.g.*, with avidin/biotin) by microscopy. Briefly, for Northern blot analysis, RNA is isolated from cells and electrophoresed on agarose gels to fractionate the RNA according to size followed by transfer of the RNA from the gel to a solid support (*e.g.*, nitrocellulose or a nylon membrane). The immobilized RNA is then probed with a labeled oligo-deoxyribonucleotide probe or DNA probe to detect RNA species complementary to the probe used. Northern blots are a standard tool of molecular biologists.

The term "promoter," "promoter element," or "promoter sequence" as used herein, refers to a DNA sequence which when ligated to a nucleotide sequence of interest is capable of controlling the transcription of the nucleotide sequence of interest into mRNA. A promoter is typically, though not necessarily, located 5' (*i.e.*, upstream) of a nucleotide sequence of interest whose transcription into mRNA it controls, and provides a site for

specific binding by RNA polymerase and other transcription factors for initiation of transcription.

Promoters may be constitutive or regulatable. The term "constitutive" when made in reference to a promoter means that the promoter is capable of directing transcription of an operably linked nucleic acid sequence in the absence of a stimulus (*e.g.*, heat shock, chemicals, *etc.*). In contrast, a "regulatable" promoter is one which is capable of directing a level of transcription of an operably linked nucleic acid sequence in the presence of a stimulus (*e.g.*, heat shock, chemicals, *etc.*) which is different from the level of transcription of the operably linked nucleic acid sequence in the absence of the stimulus.

The presence of "splicing signals" on an expression vector often results in higher levels of expression of the recombinant transcript. Splicing signals mediate the removal of introns from the primary RNA transcript and consist of a splice donor and acceptor site (Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, New York [1989], pp. 16.7-16.8). A commonly used splice donor and acceptor site is the splice junction from the 16S RNA of SV40.

Efficient expression of recombinant DNA sequences in eukaryotic cells requires expression of signals directing the efficient termination and polyadenylation of the resulting transcript. Transcription termination signals are generally found downstream of the polyadenylation signal and are a few hundred nucleotides in length. The term "poly A site" or "poly A sequence" as used herein denotes a DNA sequence that directs both the termination and polyadenylation of the nascent RNA transcript. Efficient polyadenylation of the recombinant transcript is desirable as transcripts lacking a poly A tail are unstable and are rapidly degraded. The poly A signal utilized in an expression vector may be "heterologous" or "endogenous." An endogenous poly A signal is one that is found naturally at the 3' end of the coding region of a given gene in the genome. A heterologous poly A signal is one that is isolated from one gene and placed 3' of another gene. A commonly used heterologous poly A signal is the SV40 poly A signal. The SV40 poly A signal is contained on a 237 bp *Bam*HI/*Bcl*II restriction fragment and directs both termination and polyadenylation (Sambrook, *supra*, at 16.6-16.7).

Eukaryotic expression vectors may also contain "viral replicons" or "viral origins of replication." Viral replicons are viral DNA sequences that allow for the extrachromosomal replication of a vector in a host cell expressing the appropriate

74667-216

replication factors. Vectors that contain either the SV40 or polyoma virus origin of replication replicate to high "copy number" (up to 10^4 copies/cell) in cells that express the appropriate viral T antigen. Vectors that contain the replicons from bovine papillomavirus or Epstein-Barr virus replicate extrachromosomally at "low copy number" (~100 copies/cell). However, it is not intended that expression vectors be limited to any particular viral origin of replication.

As used herein, the term "long terminal repeat" of "LTR" refers to transcriptional control elements located in or isolated from the U3 region 5' and 3' of a retroviral genome. As is known in the art, long terminal repeats may be used as control elements in retroviral vectors, or isolated from the retroviral genome and used to control expression from other types of vectors.

As used herein, the term "secretion signal" refers to any DNA sequence which when operably linked to a recombinant DNA sequence encodes a signal peptide which is capable of causing the secretion of the recombinant polypeptide. In general, the signal peptides comprise a series of about 15 to 30 hydrophobic amino acid residues (*See, e.g., Zwizinski et al., J. Biol. Chem.* 255(16): 7973-77 [1980], *Gray et al., Gene* 39(2): 247-54 [1985], and *Martial et al., Science* 205: 602-607 [1979]). Such secretion signal sequences are preferably derived from genes encoding polypeptides secreted from the cell type targeted for tissue-specific expression (*e.g.,* secreted milk proteins for expression in and secretion from mammary secretory cells). Secretory DNA sequences, however, are not limited to such sequences. Secretory DNA sequences from proteins secreted from many cell types and organisms may also be used (*e.g.,* the secretion signals for t-PA, serum albumin, lactoferrin, and growth hormone, and secretion signals from microbial genes encoding secreted polypeptides such as from yeast, filamentous fungi, and bacteria).

As used herein, the terms "RNA export element" or "Pre-mRNA Processing Enhancer (PPE)" refer to 3' and 5' cis-acting post-transcriptional regulatory elements that enhance export of RNA from the nucleus. "PPE" elements include, but are not limited to Mertz sequences (described in U.S. Pat. Nos. 5,914,267 and 5,686,120) and woodchuck mRNA processing enhancer (WPRE; WO99/14310 and U.S. Pat. No. 6,136,597).

74667-216

As used herein, the term "polycistronic" refers to an mRNA encoding more than one polypeptide chain (See, e.g., WO 93/03143, WO 88/05486, and European Pat. No. 117058). Likewise, the term "arranged in polycistronic sequence" refers to the arrangement of genes encoding two different polypeptide chains in a single mRNA.

As used herein, the term "internal ribosome entry site" or "IRES" refers to a sequence located between polycistronic genes that permits the production of the expression product originating from the second gene by internal initiation of the translation of the dicistronic mRNA. Examples of internal ribosome entry sites include, but are not limited to, those derived from foot and mouth disease virus (FMDV), encephalomyocarditis virus, poliovirus and RDV (Scheper *et al.*, Biochem. 76: 801-809 [1994]; Meyer *et al.*, J. Virol. 69: 2819-2824 [1995]; Jang *et al.*, 1988, J. Virol. 62: 2636-2643 [1998]; Haller *et al.*, J. Virol. 66: 5075-5086 [1995]). Vectors incorporating IRES's may be assembled as is known in the art. For example, a retroviral vector containing a polycistronic sequence may contain the following elements in operable association: nucleotide polylinker, gene of interest, an internal ribosome entry site and a mammalian selectable marker or another gene of interest. The polycistronic cassette is situated within the retroviral vector between the 5' LTR and the 3' LTR at a position such that transcription from the 5' LTR promoter transcribes the polycistronic message cassette. The transcription of the polycistronic message cassette may also be driven by an internal promoter (e.g., cytomegalovirus promoter) or an inducible promoter, which may be preferable depending on the use. The polycistronic message cassette can further comprise a cDNA or genomic DNA (gDNA) sequence operatively associated within the polylinker. Any mammalian selectable marker can be utilized as the polycistronic message cassette mammalian selectable marker. Such mammalian selectable markers are well known to those of skill in the art and can include, but are not limited to, kanamycin/G418, hygromycin B or mycophenolic acid resistance markers.

As used herein, the term "retrovirus" refers to a retroviral particle which is capable of entering a cell (*i.e.*, the particle contains a membrane-associated protein such as an envelope protein or a viral G glycoprotein which can bind to the host cell surface and facilitate entry of the viral particle into the cytoplasm of the host cell) and integrating the retroviral genome (as a double-stranded provirus) into the genome of the

74667-216

host cell. The term "retrovirus" encompasses Oncovirinae (*e.g.*, Moloney murine leukemia virus (MoMOLV), Moloney murine sarcoma virus (MoMSV), and Mouse mammary tumor virus (MMTV), Spumavirinae, and Lentivirinae (*e.g.*, Human immunodeficiency virus, Simian immunodeficiency virus, Equine infection anemia virus, and Caprine arthritis-encephalitis virus; *See, e.g.*, U.S. Pat. Nos. 5,994,136 and 6,013,516).

As used herein, the term "retroviral vector" refers to a retrovirus that has been modified to express a gene of interest. Retroviral vectors can be used to transfer genes efficiently into host cells by exploiting the viral infectious process. Foreign or heterologous genes cloned (*i.e.*, inserted using molecular biological techniques) into the retroviral genome can be delivered efficiently to host cells which are susceptible to infection by the retrovirus. Through well known genetic manipulations, the replicative capacity of the retroviral genome can be destroyed. The resulting replication-defective vectors can be used to introduce new genetic material to a cell but they are unable to replicate. A helper virus or packaging cell line can be used to permit vector particle assembly and egress from the cell. Such retroviral vectors comprise a replication-deficient retroviral genome containing a nucleic acid sequence encoding at least one gene of interest (*i.e.*, a polycistronic nucleic acid sequence can encode more than one gene of interest), a 5' retroviral long terminal repeat (5' LTR); and a 3' retroviral long terminal repeat (3' LTR).

The term "pseudotyped retroviral vector" refers to a retroviral vector containing a heterologous membrane protein. The term "membrane-associated protein" refers to a protein (*e.g.*, a viral envelope glycoprotein or the G proteins of viruses in the Rhabdoviridae family such as VSV, Piry, Chandipura and Mokola) which are associated with the membrane surrounding a viral particle; these membrane-associated proteins mediate the entry of the viral particle into the host cell. The membrane associated protein may bind to specific cell surface protein receptors, as is the case for retroviral envelope proteins or the membrane-associated protein may interact with a phospholipid component of the plasma membrane of the host cell, as is the case for the G proteins derived from members of the Rhabdoviridae family.

The term "heterologous membrane-associated protein" refers to a membrane-associated protein which is derived from a virus which is not a member of the same viral class or family as that from which the nucleocapsid protein of the vector particle is

derived. "Viral class or family" refers to the taxonomic rank of class or family, as assigned by the International Committee on Taxonomy of Viruses.

The term "Rhabdoviridae" refers to a family of enveloped RNA viruses that infect animals, including humans, and plants. The Rhabdoviridae family encompasses the genus Vesiculovirus which includes vesicular stomatitis virus (VSV), Cocal virus, Piry virus, Chandipura virus, and Spring viremia of carp virus (sequences encoding the Spring viremia of carp virus are available under GenBank accession number U18101). The G proteins of viruses in the Vesiculovirus genera are virally-encoded integral membrane proteins that form externally projecting homotrimeric spike glycoproteins complexes that are required for receptor binding and membrane fusion. The G proteins of viruses in the Vesiculovirus genera have a covalently bound palmitic acid (C₁₆) moiety. The amino acid sequences of the G proteins from the Vesiculoviruses are fairly well conserved. For example, the Piry virus G protein share about 38% identity and about 55% similarity with the VSV G proteins (several strains of VSV are known, *e.g.*, Indiana, New Jersey, Orsay, San Juan, etc., and their G proteins are highly homologous). The Chandipura virus G protein and the VSV G proteins share about 37% identity and 52% similarity. Given the high degree of conservation (amino acid sequence) and the related functional characteristics (*e.g.*, binding of the virus to the host cell and fusion of membranes, including syncytia formation) of the G proteins of the Vesiculoviruses, the G proteins from non-VSV Vesiculoviruses may be used in place of the VSV G protein for the pseudotyping of viral particles. The G proteins of the Lyssa viruses (another genera within the Rhabdoviridae family) also share a fair degree of conservation with the VSV G proteins and function in a similar manner (*e.g.*, mediate fusion of membranes) and therefore may be used in place of the VSV G protein for the pseudotyping of viral particles. The Lyssa viruses include the Mokola virus and the Rabies viruses (several strains of Rabies virus are known and their G proteins have been cloned and sequenced). The Mokola virus G protein shares stretches of homology (particularly over the extracellular and transmembrane domains) with the VSV G proteins which show about 31% identity and 48% similarity with the VSV G proteins. Preferred G proteins share at least 25% identity, preferably at least 30% identity and most preferably at least 35% identity with the VSV G proteins. The VSV G protein from which New Jersey strain (the sequence of this G protein is provided in GenBank accession numbers M27165 and M21557) is employed as the reference VSV G protein.

As used herein, the term "lentivirus vector" refers to retroviral vectors derived from the Lentiviridae family (*e.g.*, human immunodeficiency virus, simian immunodeficiency virus, equine infectious anemia virus, and caprine arthritis-encephalitis virus) that are capable of integrating into non-dividing cells (*See, e.g.*, U.S. Pat. Nos. 5,994,136 and 6,013,516, both of which are incorporated herein by reference).

The term "pseudotyped lentivirus vector" refers to lentivirus vector containing a heterologous membrane protein (*e.g.*, a viral envelope glycoprotein or the G proteins of viruses in the Rhabdoviridae family such as VSV, Piry, Chandipura and Mokola).

As used herein, the term "transposon" refers to transposable elements (*e.g.*, Tn5, Tn7, and Tn10) that can move or transpose from one position to another in a genome. In general, the transposition is controlled by a transposase. The term "transposon vector," as used herein, refers to a vector encoding a nucleic acid of interest flanked by the terminal ends of transposon. Examples of transposon vectors include, but are not limited to, those described in U.S. Pat. Nos. 6,027,722; 5,958,775; 5,968,785; 5,965,443; and 5,719,055, all of which are incorporated herein by reference.

As used herein, the term "adeno-associated virus (AAV) vector" refers to a vector derived from an adeno-associated virus serotype, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAVX7, etc. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part, preferably the *rep* and/or *cap* genes, but retain functional flanking ITR sequences.

AAV vectors can be constructed using recombinant techniques that are known in the art to include one or more heterologous nucleotide sequences flanked on both ends (5' and 3') with functional AAV ITRs. In the practice of the invention, an AAV vector can include at least one AAV ITR and a suitable promoter sequence positioned upstream of the heterologous nucleotide sequence and at least one AAV ITR positioned downstream of the heterologous sequence. A "recombinant AAV vector plasmid" refers to one type of recombinant AAV vector wherein the vector comprises a plasmid. As with AAV vectors in general, 5' and 3' ITRs flank the selected heterologous nucleotide sequence.

AAV vectors can also include transcription sequences such as polyadenylation sites, as well as selectable markers or reporter genes, enhancer sequences, and other control elements which allow for the induction of transcription. Such control elements are described above.

74667-216

As used herein, the term "AAV virion" refers to a complete virus particle. An AAV virion may be a wild type AAV virus particle (comprising a linear, single-stranded AAV nucleic acid genome associated with an AAV capsid, *i.e.*, a protein coat), or a recombinant AAV virus particle (described below). In this regard, single-stranded AAV nucleic acid molecules (either the sense/coding strand or the antisense/anticoding strand as those terms are generally defined) can be packaged into an AAV virion; both the sense and the antisense strands are equally infectious.

As used herein, the term "recombinant AAV virion" or "rAAV" is defined as an infectious, replication-defective virus composed of an AAV protein shell encapsidating (*i.e.*, surrounding with a protein coat) a heterologous nucleotide sequence, which in turn is flanked 5' and 3' by AAV ITRs. A number of techniques for constructing recombinant AAV virions are known in the art (*See, e.g.*, U.S. Patent No. 5,173,414; WO 92/01070; WO 93/03769; Lebkowski *et al.*, *Molec. Cell. Biol.* 8:3988-3996 [1988]; Vincent *et al.*, *Vaccines* 90 [1990] (Cold Spring Harbor Laboratory Press); Carter, *Current Opinion in Biotechnology* 3:533-539 [1992]; Muzyczka, *Current Topics in Microbiol. and Immunol.* 158:97-129 [1992]; Kotin, *Human Gene Therapy* 5:793-801 [1994]; Shelling and Smith, *Gene Therapy* 1:165-169 [1994]; and Zhou *et al.*, *J. Exp. Med.* 179:1867-1875 [1994]).

Suitable nucleotide sequences for use in AAV vectors (and, indeed, any of the vectors described herein) include any functionally relevant nucleotide sequence. Thus, the AAV vectors of the present invention can comprise any desired gene that encodes a protein that is defective or missing from a target cell genome or that encodes a non-native protein having a desired biological or therapeutic effect (*e.g.*, an antiviral function), or the sequence can correspond to a molecule having an antisense or ribozyme function. Suitable genes include those used for the treatment of inflammatory diseases, autoimmune, chronic and infectious diseases, including such disorders as AIDS, cancer, neurological diseases, cardiovascular disease, hypercholesterolemia; various blood disorders including various anemias, thalassemias and hemophilia; genetic defects such as cystic fibrosis, Gaucher's Disease, adenosine deaminase (ADA) deficiency, emphysema, etc. A number of antisense oligonucleotides (*e.g.*, short oligonucleotides complementary to sequences around the translational initiation site (AUG codon) of an mRNA) that are useful in antisense therapy for cancer and for viral diseases have been described in the art. (*See, e.g.*, Han *et al.*, *Proc. Natl. Acad. Sci. USA* 88:4313-4317 [1991]; Uhlmann *et*

74667-216

al., Chem. Rev. 90:543-584 [1990]; Helene *et al.*, Biochim. Biophys. Acta. 1049:99-125 [1990]; Agarwal *et al.*, Proc. Natl. Acad. Sci. USA 85:7079-7083 [1989]; and Heikkila *et al.*, Nature 328:445-449 [1987]). For a discussion of suitable ribozymes, *see, e.g.*, Cech *et al.* (1992) J. Biol. Chem. 267:17479-17482 and U.S. Patent No. 5,225,347.

By "adeno-associated virus inverted terminal repeats" or "AAV ITRs" is meant the art-recognized palindromic regions found at each end of the AAV genome which function together in *cis* as origins of DNA replication and as packaging signals for the virus. For use with the present invention, flanking AAV ITRs are positioned 5' and 3' of one or more selected heterologous nucleotide sequences and, together with the *rep* coding region or the Rep expression product, provide for the integration of the selected sequences into the genome of a target cell.

The nucleotide sequences of AAV ITR regions are known (*See, e.g.*, Kotin, Human Gene Therapy 5:793-801 [1994]; Berns, K.I. "Parvoviridae and their Replication" in Fundamental Virology, 2nd Edition, (B.N. Fields and D.M. Knipe, eds.) for the AAV-2 sequence. As used herein, an "AAV ITR" need not have the wild-type nucleotide sequence depicted, but may be altered, *e.g.*, by the insertion, deletion or substitution of nucleotides. Additionally, the AAV ITR may be derived from any of several AAV serotypes, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAVX7, etc. The 5' and 3' ITRs which flank a selected heterologous nucleotide sequence need not necessarily be identical or derived from the same AAV serotype or isolate, so long as they function as intended, *i.e.*, to allow for the integration of the associated heterologous sequence into the target cell genome when the *rep* gene is present (either on the same or on a different vector), or when the Rep expression product is present in the target cell.

As used herein the term, the term "*in vitro*" refers to an artificial environment and to processes or reactions that occur within an artificial environment. *In vitro* environments can consist of, but are not limited to, test tubes and cell cultures. The term "*in vivo*" refers to the natural environment (*e.g.*, an animal or a cell) and to processes or reaction that occur within a natural environment.

As used herein, the term "clonally derived" refers to a cell line that it derived from a single cell.

As used herein, the term “non-clonally derived” refers to a cell line that is derived from more than one cell.

As used herein, the term “passage” refers to the process of diluting a culture of cells that has grown to a particular density or confluency (*e.g.*, 70% or 80% confluent), and then allowing the diluted cells to regrow to the particular density or confluency desired (*e.g.*, by replating the cells or establishing a new roller bottle culture with the cells).

As used herein, the term “stable,” when used in reference to genome, refers to the stable maintenance of the information content of the genome from one generation to the next, or, in the particular case of a cell line, from one passage to the next. Accordingly, a genome is considered to be stable if no gross changes occur in the genome (*e.g.*, a gene is deleted or a chromosomal translocation occurs). The term “stable” does not exclude subtle changes that may occur to the genome such as point mutations.

As used herein, the term “response,” when used in reference to an assay, refers to the generation of a detectable signal (*e.g.*, accumulation of reporter protein, increase in ion concentration, accumulation of a detectable chemical product).

As used herein, the term “membrane receptor protein” refers to membrane spanning proteins that bind a ligand (*e.g.*, a hormone or neurotransmitter). As is known in the art, protein phosphorylation is a common regulatory mechanism used by cells to selectively modify proteins carrying regulatory signals from outside the cell to the nucleus. The proteins that execute these biochemical modifications are a group of enzymes known as protein kinases. They may further be defined by the substrate residue that they target for phosphorylation. One group of protein kinases are the tyrosine kinases (TKs) which selectively phosphorylate a target protein on its tyrosine residues. Some tyrosine kinases are membrane-bound receptors (RTKs), and, upon activation by a ligand, can autophosphorylate as well as modify substrates. The initiation of sequential phosphorylation by ligand stimulation is a paradigm that underlies the action of such effectors as, for example, epidermal growth factor (EGF), insulin, platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF). The receptors for these ligands are tyrosine kinases and provide the interface between the binding of a ligand (hormone, growth factor) to a target cell and the transmission of a signal into the cell by the activation of one or more biochemical pathways. Ligand binding to a receptor tyrosine kinase activates its intrinsic enzymatic activity (*See, e.g.*, Ullrich and Schlessinger, *Cell*

61:203-212 [1990]). Tyrosine kinases can also be cytoplasmic, non-receptor-type enzymes and act as a downstream component of a signal transduction pathway.

As used herein, the term "signal transduction protein" refers to a proteins that are activated or otherwise effected by ligand binding to a membrane receptor protein or some other stimulus. Examples of signal transduction protein include adenylyl cyclase, phospholipase C, and G-proteins. Many membrane receptor proteins are coupled to G-proteins (*i.e.*, G-protein coupled receptors (GPCRs); for a review, *see* Neer, 1995, *Cell* 80:249-257 [1995]). Typically, GPCRs contain seven transmembrane domains. Putative GPCRs can be identified on the basis of sequence homology to known GPCRs.

GPCRs mediate signal transduction across a cell membrane upon the binding of a ligand to an extracellular portion of a GPCR. The intracellular portion of a GPCR interacts with a G-protein to modulate signal transduction from outside to inside a cell. A GPCR is therefore said to be "coupled" to a G-protein. G-proteins are composed of three polypeptide subunits: an α subunit, which binds and hydrolyses GTP, and a dimeric $\beta\gamma$ subunit. In the basal, inactive state, the G-protein exists as a heterotrimer of the α and $\beta\gamma$ subunits. When the G-protein is inactive, guanosine diphosphate (GDP) is associated with the α subunit of the G-protein. When a GPCR is bound and activated by a ligand, the GPCR binds to the G-protein heterotrimer and decreases the affinity of the $G\alpha$ subunit for GDP. In its active state, the G subunit exchanges GDP for guanine triphosphate (GTP) and active $G\alpha$ subunit disassociates from both the receptor and the dimeric $\beta\gamma$ subunit. The disassociated, active $G\alpha$ subunit transduces signals to effectors that are "downstream" in the G-protein signalling pathway within the cell. Eventually, the G-protein's endogenous GTPase activity returns active G subunit to its inactive state, in which it is associated with GDP and the dimeric $\beta\gamma$ subunit.

Numerous members of the heterotrimeric G-protein family have been cloned, including more than 20 genes encoding various $G\alpha$ subunits. The various G subunits have been categorized into four families, on the basis of amino acid sequences and functional homology. These four families are termed $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12}$. Functionally, these four families differ with respect to the intracellular signaling pathways that they activate and the GPCR to which they couple.

For example, certain GPCRs normally couple with $G\alpha_s$ and, through $G\alpha_s$, these GPCRs stimulate adenylyl cyclase activity. Other GPCRs normally couple with $G\alpha_q$, and through $G\alpha_q$, these GPCRs can activate phospholipase C (PLC), such as the β

isoform of phospholipase C (*i.e.*, PLC β , Sternweis and Smrcka, Trends in Biochem. Sci. 17:502-506 [1992]).

As used herein, the term “nucleic acid binding protein” refers to proteins that bind to nucleic acid, and in particular to proteins that cause increased (*i.e.*, activators or transcription factors) or decreased (*i.e.*, inhibitors) transcription from a gene.

As used herein, the term “ion channel protein” refers to proteins that control the ingress or egress of ions across cell membranes. Examples of ion channel proteins include, but are not limited to, the Na⁺-K⁺ ATPase pump, the Ca²⁺ pump, and the K⁺ leak channel.

As used herein, the term “protein kinase” refers to proteins that catalyze the addition of a phosphate group from a nucleoside triphosphate to an amino acid side chain in a protein. Kinases comprise the largest known enzyme superfamily and vary widely in their target proteins. Kinases may be categorized as protein tyrosine kinases (PTKs), which phosphorylate tyrosine residues, and protein serine/threonine kinases (STKs), which phosphorylate serine and/or threonine residues. Some kinases have dual specificity for both serine/threonine and tyrosine residues. Almost all kinases contain a conserved 250-300 amino acid catalytic domain. This domain can be further divided into 11 subdomains. N-terminal subdomains I-IV fold into a two-lobed structure which binds and orients the ATP donor molecule, and subdomain V spans the two lobes. C-terminal subdomains VI-XI bind the protein substrate and transfer the gamma phosphate from ATP to the hydroxyl group of a serine, threonine, or tyrosine residue. Each of the 11 subdomains contains specific catalytic residues or amino acid motifs characteristic of that subdomain. For example, subdomain I contains an 8-amino acid glycine-rich ATP binding consensus motif, subdomain II contains a critical lysine residue required for maximal catalytic activity, and subdomains VI through IX comprise the highly conserved catalytic core. STKs and PTKs also contain distinct sequence motifs in subdomains VI and VIII which may confer hydroxyamino acid specificity. Some STKs and PTKs possess structural characteristics of both families. In addition, kinases may also be classified by additional amino acid sequences, generally between 5 and 100 residues, which either flank or occur within the kinase domain.

Non-transmembrane PTKs form signaling complexes with the cytosolic domains of plasma membrane receptors. Receptors that signal through non-transmembrane PTKs include cytokine, hormone, and antigen-specific lymphocytic receptors. Many PTKs

74667-216

were first identified as oncogene products in cancer cells in which PTK activation was no longer subject to normal cellular controls. In fact, about one third of the known oncogenes encode PTKs. Furthermore, cellular transformation (oncogenesis) is often accompanied by increased tyrosine phosphorylation activity (*See, e.g.,* Carbonneau, H. and Tonks, Annu. Rev. Cell Biol. 8:463-93 [1992]). Regulation of PTK activity may therefore be an important strategy in controlling some types of cancer.

Examples of protein kinases include, but are not limited to, cAMP-dependent protein kinase, protein kinase C, and cyclin-dependent protein kinases (*See, e.g.,* U.S. Pat. Nos. 6,034,228; 6,030,822; 6,030,788; 6,020,306; 6,013,455; 6,013,464; and 6,015,807).

As used herein, the term "protein phosphatase" refers to proteins that remove a phosphate group from a protein. Protein phosphatases are generally divided into two groups, receptor and non-receptor type proteins. Most receptor-type protein tyrosine phosphatases contain two conserved catalytic domains, each of which encompasses a segment of 240 amino acid residues. (*See, e.g.,* Saito *et al.*, Cell Growth and Diff. 2:59-65 [1991]). Receptor protein tyrosine phosphatases can be subclassified further based upon the amino acid sequence diversity of their extracellular domains. (*See, e.g.,* Krueger *et al.*, Proc. Natl. Acad. Sci. USA 89:7417-7421 [1992]). Examples of protein phosphatases include, but are not limited to, cdc25 a, b, and c, PTP20, PTP1D, and PTP λ (*See, e.g.,* U.S. Pat. Nos. 5,976,853; 5,994,074; 6,004,791; 5,981,251; 5,976,852; 5,958,719; 5,955,592; and 5,952,212).

As used herein, the term "protein encoded by an oncogene" refers to proteins that cause, either directly or indirectly, the neoplastic transformation of a host cell. Examples of oncogenes include, but are not limited to, the following genes: *src*, *fps*, *fes*, *fgr*, *ros*, *H-ras*, *abl*, *ski*, *erbA*, *erbB*, *fms*, *fos*, *mos*, *sis*, *myc*, *myb*, *rel*, *kit*, *raf*, *K-ras*, and *ets*.

As used herein, the term "immunoglobulin" refers to proteins which bind a specific antigen. Immunoglobulins include, but are not limited to, polyclonal, monoclonal, chimeric, and humanized antibodies, Fab fragments, F(ab')₂ fragments, and includes immunoglobulins of the following classes: IgG, IgA, IgM, IgD, IgE, and secreted immunoglobulins (sIg). Immunoglobulins generally comprise two identical heavy chains (γ , α , μ , δ , or ϵ) and two light chains (κ or λ).

As used herein, the term "antigen binding protein" refers to proteins which bind to a specific antigen. "Antigen binding proteins" include, but are not limited to,

74667-216

immunoglobulins, including polyclonal, monoclonal, chimeric, and humanized antibodies; Fab fragments, F(ab')₂ fragments, and Fab expression libraries; and single chain antibodies. Various procedures known in the art are used for the production of polyclonal antibodies. For the production of an antibody, various host animals can be immunized by injection with the peptide corresponding to the desired epitope including but not limited to rabbits, mice, rats, sheep, goats, etc. In a preferred embodiment, the peptide is conjugated to an immunogenic carrier (e.g., diphtheria toxoid, bovine serum albumin (BSA), or keyhole limpet hemocyanin (KLH)). Various adjuvants are used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum*.

For preparation of monoclonal antibodies, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used (See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). These include, but are not limited to, the hybridoma technique originally developed by Köhler and Milstein (Köhler and Milstein, *Nature* 256:495-497 [1975]), as well as the trioma technique, the human B-cell hybridoma technique (See e.g., Kozbor *et al.* *Immunol. Today* 4:72 [1983]), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 [1985]).

According to the invention, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce specific single chain antibodies as desired. An additional embodiment of the invention utilizes the techniques known in the art for the construction of Fab expression libraries (Huse *et al.*, *Science* 246:1275-1281 [1989]) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibody fragments that contain the idiotype (antigen binding region) of the antibody molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')₂ fragment that can be produced by pepsin digestion of an antibody molecule; the Fab' fragments that can be generated by reducing

74667-216

the disulfide bridges of an F(ab')₂ fragment, and the Fab fragments that can be generated by treating an antibody molecule with papain and a reducing agent.

Genes encoding antigen binding proteins can be isolated by methods known in the art. In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art (*e.g.*, radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), Western Blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays, etc.), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.) etc.

As used herein, the term "reporter gene" refers to a gene encoding a protein that may be assayed. Examples of reporter genes include, but are not limited to, luciferase (*See, e.g.*, deWet *et al.*, Mol. Cell. Biol. 7:725 [1987] and U.S. Pat Nos., 6,074,859; 5,976,796; 5,674,713; and 5,618,682), green fluorescent protein (*e.g.*, GenBank Accession Number U43284; a number of GFP variants are commercially available from CLONTECH Laboratories, Palo Alto, CA), chloramphenicol acetyltransferase, β -galactosidase, alkaline phosphatase, and horse radish peroxidase.

As used herein, the term "purified" refers to molecules, either nucleic or amino acid sequences, that are removed from their natural environment, isolated or separated. An "isolated nucleic acid sequence" is therefore a purified nucleic acid sequence. "Substantially purified" molecules are at least 60% free, preferably at least 75% free, and more preferably at least 90% free from other components with which they are naturally associated.

The term "test compound" refers to any chemical entity, pharmaceutical, drug, and the like contemplated to be useful in the treatment and/or prevention of a disease, illness, sickness, or disorder of bodily function, or otherwise alter the physiological or cellular status of a sample. Test compounds comprise both known and potential therapeutic compounds. A test compound can be determined to be therapeutic by screening using the screening methods of the present invention. A "known therapeutic compound" refers to a therapeutic compound that has been shown (*e.g.*, through animal

trials or prior experience with administration to humans) to be effective in such treatment or prevention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the production of proteins in host cells, and more particularly to host cells containing multiple integrated copies of an integrating vector. The present invention utilizes integrating vectors (*i.e.*, vectors that integrate via an integrase or transposase) to create cell lines containing a high copy number of a nucleic acid encoding a gene of interest. The transfected genomes of the high copy number cells are stable through repeated passages (*e.g.*, at least 10 passages, preferably at least 50 passages, and most preferably at least 100 passages). Furthermore, the host cells of the present invention are capable of producing high levels of protein (*e.g.*, more than 1 pg/cell/day, preferably more than 10 pg/cell/day, more preferably more than 50 pg/cell/day, and most preferably more than 100 pg/cell/day.)

The genomic stability and high expression levels of the host cells of the present invention provide distinct advantages over previously described methods of cell culture. For example, mammalian cell lines containing multiple copies of genes are known in the art to be intrinsically unstable. Indeed, this instability is a recognized problem facing researchers desiring to use mammalian cell lines for various purposes, including high throughput screening assays (*See, e.g.*, Sittampalam *et al.*, Curr. Opin. Chem. Biol. 1(3):384-91 [1997]).

It is not intended that the present invention be limited to particular mechanism of action. Indeed, an understanding of the mechanism is not necessary to make and use the present invention. However, the high genomic stability and protein expression levels of the host cells of the present invention are thought to be due to unique properties of the integrating vectors (*e.g.*, retroviral vectors). For example, it is known that retroviruses are inherited elements in the germ line of many organisms. Indeed, as much as 5-10% of the mammalian genome may consist of elements contributed by reverse transcription, indicating a high degree of stability. Likewise, many of these types of vectors target active (*e.g.*, DNase I hypersensitive sites) transcriptional sites in the genome.

Many investigations have focused on the deleterious effects of retroviral and transposon integration. The property of targeting active regions of the genome has led to the use of retroviral vectors and transposon vectors in promoter trap schemes and for

74667-216

saturation mutagenesis (*See, e.g.*, U.S. Pat. Nos. 5,627,058 and 5,922,601).

In promoter trap schemes, the cells are infected with a promoterless reporter vector. If the promoterless vector integrates downstream of a promoter (*i.e.*, into a gene), the reporter gene encoded by the vector is activated. The promoter can then be cloned and further characterized.

As can be seen, these schemes rely on the disruption of an endogenous gene. Therefore, it is surprising that the methods of the present invention, which utilize integrating vectors at high multiplicities of infection that would normally be thought to lead to gene disruption, led to the development of stable cell lines that express high quantities of a protein of interest. The development of these cell lines is described more fully below. The description is divided into the following sections: I) Host Cells; II) Vectors and Methods of Transfection; and III) Uses of Transfected Host Cells.

I. Host Cells

The present invention contemplates the transfection of a variety of host cells with integrating vectors. A number of mammalian host cell lines are known in the art. In general, these host cells are capable of growth and survival when placed in either monolayer culture or in suspension culture in a medium containing the appropriate nutrients and growth factors, as is described in more detail below. Typically, the cells are capable of expressing and secreting large quantities of a particular protein of interest into the culture medium. Examples of suitable mammalian host cells include, but are not limited to Chinese hamster ovary cells (CHO-K1, ATCC CCL-61); bovine mammary epithelial cells (ATCC CRL 10274; bovine mammary epithelial cells); monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture; *see, e.g.*, Graham *et al.*, J. Gen Virol., 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., 383:44-68 [1982]); MRC 5 cells; FS4 cells; rat fibroblasts (208F cells);

74667-216

MDBK cells (bovine kidney cells); and a human hepatoma line (Hep G2).

In addition to mammalian cell lines, the present invention also contemplates the transfection of plant protoplasts with integrating vectors at a low or high multiplicity of infection. For example, the present invention contemplates a plant cell or whole plant comprising at least one integrated integrating vector, preferably a retroviral vector, and most preferably a pseudotyped retroviral vector. All plants that can be produced by regeneration from protoplasts can also be transfected using the process according to the invention (e.g., cultivated plants of the genera *Solanum*, *Nicotiana*, *Brassica*, *Beta*, *Pisum*, *Phaseolus*, *Glycine*, *Helianthus*, *Allium*, *Avena*, *Hordeum*, *Oryzae*, *Setaria*, *Secale*, *Sorghum*, *Triticum*, *Zea*, *Musa*, *Cocos*, *Cydonia*, *Pyrus*, *Malus*, *Phoenix*, *Elaeis*, *Rubus*, *Fragaria*, *Prunus*, *Arachis*, *Panicum*, *Saccharum*, *Coffea*, *Camellia*, *Ananas*, *Vitis* or *Citrus*). In general, protoplasts are produced in accordance with conventional methods (See, e.g., U.S. Pat. Nos. 4,743,548; 4,677,066, 5,149,645; and 5,508,184).

Plant tissue may be dispersed in an appropriate

medium having an appropriate osmotic potential (e.g., 3 to 8 wt. % of a sugar polyol) and one or more polysaccharide hydrolases (e.g., pectinase, cellulase, etc.), and the cell wall degradation allowed to proceed for a sufficient time to provide protoplasts. After filtration the protoplasts may be isolated by centrifugation and may then be resuspended for subsequent treatment or use. Regeneration of protoplasts kept in culture to whole plants is performed by methods known in the art (See, e.g., Evans *et al.*, *Handbook of Plant Cell Culture*, 1: 124-176, MacMillan Publishing Co., New York [1983]; Binding, *Plant Protoplasts*, p. 21-37, CRC Press, Boca Raton [1985],) and Potrykus and Shillito, *Methods in Enzymology*, Vol. 118, Plant Molecular Biology, A. and H. Weissbach eds., Academic Press, Orlando [1986]).

The present invention also contemplates the use of amphibian and insect host cell lines. Examples of suitable insect host cell lines include, but are not limited to, mosquito cell lines (e.g., ATCC CRL-1660). Examples of suitable amphibian host cell lines include, but are not limited to, toad cell lines (e.g., ATCC CCL-102).

II. Vectors and Methods for Transfection

According to the present invention, host cells such as those described above are transduced or transfected with integrating vectors. Examples of integrating vectors include, but are not limited to, retroviral vectors, lentiviral vectors, adeno-associated viral vectors, and transposon vectors. The design, production, and use of these vectors in the present invention is described below.

A. Retroviral Vectors

Retroviruses (family Retroviridae) are divided into three groups: the spumaviruses (*e.g.*, human foamy virus); the lentiviruses (*e.g.*, human immunodeficiency virus and sheep visna virus) and the oncoviruses (*e.g.*, MLV, Rous sarcoma virus).

Retroviruses are enveloped (*i.e.*, surrounded by a host cell-derived lipid bilayer membrane) single-stranded RNA viruses which infect animal cells. When a retrovirus infects a cell, its RNA genome is converted into a double-stranded linear DNA form (*i.e.*, it is reverse transcribed). The DNA form of the virus is then integrated into the host cell genome as a provirus. The provirus serves as a template for the production of additional viral genomes and viral mRNAs. Mature viral particles containing two copies of genomic RNA bud from the surface of the infected cell. The viral particle comprises the genomic RNA, reverse transcriptase and other *pol* gene products inside the viral capsid (which contains the viral *gag* gene products) which is surrounded by a lipid bilayer membrane derived from the host cell containing the viral envelope glycoproteins (also referred to as membrane-associated proteins).

The organization of the genomes of numerous retroviruses is well known to the art and this has allowed the adaptation of the retroviral genome to produce retroviral vectors. The production of a recombinant retroviral vector carrying a gene of interest is typically achieved in two stages.

First, the gene of interest is inserted into a retroviral vector which contains the sequences necessary for the efficient expression of the gene of interest (including promoter and/or enhancer elements which may be provided by the viral long terminal repeats (LTRs) or by an internal promoter/enhancer and relevant splicing signals), sequences required for the efficient packaging of the viral RNA into infectious virions (*e.g.*, the packaging signal (Psi), the tRNA primer binding site (-PBS), the 3' regulatory sequences required for reverse transcription (+PBS)) and the viral LTRs. The LTRs

contain sequences required for the association of viral genomic RNA, reverse transcriptase and integrase functions, and sequences involved in directing the expression of the genomic RNA to be packaged in viral particles. For safety reasons, many recombinant retroviral vectors lack functional copies of the genes which are essential for viral replication (these essential genes are either deleted or disabled); therefore, the resulting virus is said to be replication defective.

Second, following the construction of the recombinant vector, the vector DNA is introduced into a packaging cell line. Packaging cell lines provide proteins required in *trans* for the packaging of the viral genomic RNA into viral particles having the desired host range (*i.e.*, the viral-encoded gag, pol and env proteins). The host range is controlled, in part, by the type of envelope gene product expressed on the surface of the viral particle. Packaging cell lines may express ecotropic, amphotropic or xenotropic envelope gene products. Alternatively, the packaging cell line may lack sequences encoding a viral envelope (env) protein. In this case the packaging cell line will package the viral genome into particles which lack a membrane-associated protein (*e.g.*, an env protein). In order to produce viral particles containing a membrane associated protein which will permit entry of the virus into a cell, the packaging cell line containing the retroviral sequences is transfected with sequences encoding a membrane-associated protein (*e.g.*, the G protein of vesicular stomatitis virus (VSV)). The transfected packaging cell will then produce viral particles which contain the membrane-associated protein expressed by the transfected packaging cell line; these viral particles which contain viral genomic RNA derived from one virus encapsidated by the envelope proteins of another virus are said to be pseudotyped virus particles.

The retroviral vectors of the present invention can be further modified to include additional regulatory sequences. As described above, the retroviral vectors of the present invention include the following elements in operable association: a) a 5' LTR; b) a packaging signal; c) a 3' LTR and d) a nucleic acid encoding a protein of interest located between the 5' and 3' LTRs. In some embodiments of the present invention, the nucleic acid of interest may be arranged in opposite orientation to the 5' LTR when transcription from an internal promoter is desired. Suitable internal promoters include, but are not limited to, the alpha-lactalbumin promoter, the CMV promoter (human or ape), and the thymidine kinase promoter.

74667-216

In other embodiments of the present invention, where secretion of the protein of interest is desired, the vectors are modified by including a signal peptide sequence in operable association with the protein of interest. The sequences of several suitable signal peptides are known to those in the art, including, but not limited to, those derived from tissue plasminogen activator, human growth hormone, lactoferrin, alpha-casein, and alpha-lactalbumin.

In other embodiments of the present invention, the vectors are modified by incorporating an RNA export element (*See, e.g.*, U.S. Pat. Nos. 5,914,267; 6,136,597; and 5,686,120; and WO99/14310) either 3' or 5' to the nucleic acid sequence encoding the protein of interest. It is contemplated that the use of RNA export elements allows high levels of expression of the protein of interest without incorporating splice signals or introns in the nucleic acid sequence encoding the protein of interest.

In still other embodiments, the vector further comprises at least one internal ribosome entry site (IRES) sequence. The sequences of several suitable IRES's are available, including, but not limited to, those derived from foot and mouth disease virus (FMDV), encephalomyocarditis virus, and poliovirus. The IRES sequence can be interposed between two transcriptional units (*e.g.*, nucleic acids encoding different proteins of interest or subunits of a multisubunit protein such as an antibody) to form a polycistronic sequence so that the two transcriptional units are transcribed from the same promoter.

The retroviral vectors of the present invention may also further comprise a selectable marker allowing selection of transformed cells. A number of selectable markers find use in the present invention, including, but not limited to the bacterial aminoglycoside 3' phosphotransferase gene (also referred to as the *neo* gene) that confers resistance to the drug G418 in mammalian cells, the bacterial hygromycin G phosphotransferase (*hyg*) gene that confers resistance to the antibiotic hygromycin and the bacterial xanthine-guanine phosphoribosyl transferase gene (also referred to as the *gpt* gene) that confers the ability to grow in the presence of mycophenolic acid. In some embodiments, the selectable marker gene is provided as part of polycistronic sequence that also encodes the protein of interest.

In still other embodiments of the present invention, the retroviral vectors may comprise recombination elements recognized by a recombination system (*e.g.*, the

74667-216

cre/loxP or flp recombinase systems, *see, e.g.*, Hoess *et al.*, Nucleic Acids Res. 14:2287-2300 [1986], O'Gorman *et al.*, Science 251:1351-55 [1991], van Deursen *et al.*, Proc. Natl. Acad. Sci. USA 92:7376-80 [1995], and U.S. Pat. No. 6,025,192).

After integration of the vectors into the genome of the host cell, the host cell can be transiently transfected (*e.g.*, by electroporation, lipofection, or microinjection) with either a recombinase enzyme (*e.g.*, Cre recombinase) or a nucleic acid sequence encoding the recombinase enzyme and one or more nucleic acid sequences encoding a protein of interest flanked by sequences recognized by the recombination enzyme so that the nucleic acid sequence is inserted into the integrated vector.

Viral vectors, including recombinant retroviral vectors, provide a more efficient means of transferring genes into cells as compared to other techniques such as calcium phosphate-DNA co-precipitation or DEAE-dextran-mediated transfection, electroporation or microinjection of nucleic acids. It is believed that the efficiency of viral transfer is due in part to the fact that the transfer of nucleic acid is a receptor-mediated process (*i.e.*, the virus binds to a specific receptor protein on the surface of the cell to be infected). In addition, the virally transferred nucleic acid once inside a cell integrates in controlled manner in contrast to the integration of nucleic acids which are not virally transferred; nucleic acids transferred by other means such as calcium phosphate-DNA co-precipitation are subject to rearrangement and degradation.

The most commonly used recombinant retroviral vectors are derived from the amphotropic Moloney murine leukemia virus (MoMLV) (*See e.g.*, Miller and Baltimore Mol. Cell. Biol. 6:2895 [1986]). The MoMLV system has several advantages: 1) this specific retrovirus can infect many different cell types, 2) established packaging cell lines are available for the production of recombinant MoMLV viral particles and 3) the transferred genes are permanently integrated into the target cell chromosome. The established MoMLV vector systems comprise a DNA vector containing a small portion of the retroviral sequence (*e.g.*, the viral long terminal repeat or "LTR" and the packaging or "psi" signal) and a packaging cell line. The gene to be transferred is inserted into the DNA vector. The viral sequences present on the DNA vector provide the signals necessary for the insertion or packaging of the vector RNA into the viral particle and for the expression of the inserted gene. The packaging cell line provides the proteins required for particle assembly (Markowitz *et al.*, J. Virol. 62:1120 [1988]).

74667-216

Despite these advantages, existing retroviral vectors based upon MoMLV are limited by several intrinsic problems: 1) they do not infect non-dividing cells (Miller *et al.*, Mol. Cell. Biol. 10:4239 [1990]), except, perhaps, oocytes; 2) they produce low titers of the recombinant virus (Miller and Rosman, BioTechniques 7: 980 [1980] and Miller, Nature 357: 455 [1990]); and 3) they infect certain cell types (*e.g.*, human lymphocytes) with low efficiency (Adams *et al.*, Proc. Natl. Acad. Sci. USA 89:8981 [1992]). The low titers associated with MoMLV-based vectors have been attributed, at least in part, to the instability of the virus-encoded envelope protein. Concentration of retrovirus stocks by physical means (*e.g.*, ultracentrifugation and ultrafiltration) leads to a severe loss of infectious virus.

The low titer and inefficient infection of certain cell types by MoMLV-based vectors has been overcome by the use of pseudotyped retroviral vectors which contain the G protein of VSV as the membrane associated protein. Unlike retroviral envelope proteins which bind to a specific cell surface protein receptor to gain entry into a cell, the VSV G protein interacts with a phospholipid component of the plasma membrane (Mastromarino *et al.*, J. Gen. Virol. 68:2359 [1977]). Because entry of VSV into a cell is not dependent upon the presence of specific protein receptors, VSV has an extremely broad host range. Pseudotyped retroviral vectors bearing the VSV G protein have an altered host range characteristic of VSV (*i.e.*, they can infect almost all species of vertebrate, invertebrate and insect cells). Importantly, VSV G-pseudotyped retroviral vectors can be concentrated 2000-fold or more by ultracentrifugation without significant loss of infectivity (Burns *et al.* Proc. Natl. Acad. Sci. USA 90:8033 [1993]).

The present invention is not limited to the use of the VSV G protein when a viral G protein is employed as the heterologous membrane-associated protein within a viral particle (*See, e.g.*, U.S. Pat. No. 5,512,421).

The G proteins of viruses in the Vesiculovirus genera other than VSV, such as the Piry and Chandipura viruses, that are highly homologous to the VSV G protein and, like the VSV G protein, contain covalently linked palmitic acid (Brun *et al.* Intervirology 38:274 [1995] and Masters *et al.*, Virol. 171:285 (1990)). Thus, the G protein of the Piry and Chandipura viruses can be used in place of the VSV G protein for the pseudotyping of viral particles. In addition, the VSV G proteins of viruses within the Lyssa virus genera such as Rabies and Mokola viruses show a high degree of conservation (amino acid sequence as well as functional conservation) with the VSV G proteins. For example, the

Mokola virus G protein has been shown to function in a manner similar to the VSV G protein (*i.e.*, to mediate membrane fusion) and therefore may be used in place of the VSV G protein for the pseudotyping of viral particles (Mebatsion *et al.*, J. Virol. 69:1444 [1995]). Viral particles may be pseudotyped using either the Piry, Chandipura or Mokola G protein as described in Example 2, with the exception that a plasmid containing sequences encoding either the Piry, Chandipura or Mokola G protein under the transcriptional control of a suitable promoter element (*e.g.*, the CMV intermediate-early promoter; numerous expression vectors containing the CMV IE promoter are available, such as the pcDNA3.1 vectors (Invitrogen)) is used in place of pHCMV-G. Sequences encoding other G proteins derived from other members of the Rhabdoviridae family may be used; sequences encoding numerous rhabdoviral G proteins are available from the GenBank database.

The majority of retroviruses can transfer or integrate a double-stranded linear form of the virus (the provirus) into the genome of the recipient cell only if the recipient cell is cycling (*i.e.*, dividing) at the time of infection. Retroviruses which have been shown to infect dividing cells exclusively, or more efficiently, include MLV, spleen necrosis virus, Rous sarcoma virus and human immunodeficiency virus (HIV; while HIV infects dividing cells more efficiently, HIV can infect non-dividing cells).

It has been shown that the integration of MLV virus DNA depends upon the host cell's progression through mitosis and it has been postulated that the dependence upon mitosis reflects a requirement for the breakdown of the nuclear envelope in order for the viral integration complex to gain entry into the nucleus (Roe *et al.*, EMBO J. 12:2099 [1993]). However, as integration does not occur in cells arrested in metaphase, the breakdown of the nuclear envelope alone may not be sufficient to permit viral integration; there may be additional requirements such as the state of condensation of the genomic DNA (Roe *et al.*, *supra*).

B. Lentiviral Vectors

The present invention also contemplates the use of lentiviral vectors to generate high copy number cell lines. The lentiviruses (*e.g.*, equine infectious anemia virus, caprine arthritis-encephalitis virus, human immunodeficiency virus) are a subfamily of retroviruses that are able to integrate into non-dividing cells. The lentiviral genome and the proviral DNA have the three genes found in all retroviruses: *gag*, *pol*, and *env*, which

74667-216

are flanked by two LTR sequences. The gag gene encodes the internal structural proteins (*e.g.*, matrix, capsid, and nucleocapsid proteins); the pol gene encodes the reverse transcriptase, protease, and integrase proteins; and the env gene encodes the viral envelope glycoproteins. The 5' and 3' LTRs control transcription and polyadenylation of the viral RNAs. Additional genes in the lentiviral genome include the *vif*, *vpr*, *tat*, *rev*, *vpu*, *nef*, and *vpx* genes.

A variety of lentiviral vectors and packaging cell lines are known in the art and find use in the present invention (*See, e.g.*, U.S. Pat. Nos. 5,994,136 and 6,013,516). Furthermore, the VSV G protein has also been used to pseudotype retroviral vectors based upon the human immunodeficiency virus (HIV) (Naldini *et al.*, Science 272:263 [1996]). Thus, the VSV G protein may be used to generate a variety of pseudotyped retroviral vectors and is not limited to vectors based on MoMLV. The lentiviral vectors may also be modified as described above to contain various regulatory sequences (*e.g.*, signal peptide sequences, RNA export elements, and IRES's). After the lentiviral vectors are produced, they may be used to transfect host cells as described above for retroviral vectors.

C. Adeno-Associated Viral Vectors

The present invention also contemplates the use of adeno associated virus (AAV) vectors to generate high copy number cell lines. AAV is a human DNA parvovirus which belongs to the genus *Dependovirus*. The AAV genome is composed of a linear, single-stranded DNA molecule which contains approximately 4680 bases. The genome includes inverted terminal repeats (ITRs) at each end which function in *cis* as origins of DNA replication and as packaging signals for the virus. The internal nonrepeated portion of the genome includes two large open reading frames, known as the AAV *rep* and *cap* regions, respectively. These regions code for the viral proteins involved in replication and packaging of the virion. A family of at least four viral proteins are synthesized from the AAV *rep* region, Rep 78, Rep 68, Rep 52 and Rep 40, named according to their apparent molecular weight. The AAV *cap* region encodes at least three proteins, VP1, VP2 and VP3 (for a detailed description of the AAV genome, *see e.g.*, Muzyczka, Current Topics Microbiol. Immunol. 158:97-129 [1992]; Kotin, Human Gene Therapy 5:793-801 [1994]).

74667-216

AAV requires coinfection with an unrelated helper virus, such as adenovirus, a herpesvirus or vaccinia, in order for a productive infection to occur. In the absence of such coinfection, AAV establishes a latent state by insertion of its genome into a host cell chromosome. Subsequent infection by a helper virus rescues the integrated copy which can then replicate to produce infectious viral progeny. Unlike the non-pseudotyped retroviruses, AAV has a wide host range and is able to replicate in cells from any species so long as there is coinfection with a helper virus that will also multiply in that species. Thus, for example, human AAV will replicate in canine cells coinfecting with a canine adenovirus. Furthermore, unlike the retroviruses, AAV is not associated with any human or animal disease, does not appear to alter the biological properties of the host cell upon integration and is able to integrate into nondividing cells. It has also recently been found that AAV is capable of site-specific integration into a host cell genome.

In light of the above-described properties, a number of recombinant AAV vectors have been developed for gene delivery (*See, e.g.*, U.S. Patent Nos. 5,173,414; 5,139,941; WO 92/01070 and WO 93/03769; Lebkowski *et al.*, *Molec. Cell. Biol.* 8:3988-3996 [1988]; Carter, B.J., *Current Opinion in Biotechnology* 3:533-539 [1992]; Muzyczka, *Current Topics in Microbiol. and Immunol.* 158:97-129 [1992]; Kotin, R.M. (1994) *Human Gene Therapy* 5:793-801; Shelling and Smith, *Gene Therapy* 1:165-169 [1994]; and Zhou *et al.*, *J. Exp. Med.* 179:1867-1875 [1994]).

Recombinant AAV virions can be produced in a suitable host cell which has been transfected with both an AAV helper plasmid and an AAV vector. An AAV helper plasmid generally includes AAV *rep* and *cap* coding regions, but lacks AAV ITRs. Accordingly, the helper plasmid can neither replicate nor package itself. An AAV vector generally includes a selected gene of interest bounded by AAV ITRs which provide for viral replication and packaging functions. Both the helper plasmid and the AAV vector bearing the selected gene are introduced into a suitable host cell by transient transfection. The transfected cell is then infected with a helper virus, such as an adenovirus, which transactivates the AAV promoters present on the helper plasmid that direct the transcription and translation of AAV *rep* and *cap* regions. Recombinant AAV virions harboring the selected gene are formed and can be purified from the preparation. Once the AAV vectors are produced, they may be used to transfect (*See, e.g.*, U.S. Pat.

74667-216

5,843,742) host cells at the desired multiplicity of infection to produce high copy number host cells. As will be understood by those skilled in the art, the AAV vectors may also be modified as described above to contain various regulatory sequences (*e.g.*, signal peptide sequences, RNA export elements, and IRES's).

D. Transposon Vectors

The present invention also contemplates the use of transposon vectors to generate high copy number cell lines. Transposons are mobile genetic elements that can move or transpose from one location another in the genome. Transposition within the genome is controlled by a transposase enzyme that is encoded by the transposon. Many examples of transposons are known in the art, including, but not limited to, Tn5 (*See e.g.*, de la Cruz *et al.*, J. Bact. 175: 6932-38 [1993], Tn7 (*See e.g.*, Craig, Curr. Topics Microbiol. Immunol. 204: 27-48 [1996]), and Tn10 (*See e.g.*, Morisato and Kleckner, Cell 51:101-111 [1987]). The ability of transposons to integrate into genomes has been utilized to create transposon vectors (*See, e.g.*, U.S. Pat. Nos. 5,719,055; 5,968,785; 5,958,775; and 6,027,722). Because transposons are not infectious, transposon vectors are introduced into host cells via methods known in the art (*e.g.*, electroporation, lipofection, or microinjection). Therefore, the ratio of transposon vectors to host cells may be adjusted to provide the desired multiplicity of infection to produce the high copy number host cells of the present invention.

Transposon vectors suitable for use in the present invention generally comprise a nucleic acid encoding a protein of interest interposed between two transposon insertion sequences. Some vectors also comprise a nucleic acid sequence encoding a transposase enzyme. In these vectors, the one of the insertion sequences is positioned between the transposase enzyme and the nucleic acid encoding the protein of interest so that it is not incorporated into the genome of the host cell during recombination. Alternatively, the transposase enzyme may be provided by a suitable method (*e.g.*, lipofection or microinjection). As will be understood by those skilled in the art, the transposon vectors may also be modified as described above to contain various regulatory sequences (*e.g.*, signal peptide sequences, RNA export elements, and IRES's).

74667-216

E. Transfection at High Multiplicities of Infection

Once integrating vectors (*e.g.*, retroviral vectors) encoding a protein of interest have been produced, they may be used to transfect or transduce host cells (examples of which are described above in Section I). Preferably, host cells are transfected or transduced with integrating vectors at a multiplicity of infection sufficient to result in the integration of at least 1, and preferably at least 2 or more retroviral vectors. In some embodiments, multiplicities of infection of from 10 to 1,000,000 may be utilized, so that the genomes of the infected host cells contain from 2 to 100 copies of the integrated vectors, and preferably from 5 to 50 copies of the integrated vectors. In other embodiments, a multiplicity of infection of from 10 to 10,000 is utilized. When non-pseudotyped retroviral vectors are utilized for infection, the host cells are incubated with the culture medium from the retroviral producers cells containing the desired titer (*i.e.*, colony forming units, CFUs) of infectious vectors. When pseudotyped retroviral vectors are utilized, the vectors are concentrated to the appropriate titer by ultracentrifugation and then added to the host cell culture. Alternatively, the concentrated vectors can be diluted in a culture medium appropriate for the cell type. Additionally, when expression of more than one protein of interest by the host cell is desired, the host cells can be transfected with multiple vectors each containing a nucleic acid encoding a different protein of interest.

In each case, the host cells are exposed to medium containing the infectious retroviral vectors for a sufficient period of time to allow infection and subsequent integration of the vectors. In general, the amount of medium used to overlay the cells should be kept to as small a volume as possible so as to encourage the maximum amount of integration events per cell. As a general guideline, the number of colony forming units (cfu) per milliliter should be about 10^5 to 10^7 cfu/ml, depending upon the number of integration events desired.

The present invention is not limited to any particular mechanism of action. Indeed, an understanding of the mechanism of action is not necessary for practicing the present invention. However, the diffusion rate of the vectors is known to be very limited (*See, e.g.*, U.S. Pat. No. 5,866,400, for a discussion of diffusion rates). Therefore, it is expected that the actual integration rate will be lower (and in some cases much lower) than the multiplicity of infection. Applying the equations from U.S. Pat. No. 5,866,400, a titer of 10^6 cfu/ml has an average vector-vector

spacing of 1 micron. The diffusion time of a MMLV vector across 100 microns is approximately 20 minutes. Accordingly, the vector can travel approximately 300 microns in one hour. If 1000 cells are plated in a T25 flask, the cells are spaced 2.5 mm apart on average. Using these values, the only 56 viral particles would be expected to contact a given cell within an hour. The Table below provides the expected contact rate for a given number of cells in a T25 flask with a particular vector titer. However, as shown below in the examples, the actual number of integrations obtained is much lower than may be predicted by these equations.

Vector Contact Frequency As A Function of Time and Cell Spacing			
Vector Titer	Cells/T25 Flask	MOI	Contacts/Hour
10^6	1000	1,000	56
10^6	100	10,000	<56
10^5	1000	100	5.6
10^4	1000	10	0.6

Accordingly, it is contemplated that the actual integration rate is dependent not only on the multiplicity of infection, but also on the contact time (*i.e.*, the length of time the host cells are exposed to infectious vector), the confluency or geometry of the host cells being transfected, and the volume of media that the vectors are contained in. It is contemplated that these conditions can be varied as taught herein to produce host cell lines containing multiple integrated copies of integrating vectors. As demonstrated in Examples 8 and 9, MOI can be varied by either holding the number of cells constant and varying CFU's (Example 9), or by holding CFU's constant and varying cell number (Example 8).

In some embodiments, after transfection or transduction, the cells are allowed to multiply, and are then trypsinized and replated. Individual colonies are then selected to provide clonally selected cell lines. In still further embodiments, the clonally selected cell lines are screened by Southern blotting or INVADER assay to verify that the desired number of integration events has occurred. It is also contemplated that clonal selection

allows the identification of superior protein producing cell lines. In other embodiments, the cells are not clonally selected following transfection.

In some embodiments, the host cells are transfected with vectors encoding different proteins of interest. The vectors encoding different proteins of interest can be used to transfect the cells at the same time (*e.g.*, the host cells are exposed to a solution containing vectors encoding different proteins of interest) or the transfection can be serial (*e.g.*, the host cells are first transfected with a vector encoding a first protein of interest, a period of time is allowed to pass, and the host cells are then transfected with a vector encoding a second protein of interest). In some preferred embodiments, the host cells are transfected with an integrating vector encoding a first protein of interest, high expressing cell lines containing multiple integrated copies of the integrating vector are selected (*e.g.*, clonally selected), and the selected cell line is transfected with an integrating vector encoding a second protein of interest. This process may be repeated to introduce multiple proteins of interest. In some embodiments, the multiplicities of infection may be manipulated (*e.g.*, increased or decreased) to increase or decrease the expression of the protein of interest. Likewise, the different promoters may be utilized to vary the expression of the proteins of interest. It is contemplated that these transfection methods can be used to construct host cell lines containing an entire exogenous metabolic pathway or to provide host cells with an increased capability to process proteins (*e.g.*, the host cells can be provided with enzymes necessary for post-translational modification).

In still further embodiments, cell lines are serially transfected with vectors encoding the same gene. In some preferred embodiments, the host cells are transfected (*e.g.*, at an MOI of about 10 to 100,000, preferably 100 to 10,000) with an integrating vector encoding a protein of interest, cell lines containing single or multiple integrated copies of the integrating vector or expressing high levels of the desired protein are selected (*e.g.*, clonally selected), and the selected cell line is retransfected with the vector (*e.g.*, at an MOI of about 10 to 100,000, preferably 100 to 10,000). In some embodiments, cell lines comprising at least two integrated copies of the vector are identified and selected. This process may be repeated multiple times until the desired level of protein expression is obtained and may also be repeated to introduce vectors encoding multiple proteins of interest. Unexpectedly, serial transfection with the same gene results in increases in protein production from the resulting cells that are not merely additive.

III. Uses of Transfected Host Cells

The host cells transfected at a high multiplicity of infection can be used for a variety of purposes. First, the host cells find use in the production of proteins for pharmaceutical, industrial, diagnostic, and other purposes. Second, host cells expressing a particular protein or proteins find use in screening assays (e.g., high throughput screening). Third, the host cells find use in the production of multiple variants of proteins, followed by analysis of the activity of the protein variants. Each of these uses is explained in more detail below.

A. Production of Proteins

It is contemplated that the host cells of the present invention find use in the production of proteins for pharmaceutical, industrial, diagnostic, and other uses. The present invention is not limited to the production of any particular protein. Indeed, the production of a wide variety of proteins is contemplated, including, but not limited to, erythropoietin, alpha-interferon, alpha-1 proteinase inhibitor, angiogenin, antithrombin III, beta-acid decarboxylase, human growth hormone, bovine growth hormone, porcine growth hormone, human serum albumin, beta-interferon, calf intestine alkaline phosphatase, cystic fibrosis transmembrane regulator, Factor VIII, Factor IX, Factor X, insulin, lactoferrin, tissue plasminogen activator, myelin basic protein, insulin, proinsulin, prolactin, hepatitis B antigen, immunoglobulins, monoclonal antibody CTLA4 Ig, Tag 72 monoclonal antibody, Tag 72 single chain antigen binding protein, protein C, cytokines and their receptors, including, for instance tumor necrosis factors alpha and beta, their receptors and their derivatives; renin; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; von Willebrands factor; atrial natriuretic factor; lung surfactant; urokinase; bombesin; thrombin; hemopoietic growth factor; enkephalinase; human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-beta; platelet-derived growth

factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF- α and TGF- β , including TGF- β 1, TGF- β 2, TGF- β 3, TGF- β 4, or TGF- β 5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulinlike growth factor binding proteins; CD proteins such as CD-3, CD-4, CD-8, and CD-19; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon- α , - β , and - γ ; colony stimulating factors (CSFs), *e.g.*, M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.*, IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; antibodies; chimeric proteins, such as immunoadhesins, and fragments of any of the above-listed polypeptides. Nucleic acid and protein sequences for these proteins are available in public databases such as GenBank.

In some embodiments, the host cells express more than one exogenous protein. For example, the host cells may be transfected vectors encoding different proteins of interest (*e.g.*, cotransfection or infection at a multiplicity of infection of 1000 with one vector encoding a first protein of interest and a second vector encoding a second protein of interest or serial transfection or infection) so that the host cell contains at least one integrated copy of a first vector encoding a first protein of interest and at least one integrated copy of second integrating vector encoding a second protein of interest. In other embodiments, more than one protein is expressed by arranging the nucleic acids encoding the different proteins of interest in a polycistronic sequence (*e.g.*, bicistronic or tricistronic sequences). This arrangement is especially useful when expression of the different proteins of interest in about a 1:1 molar ratio is desired (*e.g.*, expressing the light and heavy chains of an antibody molecule).

In still further embodiments, ribozymes are expressed in the host cells. It is contemplated that the ribozyme can be utilized for down-regulating expression of a particular gene or used in conjunction with gene switches such as TET, ecdysone, glucocorticoid enhancer, etc. to provide host cells with various phenotypes.

The transfected host cells are cultured according to methods known in the art. Suitable culture conditions for mammalian cells are well known in the art (*See e.g.*, J. Immunol. Methods (1983)56:221-234 [1983], *Animal Cell Culture: A Practical Approach 2nd Ed.*, Rickwood, D. and Hames, B. D., eds. Oxford University Press, New York

74667-216

[1992]).

The host cell cultures of the present invention are prepared in a media suitable for the particular cell being cultured. Commercially available media such as Ham's F10 (Sigma, St. Louis, MO), Minimal Essential Medium (MEM, Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM, Sigma) are exemplary nutrient solutions. Suitable media are also described in U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 5,122,469; 4,560,655; and WO 90/03430 and WO 87/00195.

Any of these media may be

supplemented as necessary with serum, hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as gentamycin (gentamicin), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range) lipids (such as linoleic or other fatty acids) and their suitable carriers, and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. For mammalian cell culture, the osmolality of the culture medium is generally about 290-330 mOsm.

The present invention also contemplates the use of a variety of culture systems (*e.g.*, petri dishes, 96 well plates; roller bottles, and bioreactors) for the transfected host cells. For example, the transfected host cells can be cultured in a perfusion system. Perfusion culture refers to providing a continuous flow of culture medium through a culture maintained at high cell density. The cells are suspended and do not require a solid support to grow on. Generally, fresh nutrients must be supplied continuously with concomitant removal of toxic metabolites and, ideally, selective removal of dead cells. Filtering, entrapment and micro-capsulation methods are all suitable for refreshing the culture environment at sufficient rates.

As another example, in some embodiments a fed batch culture procedure can be employed. In the preferred fed batch culture the mammalian host, cells and culture medium are supplied to a culturing vessel initially and additional culture nutrients are fed, continuously or in discrete increments, to the culture during culturing, with or without periodic cell and/or product harvest before termination of culture. The fed batch culture can include, for example, a semi-continuous fed batch culture, wherein

periodically whole culture (including cells and medium) is removed and replaced by fresh medium. Fed batch culture is distinguished from simple batch culture in which all components for cell culturing (including the cells and all culture nutrients) are supplied to the culturing vessel at the start of the culturing process. Fed batch culture can be further distinguished from perfusion culturing insofar as the supernate is not removed from the culturing vessel during the process (in perfusion culturing, the cells are restrained in the culture by, *e.g.*, filtration, encapsulation, anchoring to microcarriers etc. and the culture medium is continuously or intermittently introduced and removed from the culturing vessel). In some particularly preferred embodiments, the batch cultures are performed in roller bottles.

Further, the cells of the culture may be propagated according to any scheme or routine that may be suitable for the particular host cell and the particular production plan contemplated. Therefore, the present invention contemplates a single step or multiple step culture procedure. In a single step culture the host cells are inoculated into a culture environment and the processes of the instant invention are employed during a single production phase of the cell culture. Alternatively, a multi-stage culture is envisioned. In the multi-stage culture cells may be cultivated in a number of steps or phases. For instance, cells may be grown in a first step or growth phase culture wherein cells, possibly removed from storage, are inoculated into a medium suitable for promoting growth and high viability. The cells may be maintained in the growth phase for a suitable period of time by the addition of fresh medium to the host cell culture.

Fed batch or continuous cell culture conditions are devised to enhance growth of the mammalian cells in the growth phase of the cell culture. In the growth phase cells are grown under conditions and for a period of time that is maximized for growth. Culture conditions, such as temperature, pH, dissolved oxygen (dO_2) and the like, are those used with the particular host and will be apparent to the ordinarily skilled artisan. Generally, the pH is adjusted to a level between about 6.5 and 7.5 using either an acid (*e.g.*, CO_2) or a base (*e.g.*, Na_2CO_3 or NaOH). A suitable temperature range for culturing mammalian cells such as CHO cells is between about 30° to 38° C and a suitable dO_2 is between 5-90% of air saturation.

Following the polypeptide production phase, the polypeptide of interest is recovered from the culture medium using techniques which are well established in the art. The protein of interest preferably is recovered from the culture medium as a secreted

polypeptide (*e.g.*, the secretion of the protein of interest is directed by a signal peptide sequence), although it also may be recovered from host cell lysates. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The polypeptide thereafter is purified from contaminant soluble proteins and polypeptides, with the following procedures being exemplary of suitable purification procedures: by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; and protein A Sepharose columns to remove contaminants such as IgG. A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification. Additionally, the protein of interest can be fused in frame to a marker sequence which allows for purification of the protein of interest. Non-limiting examples of marker sequences include a hexahistidine tag which may be supplied by a vector, preferably a pQE-9 vector, and a hemagglutinin (HA) tag. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (*See e.g.*, Wilson *et al.*, Cell, 37:767 [1984]). One skilled in the art will appreciate that purification methods suitable for the polypeptide of interest may require modification to account for changes in the character of the polypeptide upon expression in recombinant cell culture.

The host cells of the present invention are also useful for expressing G-protein coupled receptors (GPCRs) and other transmembrane proteins. It is contemplated that when these proteins are expressed, they are correctly inserted into the membrane in their native conformation. Thus, GPCRs and other transmembrane proteins may be purified as part of a membrane fraction or purified from the membranes by methods known in the art.

Furthermore, the vectors of the present invention are useful for co-expressing a protein of interest for which there is no assay or for which assays are difficult. In this system, a protein of interest and a signal protein are arranged in a polycistronic sequence. Preferably, an IRES sequence separates the signal protein and protein of interest (*e.g.*, a GPCR) and the genes encoding the signal protein and protein of interest are expressed as a single transcriptional unit. The present invention is not limited to any particular signal protein. Indeed, the use of a variety of signal proteins for which easy assays exist is contemplated. These signal proteins include, but are not limited to, green fluorescent

74667-216

protein, luciferase, beta-galactosidase, and antibody heavy or light chains. It is contemplated that when the signal protein and protein of interest are co-expressed from a polycistronic sequence, the presence of the signal protein is indicative of the presence of the protein of interest. Accordingly, in some embodiments, the present invention provides methods for indirectly detecting the expression of a protein of interest comprising providing a host cell transfected with a vector encoding a polycistronic sequence, wherein the polycistronic sequence comprises a signal protein and a protein of interest operably linked by an IRES, and culturing the host cells under conditions such that the signal protein and protein of interest are produced, wherein the presence of the signal protein indicates the presence of the protein of interest.

B. Screening Compounds for Activity

The present invention contemplates the use of the high copy number cell lines for screening compounds for activity, and in particular to high throughput screening of compounds from combinatorial libraries (*e.g.*, libraries containing greater than 10^4 compounds). The high copy number cell lines of the present invention can be used in a variety of screening methods. In some embodiments, the cells can be used in second messenger assays that monitor signal transduction following activation of cell-surface receptors. In other embodiments, the cells can be used in reporter gene assays that monitor cellular responses at the transcription/translation level. In still further embodiments, the cells can be used in cell proliferation assays to monitor the overall growth/no growth response of cells to external stimuli.

In second messenger assays, the host cells are preferably transfected as described above with vectors encoding cell surface receptors, ion channels, cytoplasmic receptors, or other proteins involved in signal transduction (*e.g.*, G proteins, protein kinases, or protein phosphatases) (*See, e.g.*, U.S. Pat. Nos. 5,670,113; 5,807,689; 5,876,946; and 6,027,875). The host cells are then treated with a compound or plurality of compounds (*e.g.*, from a combinatorial library) and assayed for the presence or absence of a response. It is contemplated that at least some of the compounds in the combinatorial library can serve as agonists, antagonists, activators, or inhibitors of the protein or proteins encoded by the vectors. It is also contemplated that at least some of the compounds in the combinatorial library can serve

as agonists, antagonists, activators, or inhibitors of protein acting upstream or downstream of the protein encoded by the vector in a signal transduction pathway.

By way of non-limiting example, it is known that agonist engaged transmembrane receptors are functionally linked to the modulation of several well characterized promoter/enhancer elements (*e.g.*, AP1, cAMP response element (CRE), serum response element (SRE), and nuclear factor of activated T-cells (NF-AT)). Upon activation of a $G_{\alpha s}$ coupling receptor, adenylyl cyclase is stimulated, producing increased concentrations of intracellular cAMP, stimulation of protein kinase A, phosphorylation of the CRE binding protein (CREB) and induction of promoters with CRE elements. $G_{\alpha i}$ coupling receptors dampen CRE activity by inhibition of the same signal transduction components. $G_{\alpha q}$ and some $\beta\gamma$ pairs stimulate phospholipase C (PLC), and the generation of inositol triphosphate (IP3) and diacylglycerol (DAG). A transient flux in intracellular calcium promotes induction of calcineurin and NF-AT, as well as calmodulin (CaM)-dependent kinase and CREB. Increased DAG concentrations stimulate protein kinase C (PKC) and endosomal/lysosomal acidic sphingomyelinase (aSMase); while the aSMase pathway is dominant, both induce degradation of the NF κ B inhibitor I κ B as well as NF κ B activation. In an alternative pathway, a receptor such as growth factor receptor is activated and recruits Sos to the plasma membrane, resulting in the stimulation of Ras, which in turn recruits the serine/threonine kinase Raf to the plasma membrane. Once activated, Raf phosphorylates MEK kinase, which phosphorylates and activates MAPK and the transcription factor ELK. ELK drives transcription from promoters with SRE elements, leading the synthesis of the transcription factors Fos and Jun, thus forming a transcription factor complex capable of activating AP1 sites. It is contemplated that the proteins forming the described pathways, as well as other receptors, kinases, phosphatases, and nucleic binding proteins, are targets for compounds in the combinatorial library, as well as candidates for expression in the host cells of the present invention.

In some embodiments, the second messenger assays measure fluorescent signals from reporter molecules that respond to intracellular changes (*e.g.*, Ca^{2+} concentration, membrane potential, pH, IP₃, cAMP, arachidonic acid release) due to stimulation of membrane receptors and ion channels (*e.g.*, ligand gated ion channels; *see Denyer et al.*, Drug Discov. Today 3:323-32 [1998]; and Gonzales *et al.*, Drug. Discov. Today 4:431-39 [1999]). Examples of reporter molecules include, but are not limited to, FRET

(fluorescence resonance energy transfer) systems (*e.g.*, Cuo-lipids and oxonols, EDAN/DABCYL), calcium sensitive indicators (*e.g.*, Fluo-3, FURA 2, INDO 1, and FLUO3/AM, BAPTA AM), chloride-sensitive indicators (*e.g.*, SPQ, SPA), potassium-sensitive indicators (*e.g.*, PBFI), sodium-sensitive indicators (*e.g.*, SBFI), and pH sensitive indicators (*e.g.*, BCECF).

In general, the host cells are loaded with the indicator prior to exposure to the compound. Responses of the host cells to treatment with the compounds can be detected by methods known in the art, including, but not limited to, fluorescence microscopy, confocal microscopy (*e.g.*, FCS systems), flow cytometry, microfluidic devices, FLIPR systems (*See, e.g.*, Schroeder and Neagle, J. Biomol. Screening 1:75-80 [1996]), and plate-reading systems. In some preferred embodiments, the response (*e.g.*, increase in fluorescent intensity) caused by compound of unknown activity is compared to the response generated by a known agonist and expressed as a percentage of the maximal response of the known agonist. The maximum response caused by a known agonist is defined as a 100% response. Likewise, the maximal response recorded after addition of an agonist to a sample containing a known or test antagonist is detectably lower than the 100% response.

The cells are also useful in reporter gene assays. Reporter gene assays involve the use of host cells transfected with vectors encoding a nucleic acid comprising transcriptional control elements of a target gene (*i.e.*, a gene that controls the biological expression and function of a disease target) spliced to a coding sequence for a reporter gene. Therefore, activation of the target gene results in activation of the reporter gene product. Examples of reporter genes finding use in the present invention include, but are not limited to, chloramphenicol transferase, alkaline phosphatase, firefly and bacterial luciferases, β -galactosidase, β -lactamase, and green fluorescent protein. The production of these proteins, with the exception of green fluorescent protein, is detected through the use of chemiluminescent, colorimetric, or bioluminescent products of specific substrates (*e.g.*, X-gal and luciferin). Comparisons between compounds of known and unknown activities may be conducted as described above.

C. Comparison of Variant Protein Activity

The present invention also contemplates the use of the high copy number host cells to produce variants of proteins so that the activity of the variants can be compared. In some embodiments, the variants differ by a single nucleotide polymorphism (SNP) causing a single amino acid difference. In other embodiments, the variants contain multiple amino acid substitutions. In some embodiments, the activity of the variant proteins are assayed *in vivo* or in cell extracts. In other embodiments, the proteins are purified and assayed *in vitro*. It is also contemplated that in some embodiments the variant proteins are fused to a sequence that allows easy purification (*e.g.*, a his-tag sequence) or to a reporter gene (*e.g.*, green fluorescent protein). Activity of the proteins may be assayed by appropriate methods known in the art (*e.g.*, conversion of a substrate to a product). In some preferred embodiments, the activity of a wild-type protein is determined, and the activity of variant versions of the wild-type proteins are expressed as a percentage of the activity of the wild-type protein. Furthermore, the intracellular activity of variant proteins may be compared by constructing a plurality of host cells lines, each of which expresses a different variant of the wild-type protein. The activity of the variant proteins (*e.g.*, variants of proteins involved in signal transduction pathways) may then be compared using the reporter systems for second messenger assays described above. Therefore, in some embodiments, the direct or indirect response (*e.g.*, through downstream or upstream activation of signal transduction pathway) of variant proteins to stimulation or binding by agonists or antagonists is compared. In some preferred embodiments, the response of a wild-type protein is determined, and the responses of variant versions of the wild-type proteins are expressed as a percentage of the response of the wild-type protein.

EXPERIMENTAL

The following examples serve to illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

In the experimental disclosure which follows, the following abbreviations apply: M (molar); mM (millimolar); μ M (micromolar); nM (nanomolar); mol (moles); mmol (millimoles); μ mol (micromoles); nmol (nanomoles); gm (grams); mg (milligrams); μ g (micrograms); pg (picograms); L (liters); ml (milliliters); μ l (microliters); cm

74667-216

(centimeters); mm (millimeters); μ m (micrometers); nm (nanometers); °C (degrees Centigrade); AMP (adenosine 5'-monophosphate); BSA (bovine serum albumin); cDNA (copy or complimentary DNA); CS (calf serum); DNA (deoxyribonucleic acid); ssDNA (single stranded DNA); dsDNA (double stranded DNA); dNTP (deoxyribonucleotide triphosphate); LH (luteinizing hormone); NIH (National Institutes of Health, Bethesda, MD); RNA (ribonucleic acid); PBS (phosphate buffered saline); *g* (gravity); OD (optical density); HEPES (N-[2-Hydroxyethyl]piperazine-N-[2-ethanesulfonic acid]); HBS (HEPES buffered saline); PBS (phosphate buffered saline); SDS (sodium dodecylsulfate); Tris-HCl (tris[Hydroxymethyl]aminomethane-hydrochloride); Klenow (DNA polymerase I large (Klenow) fragment); rpm (revolutions per minute); EGTA (ethylene glycol-bis(β -aminoethyl ether) N, N, N', N'-tetraacetic acid); EDTA (ethylenediaminetetracetic acid); bla (β -lactamase or ampicillin-resistance gene); ORI (plasmid origin of replication); lacI (lac repressor); X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside); ATCC (American Type Culture Collection, Rockville, MD); GIBCO/BRL (GIBCO/BRL, Grand Island, NY); Perkin-Elmer (Perkin-Elmer, Norwalk, CT); and Sigma (Sigma Chemical Company, St. Louis, MO).

Example 1

Vector Construction

The following Example describes the construction of vectors used in the experiments below.

A. CMV MN14

The CMV MN14 vector (SEQ ID NO:4; MN14 antibody is described in U.S. Pat. No. 5,874,540) comprises the following elements, arranged in 5' to 3' order: CMV promoter; MN14 heavy chain signal peptide, MN14 antibody heavy chain; IRES from encephalomyocarditis virus; bovine α -lactalbumin signal peptide; MN 14 antibody light chain; and 3' MoMuLV LTR. In addition to sequences described in SEQ ID NO: 4, the CMV MN14 vector further comprises a 5' MoMuLV LTR, a MoMuLV extended viral packaging signal, and a neomycin phosphotransferase gene (these additional elements are provided in SEQ ID NO:7; the 5' LTR is derived from Moloney Murine Sarcoma Virus in each of the constructs described herein, but is converted to the MoMuLV 5' LTR when integrated).

This construct uses the 5' MoMuLV LTR to control production of the neomycin phosphotransferase gene. The expression of MN14 antibody is controlled by the CMV promoter. The MN14 heavy chain gene and light chain gene are attached together by an IRES sequence. The CMV promoter drives production of a mRNA containing the heavy chain gene and the light chain gene attached by the IRES. Ribosomes attach to the mRNA at the CAP site and at the IRES sequence. This allows both heavy and light chain protein to be produced from a single mRNA. The mRNA expression from the LTR as well as from the CMV promoter is terminated and poly adenylated in the 3' LTR. The construct was cloned by similar methods as described in section B below.

The IRES sequence (SEQ ID NO:3) comprises a fusion of the IRES from the plasmid pLXIN (Clontech) and the bovine α -lactalbumin signal peptide. The initial ATG of the signal peptide was attached to the IRES to allow the most efficient translation initiation from the IRES. The 3' end of the signal peptide provides a multiple cloning site allowing easy attachment of any protein of interest to create a fusion protein with the signal peptide. The IRES sequence can serve as a translational enhancer as well as creating a second translation initiation site that allows two proteins to be produced from a single mRNA.

The IRES-bovine α -lactalbumin signal peptide was constructed as follows. The portion of the plasmid pLXIN (Clontech, Palo Alto, CA) containing the ECMV IRES was PCR amplified using the following primers.

Primer 1 (SEQ ID NO: 35):

5' GATCCACTAGTAACGGCCGCCAGAATTCGC 3'

Primer 2 (SEQ ID NO: 36):

5' CAGAGAGACAAAGGAGGCCATATTATCATCGTGTTTTTCAAAG 3'

Primer 2 attaches a tail corresponding to the start of the bovine α -lactalbumin signal peptide coding region to the IRES sequence. In addition, the second triplet codon of the α -lactalbumin signal peptide was mutated from ATG to GCC to allow efficient translation from the IRES sequence. This mutation results in a methionine to alanine change in the protein sequence. This mutation was performed because the IRES prefers an alanine as the second amino acid in the protein chain. The resulting IRES PCR

product contains an EcoRI site on the 5' end of the fragment (just downstream of Primer 1 above).

Next, the α -lactalbumin signal peptide containing sequence was PCR amplified from the α -LA Signal Peptide vector construct using the following primers.

Primer 3 (SEQ ID NO: 14):

5' CTTTGAAAAACACGATGATAATATGGCCTCCTTTGTCTCTCTG 3'

Primer 4 (SEQ ID NO: 15):

5' TTCGCGAGCTCGAGATCTAGATATCCCATG 3'

Primer 3 attaches a tail corresponding to the 3' end of the IRES sequence to the α -lactalbumin signal peptide coding region. As stated above, the second triplet codon of the bovine α -lactalbumin signal peptide was mutated to allow efficient translation from the IRES sequence. The resulting signal peptide PCR fragment contains NaeI, NcoI, EcoRV, XbaI, BglII and XhoI sites on the 3' end.

After the IRES and signal peptide were amplified individually using the primers shown above, the two reaction products were mixed and PCR was performed using primer 1 and primer 4. The resultant product of this reaction is a spliced fragment that contains the IRES attached to the full length α -lactalbumin signal peptide. The ATG encoding the start of the signal peptide is placed at the same location as the ATG encoding the start of the neomycin phosphotransferase gene found in the vector pLXIN. The fragment also contains the EcoRI site on the 5' end and NaeI, NcoI, EcoRV, XbaI, BglII and XhoI sites on the 3' end.

The spliced IRES/ α -lactalbumin signal peptide PCR fragment was digested with EcoRI and XhoI. The α -LA Signal Peptide vector construct was also digested with EcoRI and XhoI. These two fragments were ligated together to give the pIRES construct.

The IRES/ α -lactalbumin signal peptide portion of the pIRES vector was sequenced and found to contain mutations in the 5' end of the IRES. These mutations occur in a long stretch of C's and were found in all clones that were isolated.

To repair this problem, pLXIN DNA was digested with EcoRI and BsmFI. The 500bp band corresponding to a portion of the IRES sequence was isolated. The mutated

IRES/ α -lactalbumin signal peptide construct was also digested with EcoRI and BsmFI and the mutated IRES fragment was removed. The IRES fragment from pLXIN was then substituted for the IRES fragment of the mutated IRES/ α -lactalbumin signal peptide construct. The IRES/ α -LA signal peptide portion of resulting plasmid was then verified by DNA sequencing.

The resulting construct was found to have a number of sequence differences when compared to the expected pLXIN sequence obtained from Clontech. We also sequenced the IRES portion of pLXIN purchased from Clontech to verify its sequence. The differences from the expected sequence also appear to be present in the pLXIN plasmid that we obtained from Clontech. Four sequence differences were identified:

bp 347 T - was G in pLXIN sequence

bp 786-788 ACG - was GC in LXIN sequence.

B. CMV LL2

The CMV LL2 (SEQ ID NO:5; LL2 antibody is described in U.S. Pat. No. 6,187,287, incorporated herein by reference) construct comprises the following elements, arranged in 5' to 3' order: 5' CMV promoter (Clontech), LL2 heavy chain signal peptide, LL2 antibody heavy chain; IRES from encephalomyocarditis virus; bovine α -LA signal peptide; LL2 antibody light chain; and 3' MoMuLV LTR. In addition to sequences described in SEQ ID NO:5, the CMV LL2 vector further comprises a 5' MoMuLV LTR, a MoMuLV extended viral packaging signal, and a neomycin phosphotransferase gene (these additional elements are provided in SEQ ID NO:7).

This construct uses the 5' MoMuLV LTR to control production of the neomycin phosphotransferase gene. The expression of LL2 antibody is controlled by the CMV promoter (Clontech). The LL2 heavy chain gene and light chain gene are attached together by an IRES sequence. The CMV promoter drives production of a mRNA containing the heavy chain gene and the light chain gene attached by the IRES. Ribosomes attach to the mRNA at the CAP site and at the IRES sequence. This allows both heavy and light chain protein to be produced from a single mRNA. The mRNA expression from the LTR as well as from the CMV promoter is terminated and polyadenylated in the 3' LTR.

The IRES sequence (SEQ ID NO:3) comprises a fusion of the IRES from the plasmid pLXIN (Clontech) and the bovine alpha-lactalbumin signal peptide. The initial

ATG of the signal peptide was attached to the IRES to allow the most efficient translation initiation from the IRES. The 3' end of the signal peptide provides a multiple cloning site allowing easy attachment of any protein of interest to create a fusion protein with the signal peptide. The IRES sequence can serve as a translational enhancer as well as creating a second translation initiation site that allows two proteins to be produced from a single mRNA.

The LL2 light chain gene was attached to the IRES α -lactalbumin signal peptide as follows. The LL2 light chain was PCR amplified from the vector pCRLL2 using the following primers.

Primer 1 (SEQ ID NO: 16):

5' CTACAGGTGTCCACGTCGACATCCAGCTGACCCAG 3'

Primer 2 (SEQ ID NO: 17):

5' CTGCAGAATAGATCTCTAACACTCTCCCCTGTTG 3'

These primers add a HincII site right at the start of the coding region for mature LL2 light chain. Digestion of the PCR product with HincII gives a blunt end fragment starting with the initial GAC encoding mature LL2 on the 5' end. Primer 2 adds a BglII site to the 3' end of the gene right after the stop codon. The resulting PCR product was digested with HincII and BglII and cloned directly into the IRES-Signal Peptide plasmid that was digested with NaeI and BglII.

The Kozak sequence of the LL2 heavy chain gene was then modified. The vector pCRMN14HC was digested with XhoI and AvrII to remove about a 400 bp fragment. PCR was then used to amplify the same portion of the LL2 heavy chain construct that was removed by the XhoI-AvrII digestion. This amplification also mutated the 5' end of the gene to add a better Kozak sequence to the clone. The Kozak sequence was modified to resemble the typical IgG Kozak sequence. The PCR primers are shown below.

Primer 1 (SEQ ID NO: 18):

5'CAGTGTGATCTCGAGAATTCAGGACCTCACCATGGGATGGAGCTGTATCAT 3'

Primer 2 (SEQ ID NO: 19):

5'AGGCTGTATTGGTGGATTCGTCT 3'

The PCR product was digested with XhoI and AvrII and inserted back into the previously digested plasmid backbone.

The "good" Kozak sequence was then added to the light chain gene. The "good" Kozak LL2 heavy chain gene construct was digested with EcoRI and the heavy chain gene containing fragment was isolated. The IRES α -Lactalbumin Signal Peptide LL2 light chain gene construct was also digested with EcoRI. The heavy chain gene was then cloned into the EcoRI site of IRES light chain construct. This resulted in the heavy chain gene being placed at the 5' end of the IRES sequence.

Next, a multiple cloning site was added into the LNCX retroviral backbone plasmid. The LNCX plasmid was digested with HindIII and ClaI. Two oligonucleotide primers were produced and annealed together to create a double stranded DNA multiple cloning site. The following primers were annealed together.

Primer 1 (SEQ ID NO: 20):

5'AGCTTCTCGAGTTAACAGATCTAGGCCTCCTAGGTCGACAT 3'

Primer 2 (SEQ ID NO: 21): 5'

CGATGTCGACCTAGGAGGCCTAGATCTGTAACTCGAGA 3'

After annealing, the multiple cloning site was ligated into LNCX to create LNC-MCS.

Next, the double chain gene fragment was ligated into the retroviral backbone gene construct. The double chain gene construct created above was digested with SalI and BglII and the double chain containing fragment was isolated. The retroviral expression plasmid LNC-MCS was digested with XhoI and BglII. The double chain fragment was then cloned into the LNC-MCS retroviral expression backbone.

Next, an RNA splicing problem in the construct was corrected. The construct was digested with NsiI. The resulting fragment was then partially digested with EcoRI. The fragments resulting from the partial digest that were approximately 9300 base pairs in size were gel purified. A linker was created to mutate the splice donor site at the 3' end of the LL2 heavy chain gene. The linker was again created by annealing two

oligonucleotide primers together to form the double stranded DNA linker. The two primers used to create the linker are shown below.

Primer 1 (SEQ ID NO: 22):

5'CGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCCGGGA
AATGAAAGCCG 3'

Primer 2 (SEQ ID NO: 23):

5'AATTCGGCTTTCATTTCCCGGGAGACAGGGAGAGGCTCTTCTGCGTGTAAGTGG
TTGTGCAGAGCCTCGTGCA 3'

After annealing the linker was substituted for the original NsiI/EcoRI fragment that was removed during the partial digestion.

C. MMTV MN14

The MMTV MN14 (SEQ ID NO:6) construct comprises the following elements, arranged in 5' to 3' order: 5' MMTV promoter; double mutated PPE sequence; MN 14 antibody heavy chain; IRES from encephalomyocarditis virus; bovine α LA signal peptide MN 14 antibody light chain; WPRE sequence; and 3' MoMuLV LTR. In addition to the sequences described in SEQ ID NO:6, the MMTV MN14 vector further comprises a MoMuLV LTR, MoMuLV extended viral packaging signal; neomycin phosphotransferase gene located 5' of the MMTV promoter (these additional elements are provided in SEQ ID NO: 7).

This construct uses the 5' MoMuLV LTR to control production of the neomycin phosphotransferase gene. The expression of MN14 antibody is controlled by the MMTV promoter (Pharmacia). The MN14 heavy chain gene and light chain gene are attached together by an IRES/ bovine α -LA signal peptide sequence (SEQ ID NO: 3). The MMTV promoter drives production of a mRNA containing the heavy chain gene and the light chain gene attached by the IRES/bovine α -LA signal peptide sequence. Ribosomes attach to the mRNA at the CAP site and at the IRES/ bovine α -LA signal peptide sequence. This allows both heavy and light chain protein to be produced from a single mRNA. In addition, there are two genetic elements contained within the mRNA to aid in export of the mRNA from the nucleus to the cytoplasm and aid in poly-adenylation of

the mRNA. The PPE sequence is contained between the RNA CAP site and the start of the MN14 protein coding region, the WPRE is contained between the end of MN14 protein coding and the poly-adenylation site. The mRNA expression from the LTR as well as from the MMTV promoter is terminated and poly-adenylated in the 3' LTR.

ATG sequences within the PPE element (SEQ ID NO:2) were mutated to prevent potential unwanted translation initiation. Two copies of this mutated sequence were used in a head to tail array. This sequence is placed just downstream of the promoter and upstream of the Kozak sequence and signal peptide-coding region. The WPRE is isolated from woodchuck hepatitis virus and also aids in the export of mRNA from the nucleus and creating stability in the mRNA. If this sequence is included in the 3' untranslated region of the RNA, level of protein expression from this RNA increases up to 10-fold.

D. α -LA MN14

The α -LA MN14 (SEQ ID NO:7) construct comprises the following elements, arranged in 5' to 3' order: 5' MoMuLV LTR, MoMuLV extended viral packaging signal, neomycin phosphotransferase gene, bovine/human alpha-lactalbumin hybrid promoter, double mutated PPE element, MN14 heavy chain signal peptide, MN14 antibody heavy chain, IRES from encephalomyocarditis virus/bovine α LA signal peptide, MN14 antibody light chain, WPRE sequence; and 3' MoMuLV LTR.

This construct uses the 5' MoMuLV LTR to control production of the neomycin phosphotransferase gene. The expression of MN14 antibody is controlled by the hybrid α -LA promoter (SEQ ID NO:1). The MN14 heavy chain gene and light chain gene are attached together by an IRES sequence/ bovine α -LA signal peptide (SEQ ID NO:3). The α -LA promoter drives production of a mRNA containing the heavy chain gene and the light chain gene attached by the IRES. Ribosomes attach to the mRNA at the CAP site and at the IRES sequence. This allows both heavy and light chain protein to be produced from a single mRNA.

In addition, there are two genetic elements contained within the mRNA to aid in export of the mRNA from the nucleus to the cytoplasm and aid in poly-adenylation of the mRNA. The mutated PPE sequence (SEQ ID NO:2) is contained between the RNA CAP site and the start of the MN14 protein coding region. ATG sequences within the PPE element (SEQ ID NO:2) were mutated to prevent potential unwanted translation

initiation. Two copies of this mutated sequence were used in a head to tail array. This sequence is placed just downstream of the promoter and upstream of the Kozak sequence and signal peptide-coding region. The WPRE was isolated from woodchuck hepatitis virus and also aids in the export of mRNA from the nucleus and creating stability in the mRNA. If this sequence is included in the 3' untranslated region of the RNA, level of protein expression from this RNA increases up to 10-fold. The WPRE is contained between the end of MN14 protein coding and the poly-adenylation site. The mRNA expression from the LTR as well as from the bovine/human alpha-lactalbumin hybrid promoter is terminated and poly adenylated in the 3' LTR.

The bovine/human alpha-lactalbumin hybrid promoter (SEQ ID NO:1) is a modular promoter /enhancer element derived from human and bovine alpha-lactalbumin promoter sequences. The human portion of the promoter is from +15 relative to transcription start point (tsp) to -600 relative to the tsp. The bovine portion is then attached to the end of the human portion and corresponds to -550 to -2000 relative to the tsp. The hybrid was developed to remove poly-adenylation signals that were present in the bovine promoter and hinder retroviral RNA production. It was also developed to contain genetic control elements that are present in the human gene, but not the bovine.

For construction of the bovine/human α -lactalbumin promoter, human genomic DNA was isolated and purified. A portion of the human α -lactalbumin promoter was PCR amplified using the following two primers:

Primer 1 (SEQ ID NO: 24):

5'AAAGCATATGTTCTGGGCCTTGTTACATGGCTGGATTGGTT 3'

Primer 2 (SEQ ID NO: 25):

5'TGAATTCGGCGCCCCCAAGAACCTGAAATGGAAGCATCACTCA
GTTTCATATAT 3'

This two primers created a NdeI site on the 5' end of the PCR fragment and a EcoRI site on the 3' end of the PCR fragment.

The human PCR fragment created using the above primers was double digested with the restriction enzymes NdeI and EcoRI. The plasmid pKBaP-1 was also double digested with NdeI and EcoRI. The plasmid pKBaP-1 contains the bovine α -lactalbumin 5' flanking region attached to a multiple cloning site. This plasmid allows attachment of

various genes to the bovine α -lactalbumin promoter.

Subsequently, the human fragment was ligated/substituted for the bovine fragment of the promoter that was removed from the pKBaP-1 plasmid during the double digestion. The resulting plasmid was confirmed by DNA sequencing to be a hybrid of the Bovine and Human α -lactalbumin promoter/regulatory regions.

Attachment of the MN14 light chain gene to the IRES α -lactalbumin signal peptide was accomplished as follows. The MN14 light chain was PCR amplified from the vector pCRMN14LC using the following primers.

Primer 1 (SEQ ID NO: 26): 5' CTACAGGTGTCCACGTCGACATCCAGCTGACCCAG 3'

Primer 2 (SEQ ID NO: 27): 5' CTGCAGAATAGATCTCTAACACTCTCCCCTGTTG 3'

These primers add a HincII site right at the start of the coding region for mature MN14 light chain. Digestion of the PCR product with HincII gives a blunt end fragment starting with the initial GAC encoding mature MN14 on the 5' end. Primer 2 adds a BglII site to the 3' end of the gene right after the stop codon. The resulting PCR product was digested with HincII and BglII and cloned directly into the IRES-Signal Peptide plasmid that was digested with NaeI and BglII.

Next, the vector pCRMN14HC was digested with XhoI and NruI to remove about a 500 bp fragment. PCR was then used to amplify the same portion of the MN14 heavy chain construct that was removed by the XhoI-NruI digestion. This amplification also mutated the 5' end of the gene to add a better Kozak sequence to the clone. The Kozak sequence was modified to resemble the typical IgG Kozak sequence. The PCR primers are shown below.

Primer 1 (SEQ ID NO: 28):

5'CAGTGTGATCTCGAGAATTCAGGACCTCACCATGGGATGGAGCTGTATCAT 3'

Primer 2 (SEQ ID NO: 29):

5'GTGTCTTCGGGTCTCAGGCTGT 3'

The PCR product was digested with XhoI and NruI and inserted back into the previously digested plasmid backbone.

Next, the “good” Kozak MN14 heavy chain gene construct was digested with EcoRI and the heavy chain gene containing fragment was isolated. The IRES α -Lactalbumin Signal Peptide MN14 light chain gene construct was also digested with EcoRI. The heavy chain gene was then cloned into the EcoRI site of IRES light chain construct. This resulted in the heavy chain gene being placed at the 5' end of the IRES sequence.

A multiple cloning site was then added to the LNCX retroviral backbone plasmid. The LNCX plasmid was digested with HindIII and ClaI. Two oligonucleotide primers were produced and annealed together to create a double stranded DNA multiple cloning site. The following primers were annealed together.

Primer 1 (SEQ ID NO: 30):

5' AGCTTCTCGAGTTAACAGATCTAGGCCTCCTAGGTCGACAT 3'

Primer 2 (SEQ ID NO: 31):

5' CGATGTCGACCTAGGAGGCCTAGATCTGTAACTCGAGA 3'

After annealing the multiple cloning site was ligated into LNCX to create LNC-MCS.

The double chain gene fragment was then inserted into a retroviral backbone gene construct. The double chain gene construct created in step 3 was digested with SalI and BglII and the double chain containing fragment was isolated. The retroviral expression plasmid LNC-MCS was digested with XhoI and BglII. The double chain fragment was then cloned into the LNC-MCS retroviral expression backbone.

Next, a RNA splicing problem in the construct was repaired. The construct was digested with NsiI. The resulting fragment was then partially digested with EcoRI. The fragments resulting from the partial digest that were approximately 9300 base pairs in size, were gel purified. A linker was created to mutate the splice donor site at the 3' end of the MN14 heavy chain gene. The linker was again created by annealing two oligonucleotide primers together to form the double stranded DNA linker. The two primers used to create the linker are shown below.

Primer 1 (SEQ ID NO: 32):

5'CGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCCGGGA
AATGAAAGCCG 3'

Primer 2 (SEQ ID NO: 33):

5'AATTCGGCTTTCATTTCCCGGGAGACAGGGAGAGGCTCTTCTGCGTGTAAGTGG
TTGTGCAGAGCCTCGTGCA 3'

After annealing the linker was substituted for the original NsiI/EcoRI fragment that was removed during the partial digestion.

Next, the mutated double chain fragment was inserted into the α -Lactalbumin expression retroviral backbone LN α -LA-Mertz-MCS. The gene construct produced above was digested with BamHI and BglII and the mutated double chain gene containing fragment was isolated. The LN α -LA-Mertz-MCS retroviral backbone plasmid was digested with BglII. The BamHI/BglII fragment was then inserted into the retroviral backbone plasmid.

A WPRE element was then inserted into the gene construct. The plasmid BluescriptII SK+ WPRE-B11 was digested with BamHI and HincII to remove the WPRE element and the element was isolated. The vector created above was digested with BglII and HpaI. The WPRE fragment was ligated into the BglII and HpaI sites to create the final gene construct.

E. α -LA Bot

The α -LA Bot (SEQ ID NO:8, botulinum toxin antibody) construct comprises the following elements, arranged in 5' to 3' order: bovine/human alpha-lactalbumin hybrid promoter, mutated PPE element, cc49 signal peptide, botulinum toxin antibody light chain, IRES from encephalomyocarditis virus/ bovine α -LA signal peptide, botulinum toxin antibody heavy chain, WPRE sequence, and 3' MoMuLV LTR. In addition, the α -LA botulinum toxin antibody vector further comprises a 5' MoMuLV LTR, a MoMuLV extended viral packaging signal, and a neomycin phosphotransferase gene (these additional elements are provided in SEQ ID NO: 7).

This construct uses the 5' MoMuLV LTR to control production of the neomycin phosphotransferase gene. The expression of botulinum toxin antibody is controlled by

the hybrid α -LA promoter. The botulinum toxin antibody light chain gene and heavy chain gene are attached together by an IRES/ bovine α -LA signal peptide sequence. The bovine/human alpha-lactalbumin hybrid promoter drives production of a mRNA containing the light chain gene and the heavy chain gene attached by the IRES. Ribosomes attach to the mRNA at the CAP site and at the IRES sequence. This allows both light and heavy chain protein to be produced from a single mRNA.

In addition, there are two genetic elements contained within the mRNA to aid in export of the mRNA from the nucleus to the cytoplasm and aid in poly-adenylation of the mRNA. The mutated PPE sequence (SEQ ID NO:2) is contained between the RNA CAP site and the start of the MN14 protein coding region. ATG sequences within the PPE element (SEQ ID NO:2) were mutated to prevent potential unwanted translation initiation. Two copies of this mutated sequence were used in a head to tail array. This sequence was placed just downstream of the promoter and upstream of the Kozak sequence and signal peptide-coding region. The WPRE was isolated from woodchuck hepatitis virus and also aids in the export of mRNA from the nucleus and creating stability in the mRNA. If this sequence is included in the 3' untranslated region of the RNA, level of protein expression from this RNA increases up to 10-fold. The WPRE is contained between the end of MN14 protein coding and the poly-adenylation site. The mRNA expression from the LTR as well as from the bovine/human alpha-lactalbumin hybrid promoter is terminated and poly adenylated in the 3' LTR.

The bovine/human α -lactalbumin hybrid promoter (SEQ ID NO:1) is a modular promoter/enhancer element derived from human and bovine α -lactalbumin promoter sequences. The human portion of the promoter is from +15 relative to transcription start point to -600 relative to the tsp. The bovine portion is then attached to the end of the human portion and corresponds to -550 to -2000 relative to the tsp. The hybrid was developed to remove poly-adenylation signals that were present in the bovine promoter and hinder retroviral RNA production. It was also developed to contain genetic control elements that are present in the human gene, but not the bovine. Likewise, the construct contains control elements present in the bovine but not in the human.

74667-216

F. LSRNL

The LSRNL (SEQ ID NO:9) construct comprises the following elements, arranged in 5' to 3' order: 5' MoMuLV LTR, MoMuLV viral packaging signal; hepatitis B surface antigen; RSV promoter; neomycin phosphotransferase gene; and 3' MoMuLV LTR.

This construct uses the 5' MoMuLV LTR to control production of the Hepatitis B surface antigen gene. The expression of the neomycin phosphotransferase gene is controlled by the RSV promoter. The mRNA expression from the LTR as well as from the RSV promoter is terminated and poly adenylated in the 3' LTR.

G. α -LA cc49IL2

The α -LA cc49IL2 (SEQ ID NO:10; the cc49 antibody is described in U.S. Pat. Nos. 5,512,443; 5,993,813; and 5,892,019)

construct comprises the following elements, arranged in 5' to 3' order: 5' bovine/human α -lactalbumin hybrid promoter; cc49-IL2 coding region; and 3' MoMuLV LTR. This gene construct expresses a fusion protein of the single chain antibody cc49 attached to Interleukin-2. Expression of the fusion protein is controlled by the bovine/human α -lactalbumin hybrid promoter.

The bovine/human α -lactalbumin hybrid promoter (SEQ ID NO:1) is a modular promoter/enhancer element derived from human and bovine alpha-lactalbumin promoter sequences. The human portion of the promoter is from +15 relative to transcription start point to -600 relative to the tsp. The bovine portion is then attached to the end of the human portion and corresponds to -550 to -2000 relative to the tsp. The hybrid was developed to remove poly-adenylation signals that were present in the bovine promoter and hinder retroviral RNA production. It was also developed to contain genetic control elements that are present in the human gene, but not the bovine. Likewise, the construct contains control elements present in the bovine but not in the human. The 3' viral LTR provide the poly-adenylation sequence for the mRNA.

H. α -LA YP

The α -LA YP (SEQ ID NO: 11) construct comprises the following elements, arranged in 5' to 3' order: 5' bovine/human alpha-lactalbumin hybrid promoter; double mutated PPE sequence; bovine α LA signal peptide; *Yersenia pestis* antibody heavy chain

Fab coding region; EMCV IRES/ bovine α -LA signal peptide; *Yersenia pestis* antibody light chain Fab coding region; WPRE sequence; 3' MoMuLV LTR.

This gene construct will cause the expression of *Yersenia pestis* mouse Fab antibody. The expression of the gene construct is controlled by the bovine/human α -lactalbumin hybrid promoter. The PPE sequence and the WPRE sequence aid in moving the mRNA from the nucleus to the cytoplasm. The IRES sequence allows both the heavy and the light chain genes to be translated from the same mRNA. The 3' viral LTR provides the poly-adenylation sequence for the mRNA.

In addition, there are two genetic elements contained within the mRNA to aid in export of the mRNA from the nucleus to the cytoplasm and aid in poly-adenylation of the mRNA. The mutated PPE sequence (SEQ ID NO:2) is contained between the RNA CAP site and the start of the MN14 protein coding region. ATG sequences within the PPE element (SEQ ID NO:2) were mutated (bases 4, 112, 131, and 238 of SEQ ID NO: 2 were changed from a G to a T) to prevent potential unwanted translation initiation. Two copies of this mutated sequence were used in a head to tail array. This sequence was placed just downstream of the promoter and upstream of the Kozak sequence and signal peptide-coding region. The WPRE was isolated from woodchuck hepatitis virus and also aids in the export of mRNA from the nucleus and creating stability in the mRNA. If this sequence is included in the 3' untranslated region of the RNA, level of protein expression from this RNA increases up to 10-fold. The WPRE is contained between the end of MN14 protein coding and the poly-adenylation site. The mRNA expression from the LTR as well as from the bovine/human alpha-lactalbumin hybrid promoter is terminated and poly adenylated in the 3' LTR.

The bovine/human alpha-lactalbumin hybrid promoter (SEQ ID NO:1) is a modular promoter /enhancer element derived from human and bovine alpha-lactalbumin promoter sequences. The human portion of the promoter is from +15 relative to transcription start point to -600 relative to the tsp. The bovine portion is then attached to the end of the human portion and corresponds to -550 to -2000 relative to the tsp. The hybrid was developed to remove poly-adenylation signals that were present in the bovine promoter and hinder retroviral RNA production. It was also developed to contain genetic control elements that are present in the human gene, but not the bovine. Likewise, the construct contains control elements present in the bovine but not in the human.

Example 2

Generation of Cell Lines Stably Expressing the MoMLV gag and pol Proteins

Examples 2-5 describe the production of pseudotyped retroviral vectors. These methods are generally applicable to the production of the vectors described above. The expression of the fusogenic VSV G protein on the surface of cells results in syncytium formation and cell death. Therefore, in order to produce retroviral particles containing the VSV G protein as the membrane-associated protein a two-step approach was taken. First, stable cell lines expressing the gag and pol proteins from MoMLV at high levels were generated (*e.g.*, 293GP^{SD} cells). The stable cell line which expresses the gag and pol proteins produces noninfectious viral particles lacking a membrane-associated protein (*e.g.*, an envelope protein). The stable cell line was then co-transfected, using the calcium phosphate precipitation, with VSV-G and gene of interest plasmid DNAs. The pseudotyped vector generated was used to infect 293GP^{SD} cells to produce stably transformed cell lines. Stable cell lines can be transiently transfected with a plasmid capable of directing the high level expression of the VSV G protein (see below). The transiently transfected cells produce VSV G-pseudotyped retroviral vectors which can be collected from the cells over a period of 3 to 4 days before the producing cells die as a result of syncytium formation.

The first step in the production of VSV G-pseudotyped retroviral vectors, the generation of stable cell lines expressing the MoMLV *gag* and *pol* proteins is described below. The human adenovirus Ad-5-transformed embryonal kidney cell line 293 (ATCC CRL 1573) was cotransfected with the pCMVgag-pol and the gene encoding for phleomycin. pCMV gag-pol contains the MoMLV *gag* and *pol* genes under the control of the CMV promoter (pCMV gag-pol is available from the ATCC).

The plasmid DNA was introduced into the 293 cells using calcium phosphate co-precipitation (Graham and Van der Eb, *Virol.* 52:456 [1973]). Approximately 5×10^5 293 cells were plated into a 100 mm tissue culture plate the day before the DNA co-precipitate was added. Stable transformants were selected by growth in DMEM-high glucose medium containing 10% FCS and 10 μ g/ml phleomycin (selective medium). Colonies which grew in the selective medium were screened for extracellular reverse transcriptase activity (Goff *et al.*, *J. Virol.* 38:239 [1981]) and intracellular p30gag expression. The presence of p30gag expression was determined by Western blotting

using a goat-anti p30 antibody (NCI antiserum 77S000087). A clone which exhibited stable expression of the retroviral genes was selected. This clone was named 293GP^{SD} (293 gag-pol-San Diego). The 293GP^{SD} cell line, a derivative of the human Ad-5-transformed embryonal kidney cell line 293, was grown in DMEM-high glucose medium containing 10% FCS.

Example 3

Preparation of Pseudotyped Retroviral Vectors Bearing the G Glycoprotein of VSV

In order to produce VSV G protein pseudotyped retrovirus the following steps were taken. The 293GP^{SD} cell line was co-transfected with VSV-G plasmid and DNA plasmid of interest. This co-transfection generates the infectious particles used to infect 293GP^{SD} cells to generate the packaging cell lines. This Example describes the production of pseudotyped LNBOTDC virus. This general method may be used to produce any of the vectors described in Example 1.

a) Cell Lines and Plasmids

The packaging cell line, 293GP^{SD} was grown in alpha-MEM-high glucose medium containing 10% FCS. The titer of the pseudo-typed virus may be determined using either 208F cells (Quade, Virol. 98:461 [1979]) or NIH/3T3 cells (ATCC CRL 1658); 208F and NIH/3T3 cells are grown in DMEM-high glucose medium containing 10% CS.

The plasmid LNBOTDC contains the gene encoding BOTD under the transcriptional control of cytomegalovirus intermediate-early promoter followed by the gene encoding neomycin phosphotransferase (Neo) under the transcriptional control of the LTR promoter. The plasmid pHCMV-G contains the VSV G gene under the transcriptional control of the human cytomegalovirus intermediate-early promoter (Yee *et al.*, Meth. Cell Biol. 43:99 [1994]).

b) Production of stable packaging cell lines, pseudotyped vector and Titering of Pseudotyped LNBOTDC Vector

LNBOTDC DNA (SEQ ID NO: 13) was co-transfected with pHCMV-G DNA into the packaging line 293GP^{SD} to produce LNBOTDC virus. The resulting LNBOTDC virus was then used to infect 293GP^{SD} cells to transform the cells. The procedure for producing pseudotyped LNBOTDC virus was carried out as described (Yee *et al.*, Meth. Cell Biol. 43:99 [1994]).

This is a retroviral gene construct that upon creation of infectious replication defective retroviral vector will cause the insertion of the sequence described above into the cells of interest. Upon insertion the CMV regulatory sequences control the expression of the botulinum toxin antibody heavy and light chain genes. The IRES sequence allows both the heavy and the light chain genes to be translated from the same mRNA. The 3' viral LTR provides the poly-adenylation sequence for the mRNA.

Both heavy and light chain protein for botulinum toxin antibody are produced from this signal mRNA. The two proteins associated to form active botulinum toxin antibody. The heavy and light chain proteins also appear to be formed in an equal molar ratio to each other.

Briefly, on day 1, approximately 5×10^4 293GP^{SD} cells were placed in a 75 cm² tissue culture flask. On the following day (day 2), the 293GP^{SD} cells were transfected with 25 µg of pLNBOTDC plasmid DNA and 25 µg of VSV-G plasmid DNA using the standard calcium phosphate co-precipitation procedure (Graham and Van der Eb, Virol. 52:456 [1973]). A range of 10 to 40 µg of plasmid DNA may be used. Because 293GP^{SD} cells may take more than 24 hours to attach firmly to tissue culture plates, the 293GP^{SD} cells may be placed in 75 cm² flasks 48 hours prior to transfection. The transfected 293GP^{SD} cells provide pseudotyped LNBOTDC virus.

On day 3, approximately 1×10^5 293GP^{SD} cells were placed in a 75 cm² tissue culture flask 24 hours prior to the harvest of the pseudotyped virus from the transfected 293GP^{SD} cells. On day 4, culture medium was harvested from the transfected 2093GP^{SD} cells 48 hours after the application of the pLNBOTDC and VSV-G DNA. The culture medium was filtered through a 0.45 µm filter and polybrene was added to a final concentration of 8 µg/ml. The culture medium containing LNBOTDC virus was used to infect the 293GP^{SD} cells as follows. The culture medium was removed from the 293GP^{SD} cells and was replaced with the LNBOTDC virus containing culture medium.

Polybrene was added to the medium following addition to cells. The virus containing medium was allowed to remain on the 293GP^{SD} cells for 24 hours. Following the 16 hour infection period (on day 5), the medium was removed from the 293GP^{SD} cells and was replaced with fresh medium containing 400 µg/ml G418 (GIBCO/BRL). The medium was changed approximately every 3 days until G418-resistant colonies appeared approximately two weeks later.

The G418-resistant 293 colonies were plated as single cells in 96 wells. Sixty to one hundred G418-resistant colonies were screened for the expression of the BOTDC antibody in order to identify high producing clones. The top 10 clones in 96-well plates were transferred 6-well plates and allowed to grow to confluency.

The top 10 clones were then expanded to screen for high titer production. Based on protein expression and titer production, 5 clonal cell lines were selected. One line was designated the master cell bank and the other 4 as backup cell lines. Pseudotyped vector was generated as follows. Approximately 1×10^6 293GP^{SD}/LNBOTDC cells were placed into a 75cm² tissue culture flask. Twenty-four hours later, the cells were transfected with 25 µg of pHCMV-G plasmid DNA using calcium phosphate co-precipitation. Six to eight hours after the calcium-DNA precipitate was applied to the cells, the DNA solution was replaced with fresh culture medium (lacking G418). Longer transfection times (overnight) were found to result in the detachment of the majority of the 293GP^{SD}/LNBOTDC cells from the plate and are therefore avoided. The transfected 293GP^{SD}/LNBOTDC cells produce pseudotyped LNBOTDC virus.

The pseudotyped LNBOTDC virus generated from the transfected 293GP^{SD}/LNBOTDC cells can be collected at least once a day between 24 and 96 hr after transfection. The highest virus titer was generated approximately 48 to 72 hr after initial pHCMV-G transfection. While syncytium formation became visible about 48 hr after transfection in the majority of the transfected cells, the cells continued to generate pseudotyped virus for at least an additional 48 hr as long as the cells remained attached to the tissue culture plate. The collected culture medium containing the VSV G-pseudotyped LNBOTDC virus was pooled, filtered through a 0.45 µm filter and stored at -80°C or concentrated immediately and then stored at -80°C.

The titer of the VSV G-pseudotyped LNBOTDC virus was then determined as follows. Approximately 5×10^4 rat 208F fibroblasts cells were plated into 6 well plates. Twenty-four hours after plating, the cells were infected with serial dilutions of the

LNBOTDC virus-containing culture medium in the presence of 8 µg/ml polybrene.

Twenty four hours after infection with virus, the medium was replaced with fresh medium containing 400 µg/ml G418 and selection was continued for 14 days until G418-resistant colonies became visible. Viral titers were typically about 0.5 to 5.0 x 10⁶ colony forming units (cfu)/ml. The titer of the virus stock could be concentrated to a titer of greater than 10⁹ cfu/ml as described below.

Example 4

Concentration of Pseudotyped Retroviral Vectors

The VSV G-pseudotyped LNBOTDC viruses were concentrated to a high titer by one cycle of ultracentrifugation. However, two cycles can be performed for further concentration. The frozen culture medium collected as described in Example 2 which contained pseudotyped LNBOTDC virus was thawed in a 37°C water bath and was then transferred to Oakridge centrifuge tubes (50 ml Oakridge tubes with sealing caps, Nalge Nunc International) previously sterilized by autoclaving. The virus was sedimented in a JA20 rotor (Beckman) at 48,000 x g (20,000 rpm) at 4°C for 120 min. The culture medium was then removed from the tubes in a biosafety hood and the media remaining in the tubes was aspirated to remove the supernatant. The virus pellet was resuspended to 0.5 to 1% of the original volume of culture medium DMEM. The resuspended virus pellet was incubated overnight at 4°C without swirling. The virus pellet could be dispersed with gentle pipetting after the overnight incubation without significant loss of infectious virus. The titer of the virus stock was routinely increased 100- to 300-fold after one round of ultracentrifugation. The efficiency of recovery of infectious virus varied between 30 and 100%.

The virus stock was then subjected to low speed centrifugation in a microfuge for 5 min at 4°C to remove any visible cell debris or aggregated virions that were not resuspended under the above conditions. It was noted that if the virus stock is not to be used for injection into oocytes or embryos, this centrifugation step may be omitted.

The virus stock can be subjected to another round of ultracentrifugation to further concentrate the virus stock. The resuspended virus from the first round of centrifugation is pooled and pelleted by a second round of ultracentrifugation which is performed as described above. Viral titers are increased approximately 2000-fold after the second

round of ultracentrifugation (titers of the pseudotyped LNBOTDC virus are typically greater than or equal to 1×10^9 cfu/ml after the second round of ultracentrifugation).

The titers of the pre- and post-centrifugation fluids were determined by infection of 208F cells (NIH 3T3 or bovine mammary epithelial cells can also be employed) followed by selection of G418-resistant colonies as described above in Example 2.

Example 5

Preparation of Pseudotyped Retrovirus For Infection of Host Cells

The concentrated pseudotyped retroviruses were resuspended in 0.1X HBS (2.5 mM HEPES, pH 7.12, 14 mM NaCl, 75 μ M $\text{Na}_2\text{HPO}_4\text{-H}_2\text{O}$) and 18 μ l aliquots were placed in 0.5 ml vials (Eppendorf) and stored at -80°C until used. The titer of the concentrated vector was determined by diluting 1 μ l of the concentrated virus 10^{-7} - or 10^{-8} -fold with 0.1X HBS. The diluted virus solution was then used to infect 208F and bovine mammary epithelial cells and viral titers were determined as described in Example 2.

Example 6

Expression of MN14 by Host Cells

This Example describes the production of antibody MN14 from cells transfected with a high number of integrating vectors. Pseudotyped vector were made from the packaging cell lines for the following vectors: CMV MN14, α -LA MN14, and MMTV MN14. Rat fibroblasts (208F cells), MDBK cells (bovine kidney cells), and bovine mammary epithelial cells were transfected at a multiplicity of infection of 1000. One thousand cells were plated in a T25 flask and 10^6 colony forming units (CFU's) of vector in 3 ml media was incubated with the cells. The duration of the infection was 24 hr, followed by a media change. Following transfection, the cells were allowed to grow and become confluent.

The cell lines were grown to confluency in T25 flasks and 5ml of media was changed daily. The media was assayed daily for the presence of MN14. All of the MN14 produced is active (an ELISA to detect human IgG gave the exact same values as the CEA binding ELISA) and Western blotting has shown that the heavy and light chains

are produced at a ratio that appears to be a 1:1 ratio. In addition, a non-denaturing Western blot indicated that what appeared to be 100% of the antibody complexes were correctly formed (*See Figure 1: Lane 1, 85 ng control Mn14; Lane 2, bovine mammary cell line, α -LA promoter; Lane 3, bovine mammary cell line, CMV promoter; Lane 4, bovine kidney cell line, α -LA promoter; Lane 5, bovine kidney cell line, CMV promoter; Lane 6, 208 cell line, α -LA promoter; Lane 7, 208 cell line, CMV promoter*)).

Figure 2 is a graph showing the production of MN14 over time for four cell lines. The Y axis shows MN14 production in ng/ml of media. The X-axis shows the day of media collection for the experiment. Four sets of data are shown on the graph. The comparisons are between the CMV and α -LA promoter and between the 208 cells and the bovine mammary cells. The bovine mammary cell line exhibited the highest expression, followed by the 208F cells and MDBK cells. With respect to the constructs, the CMV driven construct demonstrated the highest level of expression, followed by the α -LA driven gene construct and the MMTV construct. At 2 weeks, the level of daily production of the CMV construct was 4.5 μ g/ml of media (22.5 mg/day in a T25 flask). The level of expression subsequently increased slowly to 40 μ g/day as the cells became very densely confluent over the subsequent week. 2.7 L of media from an α -lac-MN14 packaging cell line was processed by affinity chromatography to produce a purified stock of MN14.

Figure 3 is a western blot of a 15% SDS-PAGE gel run under denaturing conditions in order to separate the heavy and light chains of the MN14 antibody. Lane 1 shows MN14 from bovine mammary cell line, hybrid α -LA promoter; lane 2 shows MN14 from bovine mammary cell line, CMV promoter; lane 3 shows MN14 from bovine kidney cell line, hybrid α LA promoter; lane 4 shows MN14 from bovine kidney cell line, CMV promoter; lane 5 shows MN14 from rat fibroblast cell line, hybrid α -LA promoter; lane 6 shows MN14 from rat fibroblast, CMV promoter. In agreement with Figure 1 above, the results show that the heavy and light chains are produced in a ratio of approximately 1:1.

Example 7

Quantitation of Protein Produced Per Cell

This Example describes the quantitation of the amount of protein produced per cell in cell cultures produced according to the invention. Various cells (208F cells, MDBK cells, and bovine mammary cells) were plated in 25 cm² culture dishes at 1000 cells/dish. Three different vectors were used to infect the three cells types (CMV-MN14, MMTV-MN14, and α -LA-MN14) at an MOI of 1000 (titers: 2.8×10^6 , 4.9×10^6 , and 4.3×10^6 , respectively). Media was collected approximately every 24 hours from all cells. Following one month of media collection, the 208F and MDBK cells were discarded due to poor health and low MN14 expression. The cells were passaged to T25 flasks and collection of media from the bovine mammary cells was continued for approximately 2 months with continued expression of MN14. After two months in T25 flasks, the cells with CMV promoters were producing 22.5 pg/cell/day and the cells with α -LA promoters were producing 2.5 pg MN14/cell/day.

After 2 months in T25 flasks, roller bottles (850 cm²) were seeded to scale-up production and to determine if MN14 expression was stable following multiple passages. Two roller bottles were seeded with bovine mammary cells expressing MN14 from a CMV promoter and two roller bottles were seeded with bovine mammary cells expressing MN14 from the α -LA promoter. The cultures reached confluency after approximately two weeks and continue to express MN14. Roller bottle expression is shown in Table 1 below.

Table 1 Production of MN14 in Roller Bottles			
Cell Line	Promoter	MN14 Production/ Week ($\mu\text{g/ml}$)	MN14 Production/ Week - Total ($\mu\text{g/ml}$)
Bovine mammary	CMV	2.6	1 - 520
Bovine mammary	CMV	10.6	2 - 2120
Bovine mammary	CMV	8.7	3 - 1740
Bovine mammary	CMV	7.8	4 - 1560
Bovine mammary	α -LA	0.272	1 - 54.4
Bovine mammary	α -LA	2.8	2 - 560
Bovine mammary	α -LA	2.2	3 - 440
Bovine mammary	α -LA	2.3	4 - 460

Example 8

Transfection at Varied Multiplicities of Infection

This Example describes the effect of transfection at varied multiplicities of infection on protein expression. 208F rat fibroblast and bovine mammary epithelial cells (BMEC) were plated in a 25 cm² plates at varied cell numbers/25 cm². Cells were infected with either the CMV MN14 vector or the α LA MN14 vector at a MOI of 1,10, 1000, and 10,000 by keeping the number of CFUs kept constant and varying the number of cells infected.

Following infection, medium was changed daily and collected approximately every 24 hours from all cells for approximately 2 months. The results of both of the vectors in bovine mammary epithelial cells are shown in Table 2 below. Cells without data indicate cultures that became infected prior to the completion of the experiment. The "# cells" column represents the number of cells at the conclusion of the experiment. The results indicate that a higher MOI results in increased MN14 production, both in terms of the amount of protein produced per day, and the total accumulation.

Table 2 MOI vs. Protein Production						
Cell Line	Promoter	MOI	% cell Confluency	MN14 (ng/ml)	# Cells	MN14 Production /day (pg/cell)
BMEC	CMV	10000	100%	4228	4.5E5	47
BMEC	CMV	1000	100%	2832	2.0E6	7.1
BMEC	CMV	100				
BMEC	CMV	10	100%	1873	2.5E6	3.75
BMEC	CMV	1				
BMEC	α LA	10000	100%	1024	1.5E6	3.4
BMEC	α LA	1000				
BMEC	α LA	100	100%	722	1.8E6	1.9
BMEC	α LA	10	100%	421234	2.3E6	.925
BMEC	α LA	1	100%		1.9E6	.325

Example 9

Transfection at Varied Multiplicities of Infection

This experiment describes protein production from the CMV MN14 vector at a variety of MOI values. Bovine mammary cells, CHO cells, and human embryo kidney cells (293 cells) were plated in 24 well plates (2 cm²) at 100 cells/2 cm² well. Cells were infected at various dilutions with CMV MN14 to obtain MOI values of 1, 10, 100, 1000, and 10000. The CHO cells reached confluency at all MOI within 11 days of infection. However, the cells infected at a MOI of 10,000 grew more slowly. The bovine mammary and 293 cells grew slower, especially at the highest MOI of 10,000. The cells were then passaged into T25 flasks to disperse cells. Following dispersion, cells reached confluence within 1 week. the medium was collected after one week and analyzed for MN14 production. The CHO and human 293 cells did not exhibit good growth in extended culture. Thus, data were not collected from these cells. Data for bovine mammary epithelial cells are shown in Table 3 below. The results indicate that production of MN14 increased with higher MOI.

Table 3 MOI vs. Protein Production				
Cell Line	Promoter	MOI	% confluency	MN14 Production (ng/ml)
BMEC	CMV	10000	100%	1312
BMEC	CMV	1000	100%	100
BMEC	CMV	100	100%	7.23
BMEC	CMV	10	100%	0
BMEC	CMV	1	100%	0

Example 10

Expression of LL2 Antibody by Bovine Mammary Cells

This Example describes the expression of antibody LL2 by bovine mammary cells. Bovine mammary cells were infected with vector CMV LL2 (7.85×10^7 CFU/ml) at MOI's of 1000 and 10,000 and plated in 25cm² culture dishes. None of the cells survived transfection at the MOI of 10,000. At 20% confluency, 250 ng/ml of LL2 was present in the media.

Example 11

Expression of Botulinum Toxin Antibody by Bovine Mammary Cells

This Example describes the expression of Botulinum toxin antibody in bovine mammary cells. Bovine mammary cells were infected with vector α -LA Bot (2.2×10^2 CFU/ml) and plated in 25cm² culture dishes. At 100% confluency, 6 ng/ml of Botulinum toxin antibody was present in the media.

Example 12

Expression of Hepatitis B Surface Antigen by Bovine Mammary Cells

This Example describes the expression of hepatitis B surface antigen (HBsAg) in bovine mammary cells. Bovine mammary cells were infected with vector LSRNL (350 CFU/ml) and plated in 25cm² culture dishes. At 100% confluency, 20 ng/ml of HBsAg was present in the media.

Example 13

Expression of cc49IL2 Antigen Binding Protein by Bovine Mammary Cells

This Example describes the expression of cc49IL2 in bovine mammary cells. Bovine mammary cells were infected with vector cc49IL2 (3.1 X 10⁵ CFU/ml) at a MOI of 1000 and plated in 25cm² culture dishes. At 100% confluency, 10 µg/ml of cc49IL2 was present in the media.

Example 14

Expression of Multiple Proteins by Bovine Mammary Cells

This Example describes the expression of multiple proteins in bovine mammary cells. Mammary cells producing MN14 (infected with CMV-MN14 vector) were infected with cc49IL2 vector (3.1 X 10⁵ CFU/ml) at an MOI of 1000, and 1000 cells were plated in 25cm² culture plates. At 100% confluency, the cells expressed MN14 at 2.5 µg/ml and cc49IL2 at 5 µg/ml.

Example 15

Expression of Multiple Proteins by Bovine Mammary Cells

This Example describes the expression of multiple proteins in bovine mammary cells. Mammary cells producing MN14 (infected with CMV-MN14 vector) were infected with LSNRL vector (100 CFU/ml) at an MOI of 1000, and 1000 cells were plated in 25cm² culture plates. At 100% confluency, the cells expressed MN14 at 2.5 µg/ml and hepatitis surface antigen at 150 ng/ml.

Example 16

Expression of Multiple Proteins by Bovine Mammary Cells

This Example describes the expression of multiple proteins in bovine mammary cells. Mammary cells producing hepatitis B surface antigen (infected with LSRNL vector) were infected with cc49IL2 vector at an MOI of 1000, and 1000 cells were plated in 25cm² culture plates. At 100% confluency, the cells expressed MN14 at 2.4 µg/ml and hepatitis B surface antigen at 13 ng/ml. It will be understood that multiple proteins may be expressed in the other cell lines described above.

Example 17

Expression of Hepatitis B Surface Antigen and Botulinum Toxin Antibody in Bovine Mammary Cells

This Example describes the culture of transfected cells in roller bottle cultures. 208F cells and bovine mammary cells were plated in 25cm² culture dishes at 1000 cells/25cm². LSRNL or α-LA Bot vectors were used to infect each cell line at a MOI of 1000. Following one month of culture and media collection, the 208F cells were discarded due to poor growth and plating. Likewise, the bovine mammary cells infected with α-LA Bot were discarded due to low protein expression. The bovine mammary cells infected with LSRNL were passaged to seed roller bottles (850 cm²). Approximately 20 ng/ml hepatitis type B surface antigen was produced in the roller bottle cultures.

Example 18

Expression in Clonally Selected Cell Lines

This experiment describes expression of MN14 from clonally selected cell lines. Cell lines were grown to confluency in T25 flasks and 5ml of media were collected daily. The media was assayed daily for the presence of MN14. All the MN14 produced was active and Western blotting indicated that the heavy and light chains were produced at a ratio that appears to be almost exactly 1:1. In addition, a non-denaturing western blot indicated that approximately 100% of the antibody complexes were correctly formed.

After being in culture for about two months, the cells were expanded into roller bottles or plated as single cell clones in 96 well plates.

The production of MN14 in the roller bottles was analyzed for a 24 hour period to determine if additional medium changing would increase production over what was obtained with weekly medium changes. Three 24 hour periods were examined. The CMV promoter cells in 850 cm² roller bottles produced 909 ng/ml the first day, 1160 ng/ml the second day and 1112 ng/ml the third day. The α -LA promoter cells produced 401 ng/ml the first day, 477 ng/ml the second day and 463 ng/ml the third day. These values correspond well to the 8-10 mg/ml/week that were obtained for the CMV cells and the 2-3 mg/ml that were obtained for the α -LA cells. It does not appear that more frequent media changing would increase MN14 production in roller bottles.

Single cell lines were established in 96 well plates and then passaged into the same wells to allow the cells to grow to confluency. Once the cells reached confluency, they were assayed for MN14 production over a 24 hour period. The clonal production of MN14 from CMV cell lines ranged from 19 ng/ml/day to 5500 ng/ml/day. The average production of all cell clones was 1984 ng/ml/day. The α -LA cell clones yielded similar results. The clonal production of MN14 from α -LA cell lines ranged from 1 ng/ml/day to 2800 ng/ml/day. The average production of these cell clones was 622 ng/ml/day. The results are provided in Table 4 below.

Table 4 Expression in Clonal Cell Lines			
CMV Clonal Cell Line Number	MN14 Production (ng/ml)	Alpha-lactalbumin Clonal Cell Line Number	MN14 Production (ng/ml)
22	19	27	0
6	88	29	0
29	134	12	0.7
34	151	50	8
32	221	28	55

Table 4 Expression in Clonal Cell Lines			
CMV Clonal Cell Line Number	MN14 Production (ng/ml)	Alpha-lactalbumin Clonal Cell Line Number	MN14 Production (ng/ml)
23	343	43	57
27	423	8	81
4	536	13	154
41	682	48	159
45	685	7	186
40	696	36	228
11	1042	39	239
8	1044	51	275
5	1066	31	283
19	1104	54	311
48	1142	38	317
12	1224	21	318
26	1315	16	322
39	1418	47	322
37	1610	17	325
20	1830	37	367
21	1898	45	395
47	1918	25	431
35	1938	5	441

Table 4 Expression in Clonal Cell Lines			
CMV Clonal Cell Line Number	MN14 Production (ng/ml)	Alpha-lactalbumin Clonal Cell Line Number	MN14 Production (ng/ml)
15	1968	20	449
3	1976	19	454
28	1976	22	503
1	2166	55	510
16	2172	14	519
17	2188	41	565
33	2238	46	566
30	2312	23	570
38	2429	1	602
2	2503	9	609
14	2564	53	610
24	2571	56	631
9	2708	2	641
42	2729	40	643
44	2971	32	653
7	3125	24	664
43	3125	26	671
25	3650	52	684
46	3706	6	693

Table 4 Expression in Clonal Cell Lines			
CMV Clonal Cell Line Number	MN14 Production (ng/ml)	Alpha-lactalbumin Clonal Cell Line Number	MN14 Production (ng/ml)
50	3947	33	758
49	4538	42	844
18	4695	10	1014
31	4919	3	1076
10	5518	44	1077
		35	1469
		34	1596
		18	1820
		30	2021
		11	2585
		4	2800

Example 19

Estimation of Insert Copy Number

This example describes the relationship of multiplicity of infection, gene copy number, and protein expression. Three DNA assays were developed using the INVADER Assay system (Third Wave Technologies, Madison, WI). One of the assays detects a portion of the bovine α -lactalbumin 5' flanking region. This assay is specific for bovine and does not detect the porcine or human α -lactalbumin gene. This assay will detect two copies of the α -lactalbumin gene in all control bovine DNA samples and also in bovine mammary epithelial cells. The second assay detects a portion of the extended

packaging region from the MLV virus. This assay is specific for this region and does not detect a signal in the 293 human cell line, bovine mammary epithelial cell line or bovine DNA samples. Theoretically, all cell lines or other samples not infected with MLV should not produce a signal. However, since the 293GP cell line was produced with the extended packaging region of DNA, this cell line gives a signal when the assay is run. From the initial analysis, it appears that the 293GP cell line contains two copies of the extended packing region sequence that are detected by the assay. The final assay is the control assay. This assay detects a portion of the insulin-like growth factor I gene that is identical in bovine, porcine, humans and a number of other species. It is used as a control on every sample that is run in order to determine the amount of signal that is generated from this sample for a two copy gene. All samples that are tested should contain two copies of the control gene.

DNA samples can be isolated using a number of methods. Two assays are then performed on each sample. The control assay is performed along with either the bovine α -lactalbumin assay or the extended packaging region assay. The sample and the type of information needed will determine which assay is run. Both the control and the transgene detection assay are run on the same DNA sample, using the exact same quantity of DNA.

The data resulting from the assay are as follows (Counts indicate arbitrary fluorescence units):

- Extended Packaging Region or α -Lactalbumin Background counts
- Extended Packaging Region or α -Lactalbumin counts
- Internal Control background counts
- Internal Control counts

To determine net counts for the assay the background counts are subtracted from the actual counts. This occurs for both the control and transgene detection assay. Once the net counts are obtained, a ratio of the net counts for the transgene detection assay to

the net counts of the control assay can be produced. This value is an indication of the number of copies of transgene compared to the number of copies of the internal control gene (in this case IGF-I). Because the transgene detection assay and the control assay are two totally different assays, they do not behave exactly the same. This means that one does not get an exact 1:1 ratio if there are two copies of the transgene and two copies of the control gene in a specific sample. However the values are generally close to the 1:1 ratio. Also, different insertion sites for the transgene may cause the transgene assay to behave differently depending on where the insertions are located.

Therefore, although the ratio is not an exact measure of copy number, it is a good indication of relative copy number between samples. The greater the value of the ratio the greater the copy number of the transgene. Thus, a ranking of samples from lowest to highest will give a very accurate comparison of the samples to one another with regard to copy number. Table 5 provides actual data for the EPR assay:

Table 5							
Sample #	Control Counts	Control Background Counts	Net Control Counts	Transgene Counts	Transgene Background Counts	Net Transgene Counts	Net Ratio
293	116	44	72	46.3	46	0.3	0
293GP	112	44	68	104	46	58	.84
1	74	40	34	88	41	47	1.38
2	64	40	24	83	41	43	1.75
3	62	44	18	144	46	98	5.57

From this data, it can be determined that the 293 cell line has no copies of the extended packaging region/transgene. However the 293 GP cells appear to have two copies of the extended packaging region. The other three cell lines appear to have three or more copies of the extended packaging region (one or more additional copies compared to 293GP cells).

Invader Assay Gene Ratio and Cell Line Protein Production

Bovine mammary epithelial cells were infected with either the CMV driven MN14 construct or the α -lactalbumin driven MN14 construct. The cells were infected at a 1000 to 1 vector to cell ratio. The infected cells were expanded. Clonal cell lines were established for both the α -LA and CMV containing cells from this initial pooled population of cells. Approximately 50 cell lines were produced for each gene construct. Individual cells were placed in 96 well plates and then passaged into the same well to allow the cells to grow to confluency. Once the cells lines reached confluency, they were assayed for MN14 production over a 24 hour period. The clonal production of MN14 from CMV cell lines ranged from 0 ng/ml/day to 5500 ng/ml/day. The average production of all cell clones was 1984 ng/ml/day. The α -LA cell clones showed similar trends. The clonal production of MN14 from α -LA cell lines ranged from 0 ng/ml/day to 2800 ng/ml/day. The average production of these cell clones was 622 ng/ml/day.

For further analysis of these clonal lines, fifteen CMV clones and fifteen α -LA clones were selected. Five highest expressing, five low expressing and five mid-level expressing lines were chosen. These thirty cell lines were expanded and banked. DNA was isolated from most all of the thirty cell lines. The cell lines were passed into 6 well plates and grown to confluency. Once at confluency, the media was changed every 24 hours and two separate collections from each cell line were assayed for MN14 production. The results of these two assays were averaged and these numbers were used to create Tables 6 and 7 below. DNA from the cell lines was run using the Invader extended packaging region assay and the results are shown below. The Tables show the cell line number, corresponding gene ratio and antibody production.

Table 6		
CMV Clonal Cell Line Number	Invader Gene Ratio	MN14 Production (ng/ml)
6	0.19	104
7	1.62	2874
10	2.57	11202
18	3.12	7757
19	1.62	2483
21	1.53	3922
22	0	0
29	0.23	443
31	3.45	5697
32	0.27	346
34	0.37	305
38	1.47	2708
41	1.54	5434
49	2.6	7892
50	1.56	5022
Average of All Clones	1.48	3746

Table 7		
α -LA Clonal Cell Line Number	Invader Gene Ratio	MN14 Production (ng/ml)
4	4.28	3600
6	1.15	959
12	0.35	21
17	0.54	538
28	0.75	60
30	1.73	2076
31	0.74	484
34	4.04	3332
41	1.33	771
Average of All Clones	1.66	1316

The graphs (Figs. 17 and 18) show the comparison between protein expression and invader assay gene ratio. The results indicate that there is a direct correlation between invader assay gene ratio and protein production. It also appears that the protein production has not reached a maximum and if cells containing a higher invader assay gene ratio were produced, higher protein production would occur.

Invader Assay Gene Ratio and Multiple Cell Line Infections

Two packaging cell lines (293GP) produced using previously described methods were used to produce replication defective retroviral vector. One of the cell lines contains a retroviral gene construct that expresses the botulinum toxin antibody gene from the CMV promoter (LTR-Extended Viral Packaging Region-Neo Gene-CMV Promoter-Bot Light Chain Gene-IRES-Bot Heavy Chain Gene-LTR), the other cell line contains a retroviral gene construct that expresses the YP antibody gene from the CMV promoter (LTR-Extended Viral Packaging Region-Neo Gene-CMV Promoter-YP Heavy Chain Gene-IRES-YP Light Chain

Gene-WPRE-LTR). In addition to being able to produce replication defective retroviral vector, each of these cell lines also produce either botulinum toxin antibody or YP antibody.

The vector produced from these cell lines was then used to re-infect the parent cell line. This procedure was performed in order to increase the number of gene insertions and to improve antibody production from these cell lines. The botulinum toxin parent cell line was infected with a new aliquot of vector on three successive days. The titer of the vector used to perform the infection was 1×10^8 cfu/ml. Upon completion of the final 24 hour infection, clonal selection was performed on the cells and the highest protein producing line was established for botulinum toxin antibody production. A similar procedure was performed on the YP parent cell line. This cell line was also infected with a new aliquot of vector on three successive days. The titer of the YP vector aliquots was 1×10^4 . Upon completion of the final 24 hour infection, clonal selection was performed on the cells and the highest protein producing line was established for YP production.

Each of the parent cell lines and the daughter production cell lines were examined for Invader gene ratio using the extended packaging region assay and for protein production. The Bot production cell line which was generated using the highest titer vector had the highest gene ratio. It also had the highest protein production, again suggesting that gene copy number is proportional to protein production. The YP production cell line also had a higher gene ratio and produced more protein than its parent cell line, also suggesting that increasing gene copy is directly related to increases in protein production. The data is presented in Table 8.

Table 8		
Cell Line	Invader Gene Ratio	Antibody Production (Bot/YP)
Bot Parent Cell Line	1.12	4.8 mg/ml
Bot Production Cell Line	3.03	55 mg/ml
YP Parent Cell Line	1.32	4 mg/ml
YP Production Cell Line	2.04	25 mg/ml

Example 20

Transfection with Lentivirus Vectors

This example describes methods for the production of lentivirus vectors and their use to infect host cells at a high multiplicity of infection.

Replication-defective viral particles are produced by the transient cotransfection of the plasmids described in U.S. Pat. No. 6,013,516 in 293T human kidney cells. All plasmids are transformed and grown in *E. coli* HB101 bacteria following standard molecular biology procedures. For transfection of eukaryotic cells, plasmid DNA is purified twice by equilibrium centrifugation in CsCl-ethidium bromide gradients. A total of 40 µg DNA is used for the transfection of a culture in a 10 cm dish, in the following proportions: 10 µg pCMVΔR8, 20 µg pHR', and 10 µg env plasmids, either MLV/Ampho, MLV/Eco or VSV-G. 293T cells are grown in DMEM supplemented with 10% fetal calf serum and antibiotics in a 10% CO₂ incubator. Cells are plated at a density of 1.3×10⁶/10 cm dish the day before transfection. Culture medium is changed 4 to 6 hrs before transfection. Calcium phosphate-DNA complexes are prepared according to the method of Chen and Okayama (Mol. Cell. Biol., 7:2745, 1987), and incubated overnight with the cells in an atmosphere of 5% CO₂. The following morning, the medium is replaced, and the cultures returned to 10% CO₂. Conditioned medium is harvested 48 to 60 hrs after transfection, cleared of cellular debris by low speed centrifugation (300×g 10 min), and filtered through 0.45 µm low protein binding filters.

To concentrate vector particles, pooled conditioned medium harvested as described above is layered on top of a cushion of 20% sucrose solution in PBS and centrifuged in a Beckman SW28 rotor at 50,000×g for 90 min. The pellet is resuspended by incubation and gentle pipetting in 1-4 ml PBS for 30-60 min, then centrifuged again at 50,000×g for 90 min in a Beckmann SW55 rotor. The pellet is resuspended in a minimal volume (20-50 µl) of PBS and either used directly for infection or stored in frozen aliquots at -80° C.

The concentrated lentivirus vectors are titered and used to transfect an appropriate cell line (*e.g.*, 293 cells, Hela cells, rat 208F fibroblasts)) at a multiplicity of infection of 1,000. Analysis of clonally selected cell lines expressing the exogenous protein will reveal that a portion of the selected cell lines

contain more than two integrated copies of the vector. These cell lines will produce more of the exogenous protein than cell lines containing only one copy of the integrated vector.

Example 21

Expression and Assay of G-protein Coupled Receptors

This example describes the expression of a G-Protein Coupled Receptor protein (GPCR) from a retroviral vector. This example also describes the expression of a signal protein from an IRES as a marker for expression of a difficult to assay protein or a protein that has no assay such as a GPCR. The gene construct (SEQ ID NO: 34; Figure 19) comprises a G-protein-coupled receptor followed by the IRES-signal peptide-antibody light chain cloned into the MCS of pLBCX retroviral backbone. Briefly, a PvuII/PvuII fragment (3057 bp) containing the GPCR-IRES-antibody light chain was cloned into the StuI site of pLBCX. pLBCX contains the EM7 (T7) promoter, Blasticidin gene and SV40 polyA in place of the Neomycin resistance gene from pLNCX.

The gene construct was used to produce a replication defective retroviral packaging cell line and this cell line was used to produce replication defective retroviral vector. The vector produced from this cell line was then used to infect 293GP cells (human embryonic kidney cells). After infection, the cells were placed under Blasticidin selection and single cell Blasticidin resistant clones were isolated. The clones were screened for expression of antibody light chain. The top 12 light chain expressing clones were selected. These 12 light chain expressing clones were then screened for expression of the GPCR using a ligand binding assay. All twelve of the samples also expressed the receptor protein. The clonal cell lines and there expression are shown in Table 9.

Table 9		
Cell Clone Number	Antibody Light Chain Expression	GPCR Expression
4	+	+
8	+	+
13	+	+
19	+	+
20	+	+
22	+	+
24	+	+
27	+	+
30	+	+
45	+	+
46	+	+
50	+	+

Example 22

Multiple infection of 293 cells with replication defective retroviral vector

This example describes the multiple serial transfection of cells with retroviral vectors. The following gene construct was used to produce a replication defective retroviral packaging cell line.

- 5' LTR = Moloney murine sarcoma virus 5' long terminal repeat.
 EPR = Moloney murine leukemia virus extended packaging region.
 Blast = Blasticidin resistance gene.
 CMV = Human cytomegalovirus immediate early promoter.
 Gene = Gene encoding test protein
 WPRE = RNA transport element
 3' LTR = Moloney murine leukemia virus 3' LTR.

This packaging cell line was then used to produce a replication defective retroviral vector arranged as follows. The vector was produced from cells grown in T150 flasks and frozen. The frozen vector was thawed at each infection. For infection # 3 a concentrated solution of vector was used to perform the infection. All other infections were performed using non-concentrated vector. The infections were performed over a period of approximately five months by placing 5 ml of vector/media solution on a T25 flask containing 30% confluent 293 cells. Eight mg/ml of polybrene was also placed in the vector solution during infection. The vector solution was left on the cells for 24 hours and then removed. Media (DMEM with 10% fetal calf serum) was then added to the cells. Cells were grown to full confluency and passaged into a new T25 flask. The cells were then grown to 30% confluency and the infection procedure was repeated. This process was repeated 12 times and is outlined Table 10 below. After infections 1, 3, 6, 9 and 12, cells left over after passaging were used to obtain a DNA sample. The DNA was analyzed using the INVADER assay to determine an estimate of the number of vector inserts in the cells after various times in the infection procedure. The results indicate that the number of vector insertions goes up over time with the highest level being after the 12th infection. Since a value of 0.5 is approximately an average of one vector insert copy per cell, after twelve infections the average vector insert copy has yet to reach two. These data indicates that the average vector copy per cell is a little less than 1.5 copies per cell. Also, there was no real change in gene copy number from infection #6 to infection #9. Furthermore, these data indicate that transfection conducted at a standard low multiplicity of infection fail to introduce more than one copy of the retroviral vector into the cells.

Table 10		
Cell Line or Infection Number	Vector Titer (CFU/ml)	"Invader" Gene Ratio
293		0.053
Infection #1	1.05×10^3	0.39
Infection #2	1.05×10^3	
Infection #3	7.6×10^4	0.45
Infection #4	1.05×10^3	
Infection #5	1.05×10^3	
Infection #6	1.05×10^3	0.54
Infection #7	1.05×10^3	
Infection #8	1.05×10^3	
Infection #9	1.05×10^3	0.52
Infection #10	1.05×10^3	
Infection #11	1.05×10^3	
Infection #12	1.05×10^3	0.69

Example 23

Production of YP antibody

This Example demonstrates the production of *Yersinia pestis* antibody by bovine mammary epithelial cells and human kidney fibroblast cells (293 cells). Cells lines were infected with the α -LA YP vector. Both of the cell lines produced YP antibody. All of the antibody is active and the heavy and light chains are produced in a ratio approximating 1:1.

Example 24

Transduction of Plant Protoplasts

This Example describes a method for transducing plant protoplasts. Tobacco protoplasts of *Nicotiana tabacum* c.v. Petit Havanna are produced according to conventional processes from a tobacco suspension culture (Potrykus and Shillito, Methods in Enzymology, vol. 118, Plant Molecular Biology, eds. A. and H. Weissbach, Academic Press, Orlando, 1986). Completely unfolded leaves are removed under sterile conditions from 6-week-old shoot cultures and thoroughly wetted with an enzyme solution of the following composition: Enzyme solution: H₂O, 70 ml; sucrose, 13 g; macerozyme R 10, 1 g; cellulase, 2 g; "Onozuka" R 10 (Yakult Co. Ltd., Japan) Driselase (Chemische Fabrik Schweizerhalle, Switzerland), 0.13 g; and 2(n-morpholine)-ethanesulphonic acid (MES), 0.5 ml pH 6.0

Leaves are then cut into squares from 1 to 2 cm in size and the squares are floated on the above-mentioned enzyme solution. They are incubated overnight at a temperature of 26°C in the dark. This mixture is then gently shaken and incubated for a further 30 minutes until digestion is complete.

The suspension is then filtered through a steel sieve having a mesh width of 100 µm, rinsed thoroughly with 0.6M sucrose (MES, pH 5.6) and subsequently centrifuged for 10 minutes at from 4000 to 5000 rpm. The protoplasts collect on the surface of the medium which is then removed from under the protoplasts, for example using a sterilized injection syringe.

The protoplasts are resuspended in a K₃ medium [sucrose (102.96 g/l; xylose (0.25 g/l); 2,4-dichlorophenoxyacetic acid (0.10 mg/l); 1-naphthylacetic acid (1.00 mg/l); 6-benzylaminopurine (0.20 mg/l); pH 5.8](Potrykus and Shillito, *supra*) that contains 0.4M sucrose.

To carry out the transformation experiments, the protoplasts are first of all washed, counted and then resuspended, at a cell density of from 1 to 2.5×10⁶ cells per ml, in a W₅ medium [154 mM NaCl, 125 mM CaCl₂ x 2H₂O, 5 mM KCl, 5 mM glucose, pH 5.6), which ensures a high survival rate of the isolated protoplasts.

After incubation for 30 minutes at from 6 to 8°C, the protoplasts are then used for the transduction experiments.

The protoplasts are exposed to a pseudotyped retroviral vector (*e.g.*, a lentiviral vector) encoding a protein of interest driven by a plant specific promoter. The vector is prepared as described above and is used at an MOI of 1,000. The protoplasts are then resuspended in fresh K₃ medium (0.3 ml protoplast solution in 10 ml of fresh K₃ medium). Further incubation is carried out in 10 ml portions in 10 cm diameter petri dishes at 24°C in the dark, the population density being from 4 to 8×10₄ protoplasts per ml. After 3 days, the culture medium is diluted with 0.3 parts by volume of K₃ medium per dish and incubation is continued for a further 4 days at 24°C and 3000 lux of artificial light. After a total of 7 days, the clones that have developed from the protoplasts are embedded in nutrient medium that contains 50 mg/l of kanamycin and has been solidified with 1% agarose, and are cultured at 24°C in the dark in accordance with the "bead-type" culturing method (Shillito, *et al.*, Plant Cell Reports, 2, 244-247 (1983)). The nutrient medium is replaced every 5 days by a fresh amount of the same nutrient solution. Analysis of the clones indicates that express the gene of interest.

Example 25

Stability of Vector Insertions in Cell Lines Over Time

Two cell lines that contain gene inserts of the LN-CMV-Bot vector were analyzed for there ability to maintain the vector inserts over a number of passages with and without neomycin selection. The first cell line is a bovine mammary epithelial cell line that contains a low number of insert copies. The second cell line is a 293GP line that contains multiple copies of the vector insert. At the start of the experiment, cell cultures were split. This was at passage 10 for the bovine mammary epithelial cells and passage 8 for the 293GP cells. One sample was continually passaged in media containing the neomycin analog G418, the other culture was continually passaged in media without any antibiotic. Every 3-6 passages, cells were collected and DNA was isolated for determination of gene ratio using the INVADER assay. Cell were continually grown and passaged in T25 flasks. The results of the assays are shown below:

Table 11 Low Gene Copy Cell Line		
Cell Line and Treatment	Passage Number	INVADER Gene Ratio
BMEC/Bot #66 + G418	10	0.67
BMEC/Bot #66 - G418	10	0.89
BMEC/Bot #66 + G418	16	0.67
BMEC/Bot #66 - G418	16	0.64
BMEC/Bot #66 + G418	21	0.62
BMEC/Bot #66 - G418	21	0.58
BMEC/Bot #66 + G418	27	0.98
BMEC/Bot #66 - G418	27	0.56
BMEC/Bot #66 + G418	33	0.80
BMEC/Bot #66 - G418	33	0.53

Table 12 High Gene Copy Cell Line		
Cell Line and Treatment	Passage Number	INVADER Gene Ratio
293GP/Bot #23 + G418	8	3.46
293GP/Bot #23 - G418	8	3.73
293GP/Bot #23 + G418	14	3.28
293GP/Bot #23 - G418	14	3.13
293GP/Bot #23 + G418	17	3.12
293GP/Bot #23 - G418	17	2.91
293GP/Bot #23 + G418	22	3.6
293GP/Bot #23 - G418	22	2.58
293GP/Bot #23 + G418	28	2.78
293GP/Bot #23 - G418	28	3.44
293GP/Bot #23 + G418	36	2.6
293GP/Bot #23 - G418	36	2.98

74667-216

These data show that there are no consistent differences in gene ratio between cells treated with G418 and those not treated with antibiotic. This suggests that G418 selection is not necessary to maintain the stability of the vector gene insertions. Also, these vector inserts appear to be very stable over time.

Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology, protein fermentation, biochemistry, or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

<110> Bleck, Gregory

Bremel, Robert

Miller, Linda

York, Donna

<120> Host Cells Containing Multiple Integrating Vectors

<130> GALA-04198

<150> 60/215,925

<151> 2000-07-03

<160> 36

<170> PatentIn version 3.0

<210> 1

<211> 2101

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 1

gatcagtcct ggggtggcat tgaaaggact gatgctgaag ttgaagctcc aatactttgg	60
ccacctgatg cgaagaactg actcatgtga taagaccctg atactgggaa agattgaagg	120
caggaggaga agggatgaca gaggatggaa gagttggatg gaatcaccaa ctcgatggac	180
atgagtttga gcaagcttcc aggagttggt aatgggcagg gaagcctggc gtgctgcagt	240
ccatgggggtt gcaaagagtt ggacactact gagtgactga actgaactga tagtgtaatc	300
catggtacag aatataggat aaaaaagagg aagagtttgc cctgattctg aagagttgta	360
ggatataaaa gtttagaata cctttagttt ggaagtctta aattatttac ttaggatggg	420
taccactgc aatataagaa atcaggcttt agagactgat gtagagagaa tgagccctgg	480
cataccagaa gctaacagct attggttata gctgttataa ccaatatata accaatatat	540
tggttatata gcatgaagct tgatgccagc aatttgaagg aaccatttag aactagtatc	600
ctaaactcta catgttccag gacactgatc ttaaagctca ggttcagaat cttgttttat	660
aggctctagg tgtatattgt ggggcttccc tgggtggctca gatggtaaag tgtctgcctg	720
caatgtgggt gatctgggtt cgatccctgg cttgggaaga tcccctggag aaggaaatgg	780
caaccactc tagtactctt acctggaaaa ttccatggac agaggagcct tgtaagctac	840
agtccatggg attgcaaaga gttgaacaca actgagcaac taagcacagc acagtacagt	900
atacacctgt gaggtgaagt gaagtgaagg ttcaatgcag ggtctcctgc attgcagaaa	960

WO 02/02738

PCT/US01/20710

gattcttttac catctgagcc accaggggaag cccaagaata ctggagtggg tagcctattc 1020
 cttctccagg ggatcttccc atcccaggaa ttgaactgga gtctcctgca tttcagggtg 1080
 attcttcacc agctgaacta ccagggtggat actactccaa tattaagtg cttaaagtcc 1140
 agttttccca cctttcccaa aaagggtggg tcactctttt ttaaccttct gtggcctact 1200
 ctgaggctgt ctacaagctt atatatattat gaacacattt attgcaagtt gttagtttta 1260
 gatttacaat gtggtatctg gctatttagt ggtattgggtg gttggggatg gggaggctga 1320
 tagcatctca gagggcagct agatactgtc atacacactt ttcaagttct ccatttttgt 1380
 gaaatagaaa gtctctggat ctaagttata tgtgattctc agtctctgtg gtcataattct 1440
 attctactcc tgaccactca acaaggaacc aagatatcaa gggacacttg ttttgtttca 1500
 tgcctggggtt gagtgggcca tgacatatgt tctgggcctt gttacatggc tggattgggtt 1560
 ggacaagtgc cagctctgat cctgggactg tggcatgtga tgacatacac cccctctcca 1620
 cattctgcat gtctctaggg gggaaggggg aagctcggta tagaaccttt attgtatttt 1680
 ctgattgcct cacttcttat attgccccca tgcccttctt tgttcctcaa gtaaccagag 1740
 acagtgcctc ccagaaccaa ccctacaaga aacaaagggc taaacaaagc caaatgggaa 1800
 gcaggatcat ggtttgaact ctttctggcc agagaacaat acctgctatg gactagatac 1860
 tgggagaggg aaaggaaaag taggggtgaat tatggaagga agctggcagg ctcagcggtt 1920
 ctgtcttggc atgaccagtc tctcttcatt ctcttcctag atgtagggtt tggtaggaga 1980
 gccctgagg ctttctgcat gaatataaat atatgaaact gagtgatgct tccatttcag 2040
 gttcttgggg gcgccgaatt cgagctcggg acccggggat ctcgaggggg ggcccgggtac 2100
 c 2101

<210> 2

<211> 245

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 2

gattacttac tggcagggtgc tgggggcttc cgagacaatc gcgaacatct acaccacaca 60
 acaccgcctc gaccaggggtg agatatcggc cggggacgcg gcggtggtaa ttacaagcga 120
 ggatccgatt acttactggc aggtgctggg ggcttccgag acaatcgcga acatctacac 180
 cacacaacac cgctcgcacc agggtagagat atcggccggg gacgcggcgg tggtaattac 240
 aagcg 245

<210> 3

<211> 680

<212> DNA

WO 02/02738

PCT/US01/20710

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 3

```

ggaattcgcc cctctccctc cccccccct aacgttactg gccgaagccg cttggaataa      60
ggccggtgtg cgtttgtcta tatgttattt tccaccatat tgccgtcttt tggcaatgtg     120
agggcccgga aacctggccc tgtcttcttg acgagcattc ctaggggtct tccccctctc     180
gccaaaggaa tgcaaggtct gttgaatgtc gtgaaggaag cagttcctct ggaagcttct     240
tgaagacaaa caacgtctgt agcgaccctt tgcaggcagc ggaaccccc acctggcgac     300
aggtgcctct gcggccaaaa gccacgtgta taagatacac ctgcaaaggc ggcacaaccc     360
cagtgccacg ttgtgagttg gatagttgtg gaaagagtca aatggctctc ctcaagcgta     420
ttcaacaagg ggctgaagga tgcccagaag gtaccccat t tatgggatc tgatctgggg     480
cctcggtgca catgctttac atgtgtttag tgcagggtta aaaaacgtct agggcccccg     540
aaccacgggg acgtgggttt cctttgaaaa acacgatgat aatatggcct cctttgtctc     600
tctgctcctg gtaggcattc tattccatgc caccaggcc ggcgccatgg gatattctaga     660
tctcgagctc gcgaaagctt

```

<210> 4

<211> 4207

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 4

```

cggatccggc cattagccat attattcatt gggtatatag cataaatcaa tattggctat      60
tgccatttgc atacgttgta tccatatcat aatatgtaca ttatatattg ctcatgtcca     120
acattaccgc catgttgaca ttgattattg actagttatt aatagtaatc aattacgggg     180
tcattagttc atagcccata tatggagttc cgcgttacat aacttacggt aaatggcccc     240
cctggctgac cgcccaacga ccccgccca ttgacgtcaa taatgacgta tgttcccata     300
gtaacgcaa tagggacttt ccattgacgt caatgggtgg agtatattac gtaaactgcc     360
cacttggcag tacatcaagt gtatcatatg ccaagtacgc ccctattga cgtcaatgac     420
ggtaaattgg ccgcctggca ttatgcccag tacatgacct tatgggactt tcctacttgg     480
cagtacatct acgtattagt catcgctatt accatgggtga tgcggttttg gcagtacatc     540
aatgggcgtg gatagcgggt tgactcacgg ggatttccaa gtctccaccc cattgacgtc     600
aatgggagtt tgttttggca ccaaatcaa cgggactttc caaatgtcg taacaactcc     660
gcccattga cgcaaatggg cggtaggcat gtacgggtgg aggtctatat aagcagagct     720

```

WO 02/02738

PCT/US01/20710

cgttttagtga	accgtcagat	cgcctggaga	cgccatccac	gctgttttga	cctccataga	780
agacaccggg	accgatccag	cctccgcggc	cccaagcttc	tcgacggatc	cccgggaatt	840
caggacctca	ccatgggatg	gagctgtatc	atcctcttct	tggtagcaac	agctacaggt	900
gtccactccg	aggtccaact	ggtggagagc	ggtggagggtg	ttgtgcaacc	tggccgggtcc	960
ctgcgcctgt	cctgctccgc	atctggcttc	gatttcacca	catattggat	gagttgggtg	1020
agacaggcac	ctggaaaagg	tcttgagtgg	attggagaaa	ttcatccaga	tagcagtacg	1080
attaactatg	cgccgtctct	aaaggataga	tttacaatat	cgcgagacaa	cgccaagaac	1140
acattgttcc	tgcaaatgga	cagcctgaga	cccgaagaca	ccgggggtcta	tttttgtgca	1200
agcctttact	tcggcttccc	ctggtttgct	tattggggcc	aagggacccc	ggtcaccgtc	1260
tcctcagcct	ccaccaaggg	cccatcggtc	ttccccctgg	cacctctctc	caagagcacc	1320
tctgggggca	cagcggccct	gggctgcctg	gtcaaggact	acttccccga	accggtgacg	1380
gtgtcgtgga	actcaggcgc	cctgaccagc	ggcgtgcaca	ccttcccggc	tgtcctacag	1440
tcctcaggac	tctactccct	cagcagcgtg	gtgaccgtgc	cctccagcag	cttgggcacc	1500
cagacctaca	tctgcaacgt	gaatcacaa	cccagcaaca	ccaaggtgga	caagagagtt	1560
gagcccaa	cttgtgacaa	aactcacaca	tgcccaccgt	gcccagcacc	tgaactcctg	1620
gggggaccgt	cagtcttctc	cttcccccca	aaaccaagg	acaccctcat	gatctcccgg	1680
acccctgagg	tcacatgcgt	ggtggtggac	gtgagccacg	aagaccctga	ggtcaagttc	1740
aactggtacg	tggacggcgt	ggaggtgcat	aatgccaaga	caaagccgcg	ggaggagcag	1800
tacaacagca	cgtaccgtgt	ggtcagcgtc	ctcaccgtcc	tgcaccagga	ctggctgaat	1860
ggcaaggagt	acaagtgcaa	ggtctccaac	aaagccctcc	cagcccccat	cgagaaaacc	1920
atctccaaag	ccaaagggca	gccccgagaa	ccacaggtgt	acaccctgcc	cccatcccgg	1980
gaggagatga	ccaagaacca	ggtcagcctg	acctgcctgg	tcaaaggctt	ctatcccagc	2040
gacatcgccg	tggagtggga	gagcaatggg	cagccggaga	acaactacaa	gaccacgcct	2100
cccgtgctgg	actccgacgg	ctccttcttc	ctctatagca	agctcaccgt	ggacaagagc	2160
aggtggcagc	aggggaacgt	cttctcatgc	tccgtgatgc	acgaggctct	gcacaaccac	2220
tacacgcaga	agagcctctc	cctgtctccc	gggaaatgaa	agccgaattc	gcccctctcc	2280
ctcccccccc	cctaacgtta	ctggccgaag	ccgcttggaa	taaggccggg	gtgcgtttgt	2340
ctatatgtta	ttttccacca	tattgccgtc	ttttggcaat	gtgagggccc	ggaaacctgg	2400
ccctgtcttc	ttgacgagca	ttcctagggg	tctttcccct	ctcgccaaag	gaatgcaagg	2460
tctgttgaat	gtcgtgaagg	aagcagttcc	tctggaagct	tcttgaagac	aaacaacgtc	2520
tgtagcgacc	ctttgcaggc	agcggaaacc	cccacctggc	gacaggtgcc	tctgcggcca	2580
aaagccacgt	gtataagata	cacctgcaaa	ggcggcacia	cccagtgcc	acgttgtgag	2640
ttggatagtt	gtggaaagag	tcaaatggct	ctcctcaagc	gtattcaaca	aggggctgaa	2700
ggatgcccag	aaggtacccc	attgtatggg	atctgatctg	gggcctcggg	gcacatgctt	2760

WO 02/02738

PCT/US01/20710

```

tacatgtggt tagtcgaggt taaaaaaacg tctaggcccc ccgaaccacg gggacgtggt 2820
tttcctttga aaaacacgat gataatatgg cctcctttgt ctctctgctc ctggtaggca 2880
tcctattcca tgccacccag gccgacatcc agctgacca gagcccaagc agcctgagcg 2940
ccagcgtggg tgacagagt accatcacct gtaaggccag tcaggatgtg ggtacttctg 3000
tagcctggta ccagcagaag ccaggtaagg ctccaaagct gctgatctac tggacatcca 3060
cccggcacac tgggtgtgcca agcagattca gcggtagcgg tagcggtaacc gacttcacct 3120
tcaccatcag cagcctccag ccagaggaca tcgccaccta ctactgccag caatatagcc 3180
tctatcggtc gttcggccaa gggaccaagg tggaaatcaa acgaactgtg gctgcaccat 3240
ctgtcttcat cttcccggca tctgatgagc agttgaaatc tggaaactgcc tctgttgtgt 3300
gcctgctgaa taacttctat cccagagagg ccaaagtaca gtggaagggtg gataacgccc 3360
tccaatcggg taactcccag gagagtgtca cagagcagga cagcaaggac agcacctaca 3420
gcctcagcag caccctgacg ctgagcaaag cagactacga gaaacacaaa gtctacgcct 3480
gcgaagtcac ccatcagggc ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt 3540
gttagagatc taggcctcct aggtcgacat cgataaaata aaagatttta tttagtctcc 3600
agaaaaaggg gggaatgaaa gacccacct gtaggtttgg caagctagct taagtaacgc 3660
cattttgcaa ggcatggaaa aatacataac tgagaataga gaagttcaga tcaaggtcag 3720
gaacagatgg aacagctgaa tatgggccaac acaggatatc tgtggtaagc agttcctgcc 3780
ccggctcagg gccaaagaaca gatggaacag ctgaatatgg gccaaacagg atatctgtgg 3840
taagcagttc ctgccccggc tcagggccaac gaacagatgg tccccagatg cgggccagcc 3900
ctcagcagtt tctagagaac catcagatgt ttccagggtg cccaaggac ctgaaatgac 3960
cctgtgcctt atttgaacta accaatcagt tcgcttctcg cttctgttcg cgcgcttctg 4020
ctccccgagc tcaataaaaag agcccacaac ccctcactcg gggcgccagt cctccgattg 4080
actgagtcgc ccgggtaccc gtgtatccaa taaaccctct tgcagttgca tccgacttgt 4140
ggtctcgctg ttccttggga ggtctcctc tgagtgattg actaccgctc agcgggggtc 4200
tttcatt 4207

```

<210> 5

<211> 4210

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 5

```

ggatccggcc attagccata ttattcattg gttatatagc ataaatcaat attggctatt 60
ggccattgca tacgttgtat ccatatcata atatgtacat ttatatattggc tcatgtccaa 120
cattaccgcc atgttgacat tgattattga ctagttatta atagtaatca attacgggggt 180

```

WO 02/02738

PCT/US01/20710

cattagttca	tagcccatat	atggagttcc	gcgttacata	acttacggta	aatggcccgc	240
ctggctgacc	gccaacgac	ccccgccc	tgacgtcaat	aatgacgtat	gttcccatag	300
taacgccaat	agggactttc	cattgacgtc	aatgggtgga	gtatttacgg	taaactgccc	360
acttggcagt	acatcaagtg	tatcatatgc	caagtacgcc	ccctattgac	gtcaatgacg	420
gtaaatggcc	cgctggcat	tatgcccagt	acatgacctt	atgggacttt	cctacttggc	480
agtacatcta	cgtattagtc	atcgctatta	ccatggtgat	gcggttttgg	cagtacatca	540
atgggcgtgg	atagcggttt	gactcacggg	gatttccaag	tctccacccc	attgacgtca	600
atgggagttt	gttttggcac	caaatcaac	gggactttcc	aaaatgtcgt	aacaactccg	660
ccccattgac	gcaaatgggc	ggtaggcatg	tacgggtgga	ggtctatata	agcagagctc	720
gtttagtga	ccgtcagatc	gcctggagac	gccatccacg	ctgttttgac	ctccatagaa	780
gacaccggga	ccgatccagc	ctccgcggcc	ccaagcttct	cgacggatcc	ccgggaattc	840
aggacctcac	catgggatgg	agctgtatca	tcctcttctt	ggtagcaaca	gctacaggtg	900
tccactccca	ggtccagctg	gtccaatcag	gggctgaagt	caagaaacct	gggtcatcag	960
tgaaggcttc	ctgcaaggct	tctggctaca	cctttactag	ctactggctg	cactgggtca	1020
ggcaggcacc	tggacagggt	ctggaatgga	ttggatacat	taatcctagg	aatgattata	1080
ctgagtacaa	tcagaacttc	aaggacaagg	ccacaataac	tgcagacgaa	tccaccaata	1140
cagcctacat	ggagctgagc	agcctgaggt	ctgaggacac	ggcattttat	ttttgtgcaa	1200
gaagggatat	tactacgttc	tactggggcc	aaggcaccac	ggtcaccgtc	tcctcagcct	1260
ccaccaaggg	cccatcggtc	ttccccctgg	caccctcctc	caagagcacc	tctgggggca	1320
cagcggccct	gggctgcctg	gtcaaggact	acttccccga	accggtgacg	gtgtcgtgga	1380
actcaggcgc	cctgaccagc	ggcgtgcaca	ccttcccggc	tgtcctacag	tcctcaggac	1440
tctactccct	cagcagcgtg	gtgaccgtgc	cctccagcag	cttggggcacc	cagacctaca	1500
tctgcaacgt	gaatcacaag	cccagcaaca	ccaaggtgga	caagagagtt	gagcccaa	1560
cttgtgacaa	aactcacaca	tgcccaccgt	gcccagcacc	tgaactcctg	gggggaccgt	1620
cagtcttcct	cttcccccca	aaaccaagg	acaccctcat	gatctcccgg	accctgagg	1680
tcacatgcgt	ggtggtggac	gtgagccacg	aagaccctga	ggtcaagttc	aactggtacg	1740
tggacggcgt	ggaggtgcat	aatgccaaga	caaagccgcg	ggaggagcag	tacaacagca	1800
cgtaccgtgt	ggtcagcgtc	ctcaccgtcc	tgcaccagga	ctggctgaat	ggcaaggagt	1860
acaagtgcaa	ggtctccaac	aaagccctcc	cagcccccat	cgagaaaacc	atctccaaag	1920
ccaaagggca	gccccgagaa	ccacaggtgt	acaccctgcc	cccatcccgg	gaggagatga	1980
ccaagaacca	ggtcagcctg	acctgcctgg	tcaaaggctt	ctatcccagc	gacatcgccg	2040
tggagtggga	gagcaatggg	cagccggaga	acaactacaa	gaccacgcct	cccgtgctgg	2100
actccgacgg	ctccttcttc	ctctatagca	agctcaccgt	ggacaagagc	aggtggcagc	2160
aggggaacgt	cttctcatgc	tccgtgatgc	acgaggctct	gcacaaccac	tacacgcaga	2220

WO 02/02738

PCT/US01/20710

agagcctctc	cctgtctccc	gggaaatgaa	agccgaattc	gcccctctcc	ctcccccccc	2280
cctaacgtta	ctggccgaag	ccgcttgga	taaggccggt	gtgcgtttgt	ctatatgtta	2340
ttttccacca	tattgccgtc	ttttggcaat	gtgagggccc	ggaaacctgg	ccctgtcttc	2400
ttgacgagca	ttcctagggg	tctttcccct	ctcgccaaag	gaatgcaagg	tctgttgaat	2460
gtcgtgaagg	aagcagttcc	tctggaagct	tcttgaagac	aaacaacgtc	tgtagcgacc	2520
ctttgcaggc	agcggaaacc	cccacctggc	gacaggtgcc	tctgcggcca	aaagccacgt	2580
gtataagata	cacctgcaaa	ggcggcacia	ccccagtgcc	acgttgtgag	ttggatagtt	2640
gtggaaagag	tcaaattggct	ctcctcaagc	gtattcaaca	aggggctgaa	ggatgcccag	2700
aaggtacccc	attgtatggg	atctgatctg	gggcctcggt	gcacatgctt	tacatgtgtt	2760
tagtcgaggt	taaaaaacg	tctaggcccc	ccgaaccacg	gggacgtggg	tttcctttga	2820
aaaacacgat	gataatatgg	cctcctttgt	ctctctgctc	ctggtaggca	tcctattcca	2880
tgccacccag	gccgacatcc	agctgaccca	gtctccatca	tctctgagcg	catctgttgg	2940
agatagggtc	actatgagct	gtaagtccag	tcaaagtgtt	ttatacagtg	caaatacacia	3000
gaactacttg	gcctgggtacc	agcagaaaacc	agggaaagca	cctaaactgc	tgatctactg	3060
ggcatccact	agggaatctg	gtgtcccttc	gcgattctct	ggcagcggat	ctgggacaga	3120
ttttactttc	accatcagct	ctcttcaacc	agaagacatt	gcaacatatt	attgtcacca	3180
atacctctcc	tcgtggacgt	tcgggtggagg	gaccaagggtg	cagatcaaac	gaactgtggc	3240
tgcaccatct	gtcttcatct	tcccgccatc	tgatgagcag	ttgaaatctg	gaactgcctc	3300
tgttgtgtgc	ctgctgaata	acttctatcc	cagagaggcc	aaagtacagt	ggaagggtgga	3360
taacgccctc	caatcgggta	actcccagga	gagtgtcaca	gagcaggaca	gcaaggacag	3420
cacctacagc	ctcagcagca	ccctgacgct	gagcaaagca	gactacgaga	aacacaaagt	3480
ctacgcctgc	gaagtcaccc	atcagggcct	gagctcgccc	gtcacaaaga	gcttcaacag	3540
gggagagtgt	tagagatcta	ggcctcctag	gtcgacatcg	ataaaataaa	agattttatt	3600
tagtctccag	aaaaaggggg	gaatgaaaga	ccccacctgt	aggtttggca	agctagctta	3660
agtaacgcca	ttttgcaagg	catggaaaaa	tacataactg	agaatagaga	agttcagatc	3720
aaggtcagga	acagatggaa	cagctgaata	tgggccaaac	aggatatctg	tggtaagcag	3780
ttcctgcccc	ggctcagggc	caagaacaga	tggaaacagct	gaatatgggc	caaacaggat	3840
atctgtggta	agcagttcct	gccccggctc	agggccaaga	acagatggtc	cccagatgcg	3900
gtccagccct	cagcagtttc	tagagaacca	tcagatgttt	ccaggggtgcc	ccaaggacct	3960
gaaatgaccc	tgtgccttat	ttgaactaac	caatcagttc	gcttctcgct	tctgttcgcg	4020
cgcttctgct	ccccgagctc	aataaaaagag	cccacaaccc	ctcactcggg	gcgccagtcc	4080
tccgattgac	tgagtcgccc	gggtaccctg	gtatccaata	aacctctctg	cagttgcata	4140
cgacttgtgg	tctcgctggt	ccttggggagg	gtctcctctg	agtgattgac	taccctgcag	4200
gtctttcatt						4210

WO 02/02738

PCT/US01/20710

<210> 6

<211> 5732

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 6

```

cgagcttggc agaaatggtt gaactcccga gagtgtccta cacctagggg agaagcagcc      60
aaggggttgt ttcccaccaa ggacgacccg tctgcgacaca aacggatgag cccatcagac      120
aaagacatat tcattctctg ctgcaaactt ggcatagctc tgcttttgct ggggctattg      180
ggggaagttg cggttcgtgc tcgcagggct ctcacccttg actctttcaa taataactct      240
tctgtgcaag attacaatct aaacaattcg gagaactcga ccttcctcct gaggcaagga      300
ccacagccaa cttcctctta caagccgcat cgattttgtc cttcagaaat agaaataaga      360
atgcttgcta aaaattatat ttttaccaat aagaccaatc caataggtag attattagtt      420
actatgttaa gaaatgaatc attatctttt agtactatct ttactcaaat tcagaagtta      480
gaaatgggaa tagaaaatag aaagagacgc tcaacctcaa ttgaagaaca ggtgcaagga      540
ctattgacca caggcctaga agtaaaaaag ggaaaaaaga gtgtttttgt caaaatagga      600
gacaggtggt ggcaaccagg gacttatagg ggaccttaca tctacagacc aacagatgcc      660
cccttaccat atacaggaag atatgactta aattgggata ggtgggttac agtcaatggc      720
tataaagtgt tatatagatc cctccccttt cgtgaaagac tcgccagagc tagacctcct      780
tggtgtatgt tgtctcaaga aaagaaagac gacatgaaac aacaggtaca tgattatatt      840
tatctaggaa caggaatgca cttttgggga aagattttcc ataccaagga ggggacagtg      900
gctggactaa tagaacatta ttctgcaaaa acttatggca tgagttatta tgattagcct      960
tgatttgccc aaccttgccg ttcccaaggc ttaagtaagt ttttggttac aaactgttct     1020
taaaacaagg atgtgagaca agtggtttcc tgacttggtt tggatatcaa ggttctgatc     1080
tgagctctga gtgttctatt ttcttatggt cttttggaat ttatccaaat cttatgtaaa     1140
tgcttatgta aaccaagata taaaagagtg ctgatttttt gagtaaactt gcaacagtcc     1200
taacattcac ctcttggtgtg tttgtgtctg ttcgccatcc cgtctccgct cgtcacttat     1260
ccttcacttt ccagaggggtc cccccgcaga ccccggcgac cctcaggtcg gccgactgcg     1320
gcagctggcg cccgaacagg gaccctcgga taagtgacct ttgtctttat ttctactatt     1380
ttgtgttcgt cttgttttgt ctctatcttg tctggctatc atcacaagag cggaacggac     1440
tcacctcagg gaaccaagct agcccggggg cgacggatcc gattacttac tggcaggtgc     1500
tgggggcttc cgagacaatc gcgaacatct acaccacaca acaccgcctc gaccagggtg     1560
agatatcggc cggggacgcg gcggtggtaa ttacaagcga gatccgatta cttactggca     1620
ggtgctgggg gcttccgaga caatcgcgaa catctacacc acacaacacc gcctcgacca     1680

```

WO 02/02738

PCT/US01/20710

gggtgagata	tcggccggggg	acgcggcggt	ggtaattaca	agcgagatcc	ccgggaattc	1740
aggacctcac	catgggatgg	agctgtatca	tcctcttctt	ggtagcaaca	gctacaggtg	1800
tccactccga	ggtccaactg	gtggagagcg	gtggaggtgt	tgtgcaacct	ggccgggtccc	1860
tgcgcctgtc	ctgctccgca	tctggcttcg	atttcaccac	atattggatg	agttgggtga	1920
gacaggcacc	tggaaaaggt	cttgagtggg	ttggagaaat	tcattccagat	agcagtacga	1980
ttactatgc	gccgtctcta	aaggatagat	ttacaatatc	gcgagacaac	gccaagaaca	2040
cattgttcct	gcaaattggac	agcctgagac	ccgaagacac	cgggggtctat	ttttgtgcaa	2100
gcctttactt	cggcttcccc	tggtttgctt	attggggcca	agggaccccg	gtcaccgtct	2160
cctcagcctc	caccaagggc	ccatcggtct	tccccctggc	accctcctcc	aagagcacct	2220
ctggggggcac	agcggccctg	ggctgcctgg	tcaaggacta	cttccccgaa	ccggtgacgg	2280
tgtcgtggaa	ctcaggcgcc	ctgaccagcg	gcgtgcacac	cttcccggct	gtcctacagt	2340
cctcaggact	ctactccctc	agcagcgtgg	tgaccgtgcc	ctccagcagc	ttgggcaccc	2400
agacctacat	ctgcaacgtg	aatcacaagc	ccagcaacac	caaggtggac	aagagagttg	2460
agcccaaata	ttgtgacaaa	actcacacat	gcccaccgtg	cccagcacct	gaactcctgg	2520
ggggaccgtc	agtcttcctc	ttccccccaa	aaccaagga	caccctcatg	atctcccgga	2580
cccctgaggt	cacatgcgtg	gtggtggacg	tgagccacga	agaccctgag	gtcaagttca	2640
actggtacgt	ggacggcgtg	gaggtgcata	atgccaaagc	aaagccgcgg	gaggagcagt	2700
acaacagcac	gtaccgtgtg	gtcagcgtcc	tcaccgtcct	gcaccaggac	tggctgaatg	2760
gcaaggagta	caagtgcaag	gtctccaaca	aagccctccc	agcccccatc	gagaaaacca	2820
tctccaaagc	caaagggcag	ccccgagaac	cacaggtgta	caccctgccc	ccatcccggg	2880
aggagatgac	caagaaccag	gtcagcctga	cctgcctggg	caaaggcttc	tatcccagcg	2940
acatcgccgt	ggagtgggag	agcaatgggc	agccggagaa	caactacaag	accacgcctc	3000
ccgtgctgga	ctccgacggc	tccttcttcc	tctatagcaa	gtcaccgtg	gacaagagca	3060
ggtggcagca	ggggaacgtc	ttctcatgct	ccgtgatgca	cgaggctctg	cacaaccact	3120
acacgcagaa	gagcctctcc	ctgtctcccc	ggaaatgaaa	gccgaattcg	cccctctccc	3180
tccccccccc	ctaacgttac	tggccgaagc	cgcttggaat	aaggccgggtg	tgcgtttgtc	3240
tatatgttat	tttccaccat	attgccgtct	tttggcaatg	tgaggggccc	gaaacctggc	3300
cctgtcttct	tgacgagcat	tcctaggggt	ctttcccctc	tcgccaaagg	aatgcaaggt	3360
ctgttgaatg	tcgtgaagga	agcagttcct	ctggaagctt	cttgaagaca	aacaacgtct	3420
gtagcgaccc	tttgcaggca	gcggaacccc	ccacctggcg	acaggtgcct	ctgcggccaa	3480
aagccacgtg	tataagatac	acctgcaaag	gcggcacaac	cccagtgcca	cgttgtgagt	3540
tggatagttg	tggaaagagt	caaattggctc	tcctcaagcg	tattcaacaa	ggggctgaag	3600
gatgcccaga	aggtacccca	ttgtatggga	tctgatctgg	ggcctcggtg	cacatgcttt	3660
acatgtgttt	agtcgaggtt	aaaaaaacgt	ctaggccccc	cgaaccacgg	ggacgtggtt	3720

WO 02/02738

PCT/US01/20710

ttccttttgaa	aaacacgatg	ataatatggc	ctcctttgtc	tctctgctcc	tggtaggcat	3780
cctattccat	gccacccagg	ccgacatcca	gctgacccag	agcccaagca	gcctgagcgc	3840
cagcgtgggt	gacagagtga	ccatcacctg	taaggccagt	caggatgtgg	gtacttctgt	3900
agcctggtac	cagcagaagc	caggtaaggc	tccaaagctg	ctgatctact	ggacatccac	3960
ccggcacact	ggtgtgccaa	gcagattcag	cggtagcggg	agcggtagcg	acttcacctt	4020
caccatcagc	agcctccagc	cagaggacat	cgccacctac	tactgccagc	aatatagcct	4080
ctatcggctg	ttcggccaag	ggaccaagg	ggaaatcaaa	cgaactgtgg	ctgcaccatc	4140
tgtcttcac	ttcccgccat	ctgatgagca	gttgaaatct	ggaactgcct	ctggtgtgtg	4200
cctgctgaat	aacttctatc	ccagagaggc	caaagtacag	tggaagggtg	ataacgccct	4260
ccaatcgggt	aactcccagg	agagtgtcac	agagcaggac	agcaaggaca	gcacctacag	4320
cctcagcagc	accctgacgc	tgagcaaagc	agactacgag	aaacacaaag	tctacgcctg	4380
cgaagtcacc	catcagggcc	tgagctcgcc	cgtcacaaag	agcttcaaca	ggggagagtg	4440
ttagagatcc	cccgggctgc	aggaattcga	tatcaagctt	atcgataatc	aacctctgga	4500
ttacaaaatt	tgtgaaagat	tgactgggat	tcttaactat	gttgctcctt	ttacgctatg	4560
tggatacgct	gctttaatgc	ctttgtatca	tgctattgct	tcccgtagtg	ctttcatttt	4620
ctcctccttg	tataaatcct	ggttgctgtc	tctttatgag	gagttgtggc	ccgttgctcag	4680
gcaacgtggc	gtggtgtgca	ctgtgtttgc	tgacgcaacc	cccactgggt	ggggcattgc	4740
caccacctgt	cagctccttt	ccgggacttt	cgctttcccc	ctccctattg	ccacggcgga	4800
actcatcgcc	gcctgccttg	cccgtgctg	gacaggggct	cggctgttgg	gcactgacaa	4860
ttccgtggtg	ttgtcgggga	aatcatcgtc	ctttccttgg	ctgctcgctt	gtgttgccac	4920
ctggattctg	cgcgggacgt	ccttctgcta	cgctcccttcg	gcctcgaatc	cagcggacct	4980
tccttcccgc	ggcctgctgc	cggtctctgc	gcctcttccg	cgtcttcgcc	ttcgccctca	5040
gacgagtcgg	atctcccttt	gggcccgcctc	cccgcctgat	cgataccgtc	aacatcgata	5100
aaataaaaaga	ttttatttag	tctccagaaa	aaggggggaa	tgaaagaccc	cacctgtagg	5160
tttggcaagc	tagcttaagt	aacgccattt	tgcaaggcat	ggaaaaatac	ataactgaga	5220
atagagaagt	tcagatcaag	gtcaggaaca	gatggaacag	ctgaatatgg	gccaacacag	5280
atatctgtgg	taagcagttc	ctgccccggc	tcaggggccaa	gaacagatgg	aacagctgaa	5340
tatggggccaa	acaggatatc	tgtggtaagc	agttcctgcc	ccggctcagg	gccaagaaca	5400
gatggtcccc	agatgcggtc	cagccctcag	cagtttctag	agaaccatca	gatgtttcca	5460
gggtgccccca	aggacctgaa	atgacctgt	gccttatattg	aactaaccaa	tcagttcgct	5520
tctcgcttct	gttcgcgcgc	ttctgctccc	cgagctcaat	aaaagagccc	acaaccctc	5580
actcggggcg	ccagtcctcc	gattgactga	gtcgccccggg	taccctgtga	tccaataaac	5640
cctcttgacg	ttgcatccga	cttgtggtct	cgctgttcc	tgggagggtc	tcctctgagt	5700
gattgactac	ccgtcagcgg	gggtctttca	tt			5732

WO 02/02738

PCT/US01/20710

<210> 7

<211> 9183

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 7

```

aaagacccca cccgtaggtg gcaagctagc ttaagtaacg ccactttgca aggcattggaa      60
aaatacataa ctgagaatag aaaagttcag atcaagggtca ggaacaaaga aacagctgaa      120
taccaaacag gatattctgt gtaagcgggt cctgccccgg ctcagggcca agaacagatg      180
agacagctga gtgatggggc aaacaggata tctgtggtaa gcagttcctg ccccggtctg      240
gggccaagaa cagatgggtc ccagatgcgg tccagccctc agcagtttct agtgaatcat      300
cagatgtttc caggggtgcc caaggacctg aaaatgacct tgtaccttat ttgaactaac      360
caatcagttc gcttctcgct tctgttcgcg cgcttcgctc ctccgagctc aataaaagag      420
cccacaacct ctactcggc gcgccagtct tccgatagac tgcgtcgccc gggtagccgt      480
attcccaata aagcctcttg ctgtttgcat ccgaatcgtg gtctcgctgt tccttgggag      540
ggtctcctct gagtgattga ctaccacga cgggggtctt tcatttgggg gctcgtccgg      600
gatttggaga cccctgcccc gggaccaccg acccaccacc gggaggtaag ctggccagca      660
acttatctgt gtctgtccga ttgtctagt tctatgtttg atgttatgcg cctgcgtctg      720
tactagttag ctaactagct ctgtatctgg cggaccctgt gtggaactga cgagttctga      780
acaccgggcc gcaaccctgg gagacgtccc agggactttg ggggcccgtt ttgtggcccc      840
acctgaggaa gggagtcgat gtggaatccg acccgcgcag gatatgtggg tctggtagga      900
gacgagaacc taaaacagtt cccgcctccg tctgaatttt tgctttcggg ttggaaccga      960
agccgcgcgt cttgtctgct gcagcgtgc agcatcgttc tgtgttgtct ctgtctgact     1020
gtgtttctgt atttgtctga aaattagggc cagactgtta ccactccctt aagtttgacc     1080
ttaggtcact ggaaagatgt cgagcggatc gtcacaacc agtcggtaga tgtcaagaag     1140
agacgttggg ttaccttctg ctctgcagaa tggccaacct ttaacgtcgg atggccgcga     1200
gacggcacct ttaaccgaga cctcatcacc caggttaaga tcaaggctct ttcacctggc     1260
ccgcatggac acccagacca ggtcccctac atcgtgacct gggaagcctt ggcttttgac     1320
ccccctcctt gggtaagcc ctttgtacac cctaagcctc cgctcctct tcctccatcc     1380
gccccgtctc tcccccttga acctcctcgt tcgaccccg ctcgacccct cctttatcca     1440
gccctcactc cttctctagg cgccggaatt ccgatctgat caagagacag gatgaggatc     1500
gtttcgcatt attgaacaag atggattgca cgcagggtct ccggccgctt ggggtggagag     1560
gctattcggc tatgactggg cacaacagac aatcggctgc tctgatgccg ccgtgttccg     1620
gctgtcagcg cagggggccc cggttctttt tgtcaagacc gacctgtccg gtgccctgaa     1680

```

WO 02/02738

PCT/US01/20710

tgaactgcag	gacgaggcag	cgcggtctatc	gtggctggcc	acgacgggcg	ttccttgccg	1740
agctgtgctc	gacgttgctc	ctgaagcggg	aagggaactgg	ctgctattgg	gcgaagtgcc	1800
ggggcaggat	ctcctgtcat	ctcaccttgc	tcctgccgag	aaagtatcca	tcattggctga	1860
tgcaatgcgg	cggctgcata	cgcttgatcc	ggctacctgc	ccattcgacc	accaagcgaa	1920
acatcgcctc	gagcgagcac	gtactcggat	ggaagccggg	cttgctcgatc	aggatgatct	1980
ggacgaagag	catcaggggc	tcgcgccagc	cgaactgttc	gccaggctca	aggcgccgat	2040
gcccgcaggc	gaggatctcg	tcgtgaccca	tggcgatgcc	tgcttgccga	atatcatggg	2100
ggaaaatggc	cgcttttctg	gattcatcga	ctgtggccgg	ctgggtgtgg	cggaccgcta	2160
tcaggacata	gcgttggcta	cccgtgatat	tgctgaagag	cttggcgggc	aatgggctga	2220
ccgcttcctc	gtgctttacg	gtatcgccgc	tcccgattcg	cagcgcatcg	ccttctatcg	2280
ccttcttgac	gagttcttct	gagcgggact	ctgggggttc	aaatgaccga	ccaagcgacg	2340
cccaacctgc	catcacgaga	tttcgattcc	accgccgcct	tctatgaaag	gttgggcttc	2400
ggaatcgttt	tccgggacgc	cggctggatg	atcctccagc	gcggggatct	catgctggag	2460
ttcttcgccc	accccgggct	cgatcccctc	gcgagttggg	tcagctgctg	cctgaggctg	2520
gacgacctcg	cggagttcta	ccggcagtgc	aaatccgtcg	gcattccagga	aaccagcagc	2580
ggctatccgc	gcattccatgc	ccccgaactg	caggagtggg	gaggcacgat	ggccgctttg	2640
gtcgaggcgg	atcctagaac	tagcgaaaat	gcaagagcaa	agacgaaaac	atgccacaca	2700
tgaggaatac	cgattctctc	attaacatat	tcaggccagt	tatctgggct	taaaagcaga	2760
agtccaaccc	agataacgat	catatacatg	gttctctcca	gaggttcatt	actgaacact	2820
cgtccgagaa	taacgagtgg	atcagtcctg	ggtgggtcatt	gaaaggactg	atgctgaagt	2880
tgaagctcca	atactttggc	cacctgatgc	gaagaactga	ctcatgtgat	aagaccctga	2940
tactgggaaa	gattgaaggc	aggaggagaa	gggatgacag	aggatggaag	agttggatgg	3000
aatcaccaac	tcgatggaca	tgagtttgag	caagcttcca	ggagttggta	atgggcaggg	3060
aagcctggcg	tgctgcagtc	catgggggtg	caaagagttg	gacactactg	agtgactgaa	3120
ctgaactgat	agtgtaatcc	atggtacaga	atataggata	aaaaagagga	agagtttgcc	3180
ctgattctga	agagttgtag	gatataaaaag	tttagaatac	ctttagtttg	gaagtcttaa	3240
attatttact	taggatgggt	accactgca	atataagaaa	tcaggcttta	gagactgatg	3300
tagagagaat	gagccctggc	ataccagaag	ctaacagcta	ttggttatag	ctgtttataac	3360
caatatataa	ccaatatatt	ggttatatag	catgaagctt	gatgccagca	atttgaagga	3420
accattttaga	actagtatcc	taaactctac	atgttccagg	acactgatct	taaagctcag	3480
gttcagaatc	ttgtttttata	ggctctaggg	gtatatgttg	gggcttccct	ggtggctcag	3540
atggtaaagt	gtctgcctgc	aatgtgggtg	atctgggttc	gatccctggc	ttgggaagat	3600
cccctggaga	aggaaatggc	aaccactct	agtactctta	cctggaaaat	tccatggaca	3660
gaggagcctt	gtaagctaca	gtccatggga	ttgcaaagag	ttgaacacaa	ctgagcaact	3720

WO 02/02738

PCT/US01/20710

aagcacagca	cagtacagta	tacacctgtg	aggtgaagtg	aagtgaaggt	tcaatgcagg	3780
gtctcctgca	ttgcagaaag	attctttacc	atctgagcca	ccaggggaagc	ccaagaatac	3840
tggagtgggt	agcctattcc	ttctccaggg	gatcttccca	tcccaggaat	tgaactggag	3900
tctcctgcat	ttcaggtgga	ttcttcacca	gctgaactac	caggtggata	ctactccaat	3960
attaaagtgc	ttaaagtcca	gttttcccac	ctttcccaaa	aagggtgggt	cactcttttt	4020
taaccttctg	tggcctactc	tgaggctgtc	tacaagctta	tatatattatg	aacacattta	4080
ttgcaagttg	ttagtttttag	atttacaatg	tggtatctgg	ctatttagtg	gtattgggtg	4140
ttggggatgg	ggaggctgat	agcatctcag	agggcagcta	gatactgtca	tacacacttt	4200
tcaagttctc	cattttttgtg	aaatagaaag	tctctggatc	taagttatat	gtgattctca	4260
gtctctgtgg	tcataattcta	ttctactcct	gaccactcaa	caaggaacca	agatatcaag	4320
ggacacttgt	tttgtttcat	gcctgggttg	agtgggccat	gacatatgtt	ctgggccttg	4380
ttacatggct	ggattgggtg	gacaagtgcc	agctctgatc	ctgggactgt	ggcatgtgat	4440
gacatacacc	ccctctccac	attctgcatg	tctctagggg	ggaaggggga	agctcggtat	4500
agaaccttta	ttgtattttc	tgattgcctc	acttcttata	ttgcccccat	gcccttcttt	4560
gttcctcaag	taaccagaga	cagtgcctcc	cagaaccaac	cctacaagaa	acaaagggct	4620
aaacaaagcc	aaatgggaag	caggatcatg	gtttgaactc	tttctggcca	gagaacaata	4680
cctgctatgg	actagatact	gggagaggga	aaggaaaagt	aggggtgaatt	atggaaggaa	4740
gctggcaggc	tcagcgtttc	tgtcttgga	tgaccagtct	ctcttcattc	tcttcctaga	4800
tgtagggctt	ggtaccagag	cccctgaggc	tttctgcatg	aatataaata	tatgaaactg	4860
agtgatgctt	ccatttcagg	ttcttggggg	cgccgaattc	gagctcggtg	cccggggatc	4920
tcgacggatc	cgattactta	ctggcaggtg	ctgggggctt	ccgagacaat	cgcgaaacatc	4980
tacaccacac	aacaccgcct	cgaccagggg	gagatatcgg	ccggggacgc	ggcgggtggta	5040
attacaagcg	agatccgatt	acttactggc	aggtgctggg	ggcttccgag	acaatcgcg	5100
acatctacac	cacacaacac	cgctctgacc	agggtgagat	atcggccggg	gacgcggcgg	5160
tggttaattac	aagcgagatc	cccgggaatt	caggacctca	ccatgggatg	gagctgtatc	5220
atcctcttct	tggtagcaac	agctacaggt	gtccactccg	aggtccaact	ggtggagagc	5280
ggtggaggtg	ttgtgcaacc	tggccgggtc	ctgcgcctgt	cctgctccgc	atctggcttc	5340
gatttcacca	catattggat	gagttgggtg	agacaggcac	ctggaaaagg	tcttgagtgg	5400
attggagaaa	ttcatccaga	tagcagtacg	attaactatg	cgccgtctct	aaaggataga	5460
tttacaatat	cgcgagacaa	cgccaagaac	acattgttcc	tgcaaattga	cagcctgaga	5520
cccgaagaca	ccgggggtcta	tttttgtgca	agcctttact	tcggcttccc	ctggtttgct	5580
tattggggcc	aagggaaccc	ggtcaccgtc	tcctcagcct	ccaccaaggg	cccatcggtc	5640
ttccccctgg	caccctctct	caagagcacc	tctgggggca	cagcggccct	gggctgcctg	5700
gtcaaggact	acttccccga	accggtgacg	gtgtcgtgga	actcaggcgc	cctgaccagc	5760

WO 02/02738

PCT/US01/20710

ggcgtgcaca	ccttcccggc	tgtcctacag	tcctcaggac	tctactccct	cagcagcgtg	5820
gtgaccgtgc	cctccagcag	cttgggcacc	cagacctaca	tctgcaacgt	gaatcacaag	5880
cccagcaaca	ccaaggtgga	caagagagtt	gagcccaa	cttgtgacaa	aactcacaca	5940
tgcccaccgt	gcccagcacc	tgaactcctg	gggggaccgt	cagtcttctt	cttcccccca	6000
aaacccaagg	acaccctcat	gatctcccgg	acccttgagg	tcacatgcgt	ggtggtggac	6060
gtgagccacg	aagaccctga	ggtcaagttc	aactggtacg	tggacggcgt	ggaggtgcat	6120
aatgccaaga	caaagccgcg	ggaggagcag	tacaacagca	cgtaccgtgt	ggtcagcgtc	6180
ctcaccgtcc	tgcaccagga	ctggctgaat	ggcaaggagt	acaagtgcaa	ggtctccaac	6240
aaagccctcc	cagcccccat	cgagaaaacc	atctccaaag	ccaaagggca	gccccgagaa	6300
ccacaggtgt	acaccctgcc	cccatcccgg	gaggagatga	ccaagaacca	ggtcagcctg	6360
acctgcctgg	tcaaaggctt	ctatcccagc	gacatcgccg	tggagtggga	gagcaatggg	6420
cagccggaga	acaactacaa	gaccacgcct	cccgtgctgg	actccgacgg	ctccttcttc	6480
ctctatagca	agctcaccgt	ggacaagagc	aggtggcagc	aggggaacgt	cttctcatgc	6540
tccgtgatgc	acgaggctct	gcacaaccac	tacacgcaga	agagcctctc	cctgtctccc	6600
gggaaatgaa	agccgaattc	gcccctctcc	ctccccccc	cctaacgtta	ctggccgaag	6660
ccgcttgga	taaggccggt	gtgcgtttgt	ctatatgtta	ttttccacca	tattgccgtc	6720
ttttggcaat	gtgaggggcc	ggaaacctgg	ccctgtcttc	ttgacgagca	ttcctagggg	6780
tctttcccct	ctcgccaaag	gaatgcaagg	tctgttgaat	gtcgtgaagg	aagcagttcc	6840
tctggaagct	tcttgaagac	aaacaacgtc	tgtagcgacc	ctttgcaggc	agcggaaacc	6900
cccacctggc	gacaggtgcc	tctgcggcca	aaagccacgt	gtataagata	cacctgcaaa	6960
ggcggcacaa	ccccagtgcc	acgttgtgag	ttggatagtt	gtggaaagag	tcaaattggct	7020
ctcctcaagc	gtattcaaca	aggggctgaa	ggatgcccag	aaggtacccc	attgtatggg	7080
atctgatctg	gggcctcggt	gcacatgctt	tacatgtgtt	tagtcgaggt	taaaaaaacg	7140
tctaggcccc	ccgaaccacg	gggacgtggg	tttcctttga	aaaacacgat	gataatatgg	7200
cctcctttgt	ctctctgctc	ctggtaggca	tcctattcca	tgccacccag	gccgacatcc	7260
agctgacca	gagcccaagc	agcctgagcg	ccagcgtggg	tgacagagtg	accatcacct	7320
gtaaggccag	tcaggatgtg	ggtacttctg	tagcctggta	ccagcagaag	ccaggtaagg	7380
ctccaaagct	gctgatctac	tggacatcca	cccggcacac	tgggtgtgcca	agcagattca	7440
gcggtagcgg	tagcgggtacc	gacttcacct	tcaccatcag	cagcctccag	ccagaggaca	7500
tcgccaccta	ctactgccag	caatatagcc	tctatcggtc	gttcggccaa	gggaccaagg	7560
tggaaatcaa	acgaactgtg	gctgcaccat	ctgtcttcat	cttcccggca	tctgatgagc	7620
agttgaaatc	tggaaactgcc	tctgttgtgt	gcctgctgaa	taacttctat	cccagagagg	7680
ccaaagtaca	gtggaagggtg	gataacgccc	tccaatcggg	taactcccag	gagagtgtca	7740
cagagcagga	cagcaaggac	agcacctaca	gcctcagcag	caccctgacg	ctgagcaaag	7800

WO 02/02738

PCT/US01/20710

cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc ctgagctcgc 7860
 ccgtcacaaa gagcttcaac aggggagagt gttagagatc ccccgggctg caggaattcg 7920
 atatcaagct tatcgataat caacctctgg attacaaaat ttgtgaaaga ttgactggta 7980
 ttcttaacta tggtgctcct ttacgctat gtggatacgc tgctttaatg cctttgtatc 8040
 atgctattgc ttcccgtatg gctttcattt tctcctcctt gtataaatcc tgggtgctgt 8100
 ctctttatga ggagttgtgg cccgttgtca ggcaacgtgg cgtgggtgtgc actgtgtttg 8160
 ctgacgcaac cccactgggt tggggcattg ccaccacctg tcagctcctt tccgggactt 8220
 tcgctttccc cctccctatt gccacggcgg aactcatcgc cgcctgcctt gcccgctgct 8280
 ggacaggggc tcggctgttg ggcactgaca attccgtgggt gttgtcgggg aaatcatcgt 8340
 cctttccttg gctgctcgcc tgtgttgcca cctggattct gcgcgggacg tccttctgct 8400
 acgtcccttc ggccctcaat ccagcggacc ttccttcccg cggcctgctg ccggctctgc 8460
 ggcctcttcc gcgtcttcgc cttegccttc agacgagtcg gatctccctt tgggcccct 8520
 ccccgctga tcgataccgt caacatcgat aaaataaaag attttattta gtctccagaa 8580
 aaagggggga atgaaagacc ccacctgtag gtttggcaag ctagcttaag taacgccatt 8640
 ttgcaaggca tggaaaaata cataactgag aatagagaag ttcagatcaa ggtcaggaac 8700
 agatggaaca gctgaatatg ggccaaacag gatattctgtg gtaagcagtt cctgccccgg 8760
 ctcagggcca agaacagatg gaacagctga atatgggcca aacaggatat ctgtggtaag 8820
 cagttcctgc cccggctcag ggccaagaac agatgggtccc cagatgcggt ccagccctca 8880
 gcagtttcta gagaaccatc agatgtttcc aggggtgcccc aaggacctga aatgacctg 8940
 tgccttattt gaactaacca atcagttcgc ttctcgcttc tggtcgcgcg cttctgctcc 9000
 ccgagctcaa taaaagagcc cacaaccct cactcggggc gccagtcctc cgattgactg 9060
 agtcgccccg gtaccctgtg atccaataaa ccctcttgca gttgcatccg acttgtggtc 9120
 tcgctgttcc ttgggaggggt ctctctgag tgattgacta cccgtcagcg ggggtcttcc 9180
 att 9183

<210> 8

<211> 5711

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 8

gatcagtcct gggtgggtcat tgaaaggact gatgctgaag ttgaagctcc aatactttgg 60
 ccacctgatg cgaagaactg actcatgtga taagaccctg atactgggaa agattgaagg 120
 caggaggaga agggatgaca gaggatggaa gagttggatg gaatcaccaa ctgatggac 180
 atgagtttga gcaagcttcc aggagttgggt aatgggcagg gaagcctggc gtgctgcagt 240

WO 02/02738

PCT/US01/20710

ccatgggggtt	gcaaagagtt	ggacactact	gagtgactga	actgaactga	tagtgtaatc	300
catggtacag	aatataggat	aaaaaagagg	aagagtttgc	cctgattctg	aagagttgta	360
ggatataaaa	gtttagaata	ccttttagttt	ggaagtctta	aattattttac	ttaggatggg	420
taccactgc	aatataagaa	atcaggcttt	agagactgat	gtagagagaa	tgagccctgg	480
cataccagaa	gctaacagct	attggttata	gctgttataa	ccaatatata	accaatatat	540
tggttatata	gcatgaagct	tgatgccagc	aatttgaagg	aaccatttag	aactagtatc	600
ctaaactcta	catgttccag	gacactgatc	ttaaagctca	ggttcagaat	cttgttttat	660
aggctctagg	tgtatatgt	ggggcttccc	tggtggctca	gatggtaaag	tgtctgcctg	720
caatgtgggt	gatctggggt	cgatccctgg	cttgggaaga	tcccctggag	aaggaaatgg	780
caaccactc	tagtactctt	acctggaaaa	ttccatggac	agaggagcct	tgtaagctac	840
agtccatggg	attgcaaaga	gttgaacaca	actgagcaac	taagcacagc	acagtacagt	900
atacacctgt	gaggtgaagt	gaagtgaagg	ttcaatgcag	ggtctcctgc	attgcagaaa	960
gattctttac	catctgagcc	accaggggaag	ccaagaata	ctggagtggg	tagcctattc	1020
cttctccagg	ggatcttccc	atcccaggaa	ttgaactgga	gtctcctgca	tttcagggtg	1080
attcttcacc	agctgaacta	ccaggtggat	actactcaa	tattaaagt	cttaaagtcc	1140
agttttccca	cctttcccaa	aaagggtggg	tcactctttt	ttaaccttct	gtggcctact	1200
ctgaggctgt	ctacaagctt	atatatttat	gaacacattt	attgcaagtt	gttagtttta	1260
gatttacaat	gtggtatctg	gctatttagt	ggtattgggt	gttgggggat	gggaggctga	1320
tagcatctca	gagggcagct	agatactgtc	atacacactt	ttcaagttct	ccatttttgt	1380
gaaatagaaa	gtctctggat	ctaagttata	tgtgattctc	agtctctgtg	gtcatattct	1440
attctactcc	tgaccactca	acaaggaacc	aagatatcaa	gggacacttg	ttttgtttca	1500
tgccctgggt	gagtgggcca	tgacatatgt	tctgggcctt	gttacatggc	tggattgggt	1560
ggacaagtgc	cagctctgat	cctgggactg	tggcatgtga	tgacatacac	cccctctcca	1620
cattctgcat	gtctctaggg	gggaaggggg	aagctcggt	tagaaccttt	attgtatttt	1680
ctgattgcct	cacttcttat	attgccccca	tgcccttctt	tgttcctcaa	gtaaccagag	1740
acagtgcctc	ccagaaccaa	ccctacaaga	aacaaagggc	taaacaaagc	caaattggga	1800
gcaggatcat	ggtttgaact	ctttctggcc	agagaacaat	acctgctatg	gactagatac	1860
tgggagaggg	aaaggaaaag	taggggtgaat	tatggaagga	agctggcagg	ctcagcgttt	1920
ctgtcttggc	atgaccagtc	tctcttcatt	ctcttcctag	atgtagggct	tggtaccaga	1980
gcccctgagg	ctttctgcat	gaatataaat	atatgaaact	gagtgatgct	tccatttcag	2040
gttcttgggg	gcgccgaatt	cgagctcggt	acccggggat	ctcgacggat	ccgattactt	2100
actggcaggt	gctgggggct	tccgagacaa	tcgcgaacat	ctacaccaca	caacaccgcc	2160
tcgaccaggg	tgagatatcg	gccggggacg	cggcgggtgg	aattacaagc	gagatccgat	2220
tacttactgg	caggtgctgg	gggcttccga	gacaatcgcg	aacatctaca	ccacacaaca	2280

WO 02/02738

PCT/US01/20710

ccgcctcgac	caggggtgaga	tatcgggccgg	ggacgcggcg	gtggtaatta	caagcgagat	2340
ctcgagaagc	ttgttgggaa	ttcaggccat	cgatcccgcc	gccaccatgg	aatggagctg	2400
ggtctttctc	ttcttctctg	cagtaactac	aggtgtccac	tccgacatcc	agatgaccca	2460
gtctccagcc	tccctatctg	catctgtggg	agaaactgtc	actatcacat	gtcgagcaag	2520
tgggaatatt	cacaattatt	tagcatggta	tcagcagaaa	cagggaaaat	ctcctcagct	2580
cctggtctat	aatgcaaaaa	ccttagcaga	tgggtgtgcca	tcaagggttca	gtggcagtg	2640
atcaggaaca	caatattctc	tcaagatcaa	cagcctgcag	cctgaagatt	ttgggagtta	2700
ttactgtcaa	cattttttgga	gtactccgtg	gacgttcggg	ggaggcacca	agctggaaat	2760
caaacgggct	gatgctgcac	caactgtatc	catcttccca	ccatccagtg	agcagttaac	2820
atctggaggt	gcctcagtcg	tgtgcttctt	gaacaacttc	taccccaaag	acatcaatgt	2880
caagtggaag	attgatggca	gtgaacgaca	aaatggcgtc	ctgaacagtt	ggactgatca	2940
ggacagcaaa	gacagcacct	acagcatgag	cagcaccctc	acattgacca	aggacgagta	3000
tgaacgacat	aacagctata	cctgtgaggg	cactcacaag	acatcaactt	cacccattgt	3060
caagagcttc	aacaggaatg	agtgttgaaa	gcacgcattt	cccctgaatt	cgcctctctc	3120
cctccccccc	ccctaacggt	actggccgaa	gccgcttgga	ataaggccgg	tgtgcgtttg	3180
tctatatgtt	attttccacc	atattgccgt	cttttggcaa	tgtgagggcc	cggaaacctg	3240
gccctgtctt	cttgacgagc	attcctaggg	gtctttcccc	tctcgccaaa	ggaatgcaag	3300
gtctgttgaa	tgtcgtgaag	gaagcagttc	ctctggaagc	ttcttgaaga	caaacaacgt	3360
ctgtagcgac	cctttgcagg	cagcgggaacc	ccccacctgg	cgacagggtgc	ctctgcggcc	3420
aaaagccacg	tgtataagat	acacctgcaa	aggcggcaca	accccagtg	cacgttgtga	3480
gttgatagat	tgtggaaaga	gtcaaattggc	tctcctcaag	cgtattcaac	aaggggctga	3540
aggatgcca	gaaggtaccc	cattgtatgg	gatctgatct	ggggcctcgg	tgcacatgct	3600
ttacatgtgt	ttagtcgagg	ttaaaaaaac	gtctaggccc	cccgaaccac	ggggacgtgg	3660
ttttcctttg	aaaaacacga	tgataatatg	gcctcctttg	tctctctgct	cctggtaggc	3720
atcctattcc	atgccaccca	ggccgaggtt	cagcttcagc	agtctggggc	agagcttgtg	3780
aagccagggg	cctcagtcaa	gttgtcctgc	acagcttctg	gcttcaacat	taaagacacc	3840
tttatgcact	gggtgaagca	gaggcctgaa	cagggcctgg	agtggattgg	aaggattgat	3900
cctgcgaatg	ggaatactga	atatgacccg	aagttccagg	gcaaggccac	tataacagca	3960
gacacatcct	ccaacacagt	caacctgcag	ctcagcagcc	tgacatctga	ggacactgcc	4020
gtctattact	gtgctagtgg	aggggaactg	gggtttcctt	actggggcca	agggactctg	4080
gtcactgtct	ctgcagccaa	aacgacaccc	ccatctgtct	atccactggc	ccctggatct	4140
gctgccc aaa	ctaactccat	ggtgaccctg	ggatgcctgg	tcaagggcta	tttccctgag	4200
ccagtgcag	tgacctggaa	ctctggatcc	ctgtccagcg	gtgtgcacac	cttcccagct	4260
gtcctgcagt	ttgacctcta	cactctgagc	agctcagtga	ctgtcccctc	cagcacctgg	4320

WO 02/02738

PCT/US01/20710

```

cccagcgcgaga ccgtcacctg caacgcttgcc caccgcggcca gcagcaccaa ggtggacaag 4380
aaaattgtgc ccagggattg tactagtgga ggtggaggta gccaccatca ccatcaccat 4440
taatctagag ttaagcggcc gtcgagatct cgacatcgat aatcaacctc tggattacaa 4500
aatttgtgaa agattgactg gtattcttaa ctatgttgct ctttttacgc tatgtggata 4560
cgctgcttta atgcctttgt atcatgctat tgcttcccgt atggctttca ttttctcctc 4620
cttgataaaa tcctgggttg tgtctcttta tgaggagttg tggcccgttg tcaggcaacg 4680
tggcgtggtg tgcactgtgt ttgctgacgc aacccccact ggttggggca ttgccaccac 4740
ctgtcagctc ctttccggga ctttcgcttt cccctccct attgccacgg cggaactcat 4800
cgccgcctgc cttgcccgct gctggacagg ggctcggctg ttgggcactg acaattccgt 4860
ggtgttgctg gggaaatcat cgtcctttcc ttggctgctc gcctgtgttg ccacctggat 4920
tctgcgcggg acgtccttct gctacgtccc ttccggcctc aatccagcgg accttccttc 4980
ccgcggcctg ctgccggctc tgcggcctct tccgcgtctt cgccttcgcc ctcagacgag 5040
tcggatctcc ctttgggccc cctccccgcc tgatcgataa aataaaagat tttatttagt 5100
ctccagaaaa aggggggaat gaaagacccc acctgtaggt ttggcaagct agcttaagta 5160
acgccatttt gcaaggcatg gaaaaataca taactgagaa tagagaagtt cagatcaagg 5220
tcaggaacag atggaacagc tgaatatggg ccaaacagga tatctgtggt aagcagttcc 5280
tgccccggct cagggccaag aacagatgga acagctgaat atgggccaaa caggatatct 5340
gtggtaagca gttcctgccc cggctcaggg ccaagaacag atgggtccca gatgcggtcc 5400
agccctcagc agtttctaga gaaccatcag atgtttccag ggtgccccaa ggacctgaaa 5460
tgacctgtg cttatattga actaaccaat cagtctgctt ctgccttctg ttcgcgcgct 5520
tctgctcccc gagctcaata aaagagccca caaccctca ctcggggcgc cagtcctccg 5580
attgactgag tcgcccgggt acccgtgtat ccaataaacc ctcttgagcag tgcacccgac 5640
ttgtggtctc gctgttcctt gggaggggtct cctctgagtg attgactacc cgtcagcggg 5700
ggtctttcat t 5711

```

<210> 9

<211> 5130

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 9

```

tttgaaagac cccacccgta ggtggcaagc tagcttaagt aacgccactt tgcaaggcat 60
ggaaaaatac ataactgaga atagaaaagt tcagatcaag gtcaggaaca aagaaacagc 120
tgaataccaa acaggatatc tgtggtaagc ggttcctgcc ccggctcagg gccaagaaca 180
gatgagacag ctgagtgatg ggccaaacag gatctctgtg gtaagcagtt cctgccccgg 240

```

WO 02/02738

PCT/US01/20710

ctcggggcca	agaacagatg	gtccccagat	gcggtccagc	cctcagcagt	ttctagtga	300
tcatcagatg	tttccagggt	gccccaaagg	cctgaaaatg	accctgtacc	ttatttgaac	360
taaccaatca	gttcgcttct	cgcttctgtt	cgcgcgcttc	cgctctccga	gctcaataaa	420
agagcccaca	accctcact	cggcgcgcca	gtcttccgat	agactgcgtc	gcccgggtac	480
ccgtattccc	aataaagcct	cttgctgttt	gcatccgaat	cgtggtctcg	ctgttccttg	540
ggagggtctc	ctctgagtga	ttgactaccc	acgacggggg	tctttcattt	gggggctcgt	600
ccgggatttg	gagaccctg	cccagggacc	accgaccac	caccgggagg	taagctggcc	660
agcaacttat	ctgtgtctgt	ccgattgtct	agtgtctatg	tttgatgtta	tgcgcctgcg	720
tctgtactag	ttagctaact	agctctgtat	ctggcggacc	cgtggtggaa	ctgacgagtt	780
ctgaacaccc	ggccgcaacc	ctgggagacg	tcccagggac	tttggggggc	gtttttgtgg	840
cccgaacctga	ggaaggaggt	cgatgtggaa	tccgaccccg	tcaggatatg	tggttctggt	900
aggagacgag	aacctaaaac	agttcccgcc	tccgtctgaa	tttttgcttt	cggtttgga	960
ccgaagccgc	gcgtcttgtc	tgctgcagcc	aagcttgggc	tgcaggtcga	ggactgggga	1020
ccctgcaccg	aacatggaga	acacaacatc	aggattccta	ggaccctgc	tcgtgttaca	1080
ggcgggggtt	ttcttggtga	caagaatcct	cacaatacca	cagagtctag	actcgtggtg	1140
gacttctctc	aattttctag	ggggagcacc	cacgtgtcct	ggccaaaatt	cgcagtcctc	1200
aacctccaat	cactcaccaa	cctcttgtcc	tccaatttgt	cctggctatc	gctggatgtg	1260
tctgcggcgt	tttatcatat	tcctcttcat	cctgctgcta	tgctcatct	tcttggtggt	1320
tcttctggac	taccaaggta	tggtgcccg	ttgtcctcta	cttccaggaa	catcaactac	1380
cagcacggga	ccatgcaaga	cctgcacgat	tcctgctcaa	ggaacctcta	tgtttcctc	1440
ttggtgctgt	acaaaacctt	cggacggaaa	ctgcacttgt	attcccatcc	catcatcctg	1500
ggctttcgca	agattcctat	gggagtgggc	ctcagtcctg	ttctcctggc	tcagtttact	1560
agtgccatth	gttcagtggt	tcgtagggt	ttccccact	gtttggcttt	cagttatatg	1620
gatgatgtgg	tattgggggc	caagtctgta	caacatcttg	agtccctttt	tacctctatt	1680
accaatthtc	ttttgtcttt	gggtatacat	ttaaacccta	ataaaaacca	acgttggggc	1740
tactccctta	acttcatggg	atatgtaatt	ggatgttggg	gtactttacc	gcaagaacat	1800
attgtactaa	aatcaagca	atgttttcga	aaactgcctg	taaatagacc	tattgattgg	1860
aaagtatgtc	agagacttgt	gggtcttttg	ggctttgctg	ccccttttac	acaatgtggc	1920
tatcctgcct	taatgccttt	atatgcatgt	atacaatcta	agcaggcttt	cactttctcg	1980
ccaacttaca	aggcctttct	gtgtaaacia	tatctgaacc	tttaccctcg	tgcccgga	2040
cggtcaggtc	tctgccaagt	gtttgctgac	gcaaccccca	ctggatgggg	cttggctatc	2100
ggccatagcc	gcatgcgcgg	acctttgtgg	ctcctctgcc	gatccatact	gcggaactcc	2160
tagcagcttg	ttttgctcgc	aggcggctcg	gagcgaaact	tatcggcacc	gacaactctg	2220
ttgtcctctc	tcggaaatac	acctcctthc	catggctgct	agggtgtgct	gccaactgga	2280

WO 02/02738

PCT/US01/20710

tccccctcagg	atatagtagt	ttcgctttttg	catagggagg	gggaaatgta	gtcttatgca	2340
atacacttgt	agtcttgcaa	catggtaacg	atgagtttagc	aacatgcctt	acaaggagag	2400
aaaaagcacc	gtgcatgccg	attggtggaa	gtaaggtggt	acgatcgtgc	cttattagga	2460
aggcaacaga	caggtctgac	atggattgga	cgaaccactg	aattccgcat	tgcagagata	2520
attgtattta	agtgcctagc	tcgatacagc	aaacgccatt	tttgaccatt	caccacattg	2580
gtgtgcacct	tccaaagctt	cacgctgccg	caagcactca	gggcgcaagg	gctgctaaag	2640
gaagcggaac	acgtagaaag	ccagtccgca	gaaacggtgc	tgaccccgga	tgaatgtcag	2700
ctactgggct	atctggacaa	gggaaaacgc	aagcgc aaag	agaaagcagg	tagcttgca	2760
tgggcttaca	tggcgatagc	tagactgggc	ggttttatgg	acagcaagcg	aaccggaatt	2820
gccagctggg	gcgccctctg	gtaaggttgg	gaagccctgc	aaagtaaact	ggatggcctt	2880
cttgccgcca	aggatctgat	ggcgcagggg	atcaagatct	gatcaagaga	caggatgagg	2940
atcgtttctgc	atgattgaac	aagatggatt	gcacgcaggt	tctccggccg	cttgggtgga	3000
gaggctattc	ggctatgact	gggcacaaca	gacaatcggc	tgctctgatg	ccgccgtgtt	3060
ccggctgtca	gcgcaggggc	gcccggttct	ttttgtcaag	accgacctgt	ccggtgccct	3120
gaatgaactg	caggacgagg	cagcgcggct	atcgtggctg	gccacgacgg	gcgttccttg	3180
cgcagctgtg	ctcgacgttg	tcactgaagc	gggaagggac	tggctgctat	tgggcgaagt	3240
gccggggcag	gatctcctgt	catctcacct	tgctcctgcc	gagaaagtat	ccatcatggc	3300
tgatgcaatg	cggcggctgc	atacgcttga	tccggctacc	tgcccatctg	accaccaagc	3360
gaaacatcgc	atcgagcgag	cacgtactcg	gatggaagcc	ggtcttgctg	atcaggatga	3420
tctggacgaa	gagcatcagg	ggctcgcgcc	agccgaactg	ttcgccaggc	tcaaggcgcg	3480
catgcccagc	ggcgaggatc	tcgtcgtgac	ccatggcgat	gcctgcttgc	cgaatatcat	3540
ggtggaaaat	ggccgctttt	ctggattcat	cgactgtggc	cggctgggtg	tggcggaccg	3600
ctatcaggac	atagcgttgg	ctacccgtga	tattgctgaa	gagcttggcg	gcgaatgggc	3660
tgaccgcttc	ctcgtgcttt	acggtatcgc	cgctcccgat	tcgcagcgca	tcgccttcta	3720
tcgccttctt	gacgagttct	tctgagcggg	actctggggg	tcgaaatgac	cgaccaagcg	3780
acgccc aacc	tgccatcacg	agatttcgat	tccaccgccc	ccttctatga	aaggttgggc	3840
ttcggaatcg	ttttccggga	cgcgggtgg	atgatcctcc	agcgcgggga	tctcatgctg	3900
gagttcttcg	cccaccccaa	ccctggccct	attattgggt	ggactaacca	tgggggggaat	3960
tgccgctgga	ataggaacag	ggactactgc	tctaattggc	actcagcaat	tccagcagct	4020
ccaagccgca	gtacaggatg	atctcaggga	ggttgaaaaa	tcaatctcta	acctagaaaa	4080
gtctctcact	tccctgtctg	aagttgtcct	acagaatcga	aggggcctag	acttggttatt	4140
tctaaaagaa	ggagggctgt	gtgctgctct	aaaagaagaa	tgttgcttct	atgcggacca	4200
cacaggacta	gtgagagaca	gcatggccaa	attgagagag	aggcttaatc	agagacagaa	4260
actgtttgag	tcaactcaag	gatggtttga	gggactgttt	aacagatccc	cttgggtttac	4320

WO 02/02738

PCT/US01/20710

```

caccttgata tctaccatta tgggacccct cattgtactc ctaatgattt tgctcttcgg 4380
accctgcatt cttaatcgat tagtccaatt tgttaaagac aggatatcag tgggtccaggc 4440
tctagttttg actcaacaat atcaccagct gaagcctata gagtacgagc catagataaa 4500
ataaaagatt ttatttagtc tccagaaaaa ggggggaatg aaagacccca cctgtaggtt 4560
tggcaagcta gcttaagtaa cgccattttg caaggcatgg aaaaatacat aactgagaat 4620
agagaagttc agatcaaggt caggaacaga tggaacagct gaatatgggc caaacaggat 4680
atctgtggta agcagttcct gccccggctc agggccaaga acagatggaa cagctgaata 4740
tgggccaac accgatctg tggtaagcag ttctgcccc ggctcagggc caagaacaga 4800
tgggtccccag atgcggtcca gccctcagca gtttctagag aaccatcaga tgtttccagg 4860
gtgccccaa gacctgaaat gaccctgtgc cttatttgaa ctaaccaatc agttcgcttc 4920
tcgcttctgt tcgcgcgctt ctgctccccg agctcaataa aagagccac aaccctcac 4980
tcggggcgcc agtcctccga ttgactgagt cggccgggta cccgtgtatc caataaacc 5040
tcttgcagtt gcatccgact tgtgggtctc ctgttccttg ggagggtctc ctctgagtga 5100
ttgactacc gtcagcgggg gtctttcatt 5130

```

<210> 10

<211> 4661

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 10

```

gatcagtcct ggggtggcat tgaaaggact gatgctgaag ttgaagctcc aatactttgg 60
ccacctgatg cgaagaactg actcatgtga taagaccctg atactgggaa agattgaagg 120
caggaggaga agggatgaca gaggatggaa gagttggatg gaatcaccaa ctcgatggac 180
atgagtttga gcaagcttcc aggagtgggt aatgggcagg gaagcctggc gtgctgcagt 240
ccatgggggt gcaaagagtt ggacactact gagtgactga actgaactga tagtgtaatc 300
catggtacag aatataggat aaaaaagagg aagagtttgc cctgattctg aagagttgta 360
ggatataaaa gtttagaata cctttagttt ggaagtctta aattatttac ttaggatggg 420
taccactgc aatataagaa atcaggcttt agagactgat gtagagagaa tgagccctgg 480
cataccagaa gctaacagct attggttata gctgttataa ccaatatata accaatatat 540
tggttatata gcatgaagct tgatgccagc aatttgaagg aaccatttag aactagtatc 600
ctaaactcta catgttccag gacactgatc ttaaagctca gggttcagaat cttgttttat 660
aggctctagg tgtatattgt ggggcttccc tgggtggctca gatggtaaag tgtctgcctg 720
caatgtgggt gatctgggtt cgatccctgg cttgggaaga tccctggag aaggaaatgg 780
caaccactc tagtactctt acctggaaaa ttccatggac agaggagcct tgtaagctac 840

```

WO 02/02738

PCT/US01/20710

agtccatggg	attgcaaaga	gttgaacaca	actgagcaac	taagcacagc	acagtacagt	900
atacacctgt	gaggtgaagt	gaagtgaagg	ttcaatgcag	ggtctcctgc	attgcagaaa	960
gattctttac	catctgagcc	accaggggaag	cccaagaata	ctggagtggg	tagcctattc	1020
cttctccagg	ggatcttccc	atcccaggaa	ttgaactgga	gtctcctgca	tttcagggtgg	1080
attcttcacc	agctgaacta	ccaggtggat	actactccaa	tattaaagtg	cttaaagtcc	1140
agttttccca	cctttcccaa	aaagggttggg	tcactctttt	ttaaccttct	gtggcctact	1200
ctgaggctgt	ctacaagctt	atatatttat	gaacacattt	attgcaagtt	gttagtttta	1260
gatttacaat	gtggtatctg	gctattttagt	ggtattgggtg	gttgggggatg	gggaggctga	1320
tagcatctca	gagggcagct	agatactgtc	atacacactt	ttcaagttct	ccattttttgt	1380
gaaatagaaa	gtctctggat	ctaagttata	tgtgattctc	agtctctgtg	gtcatattct	1440
attctactcc	tgaccactca	acaaggaacc	aagatatcaa	gggacacttg	ttttgtttca	1500
tgcttgggtt	gagtgggcca	tgacatatgt	tctgggcctt	gttacatggc	tggattgggtt	1560
ggacaagtgc	cagctctgat	cctgggactg	tggcatgtga	tgacatacac	cccctctcca	1620
cattctgcat	gtctctaggg	gggaaggggg	aagctcggta	tagaaccttt	attgtatttt	1680
ctgattgcct	cacttcttat	attgccccca	tgcccttctt	tgttcctcaa	gtaaccagag	1740
acagtgcttc	ccagaaccaa	ccctacaaga	aacaaagggc	taaacaaagc	caaattgggaa	1800
gcaggatcat	ggtttgaact	ctttctggcc	agagaacaat	acctgctatg	gactagatac	1860
tgggagaggg	aaaggaaaag	taggggtgaat	tatggaagga	agctggcagg	ctcagcgttt	1920
ctgtcttggc	atgaccagtc	tctcttcatt	ctcttcctag	atgtagggct	tggtaccaga	1980
gcccctgagg	ctttctgcat	gaatataaat	atatgaaact	gagtgatgct	tccatttcag	2040
gttcttgggg	gcgccgaatt	cgagctcggc	acccggggat	ctcgagaagc	tttaaccatg	2100
gaatggagct	gggtctttct	cttcttcctg	tcagtaacta	caggtgtcca	ctcccagggtt	2160
cagttgcagc	agtctgacgc	tgagttgggtg	aaacctgggg	cttcagtgaa	gatttcctgc	2220
aaggcttctg	gctacacctt	cactgaccat	gcaattcact	gggtgaaaca	gaaccctgaa	2280
cagggcctgg	aatggattgg	atatttttct	cccggaaatg	atgattttta	atacaatgag	2340
aggttcaagg	gcaaggccac	actgactgca	gacaaatcct	ccagcactgc	ctacgtgcag	2400
ctcaacagcc	tgacatctga	ggattctgca	gtgtatttct	gtacaagatc	cctgaatatg	2460
gcctactggg	gtcaaggaac	ctcagtcacc	gtctcctcag	gaggcggagg	cagcggaggc	2520
ggtggctcgg	gaggcggagg	ctcggacatt	gtgatgtcac	agtctccatc	ctccctacct	2580
gtgtcagttg	gcgagaaggt	tactttgagc	tgcaagtcca	gtcagagcct	tttatatagt	2640
ggtaatcaaa	agaactactt	ggcctgggtac	cagcagaaac	cagggcagtc	tcctaaactg	2700
ctgatttact	gggcatccgc	tagggaatct	ggggtccctg	atcgcttcac	aggcagtgga	2760
tctgggacag	atttcactct	ctccatcagc	agtgtgaaga	ctgaagacct	ggcagtttat	2820
tactgtcagc	agtattatag	ctatccctc	acgttcgggtg	ctgggaccaa	gctgggtgctg	2880

WO 02/02738

PCT/US01/20710

```

aaacggggccg ccgagcccaa atctcctgac aaaactcaca catgcccacc gtgcccagca 2940
cctgaactcc tgggggggacc gtcagtcttc ctcttcccc caaaacccaa ggacaccctc 3000
atgatctccc ggacccttga ggtcacatgc gtggtggtgg acgtgagcca cgaagaccct 3060
gaggtcaagt tcaactggta cgtggacggc gtggaggtgc ataatgcca gacaaagccg 3120
cgggaggagc agtacaacag cacgtaccgt gtggtcagcg tcctcaccgt cctgcaccag 3180
gactggctga atggcaagga gtacaagtgc aaggtctcca acaaagccct cccagcccc 3240
atcgagaaaa ccatctccaa agccaaaggg cagccccgag aaccacaggt gtacaccctg 3300
cccccatccc gggatgagct gaccaagaac caggtcagcc tgacctgcct ggtcaaaggc 3360
ttctatccca gcgacatcgc cgtggagtgg gagagcaatg ggcagccgga gaacaactac 3420
aagaccacgc ctcccgtgct ggactccgac ggctccttct tcctctacag caagctcacc 3480
gtggacaaga gcaggtggca gcaggggaac gtcttctcat gctccgtgat gcatgaggct 3540
ctgcacaacc actacacgca gaagagcctc tccctgtctc cgggtaaagg aggcggatca 3600
ggaggtggcg cacctacttc aagttctaca aagaaaacac agctacaact ggagcattta 3660
ctgctggatt tacagatgat tttgaatgga attaataatt acaagaatcc caaactcacc 3720
aggatgctca catttaagtt ttacatgccc aagaaggcca cagaactgaa acatcttcag 3780
tgtctagaag aagaactcaa acctctggag gaagtgctaa atttagctca aagcaaaaac 3840
tttcaactaa gacccaggga cttaatcagc aatatcaacg taatagttct ggaactaaag 3900
ggatctgaaa caacattcat gtgtgaatat gctgatgaga cagcaaccat tgtagaattt 3960
ctgaacagat ggattacctt ttgtcaaagc atcatctcaa cactaacttg aagcttgta 4020
acatcgataa aataaaagat tttatttagt ctccagaaaa agggggggaat gaaagacccc 4080
acctgtaggt ttggcaagct agcttaagta acgccatttt gcaaggcatg gaaaaataca 4140
taactgagaa tagagaagtt cagatcaagg tcaggaacag atggaacagc tgaatatggg 4200
ccaaacagga tatctgtggt aagcagttcc tgccccggct cagggccaaag aacagatgga 4260
acagctgaat atggggccaaa caggatatct gtggtaagca gttcctgccc cggctcaggg 4320
ccaagaacag atggtcccca gatgcggtcc agccctcagc agtttctaga gaaccatcag 4380
atgtttccag ggtgccccaa ggacctgaaa tgaccctgtg ccttatattga actaaccaat 4440
cagttcgctt ctcgcttctg ttcgcgcgct tctgctcccc gagctcaata aaagagccca 4500
caaccctca ctcgggggcgc cagtcctccg attgactgag tcgccccgggt acccgtgtat 4560
ccaataaacc ctcttgcagt tgcacccgac ttgtggtctc gctgttcctt gggaggggtct 4620
cctctgagtg attgactacc cgtcagcggg ggtctttcat t 4661

```

<210> 11

<211> 5691

<212> DNA

<213> Artificial Sequence

WO 02/02738

PCT/US01/20710

<220>

<223> Synthetic

<400> 11

gatcagtcct	gggtgggtcat	tgaaaggact	gatgctgaag	ttgaagctcc	aatacttttg	60
ccacctgatg	cgaagaactg	actcatgtga	taagaccctg	atactgggaa	agattgaagg	120
caggaggaga	agggatgaca	gaggatggaa	gagttggatg	gaatcaccaa	ctcgatggac	180
atgagtttga	gcaagcttcc	aggagttggt	aatgggcagg	gaagcctggc	gtgctgcagt	240
ccatgggggtt	gcaaagagtt	ggacactact	gagtgaactga	actgaactga	tagtgtaatc	300
catggtacag	aatataggat	aaaaaagagg	aagagtttgc	cctgattctg	aagagttgta	360
ggatataaaa	gtttagaata	cctttagttt	ggaagtctta	aattattttac	ttaggatggg	420
taccactgc	aatataagaa	atcaggcttt	agagactgat	gtagagagaa	tgagccctgg	480
cataccagaa	gctaacagct	attgggtata	gctgttataa	ccaatatata	accaatatat	540
tggttatata	gcatgaagct	tgatgccagc	aatttgaagg	aaccatttag	aactagtatc	600
ctaaactcta	catgttccag	gacactgatc	ttaaagctca	ggttcagaat	cttgttttat	660
aggctctagg	tgtatatattg	ggggcttccc	tgggtggctca	gatggtaaag	tgtctgcctg	720
caatgtgggt	gatctggggt	cgatccctgg	cttgggaaga	tcccctggag	aaggaaatgg	780
caaccactc	tagtactctt	acctggaaaa	ttccatggac	agaggagcct	tgtaagctac	840
agtccatggg	attgcaaaga	gttgaacaca	actgagcaac	taagcacagc	acagtacagt	900
atacacctgt	gaggtgaagt	gaagtgaagg	ttcaatgcag	ggtctcctgc	attgcagaaa	960
gattctttac	catctgagcc	accagggaag	ccaagaata	ctggagtggg	tagcctattc	1020
cttctccagg	ggatcttccc	atcccaggaa	ttgaactgga	gtctcctgca	tttcaggtgg	1080
attcttcacc	agctgaacta	ccaggtggat	actactccaa	tattaaagtg	cttaaagtcc	1140
agttttccca	cctttcccaa	aaagggtggg	tcactctttt	ttaaccttct	gtggcctact	1200
ctgaggctgt	ctacaagctt	atatatttat	gaacacattt	attgcaagtt	gttagtttta	1260
gatttacaat	gtggtatctg	gctatttagt	ggtattggtg	gttggggatg	gggaggctga	1320
tagcatctca	gagggcagct	agatactgtc	atacacactt	ttcaagttct	ccatTTTTgt	1380
gaaatagaaa	gtctctggat	ctaagttata	tgtgattctc	agtctctgtg	gtcatattct	1440
attctactcc	tgaccactca	acaaggaacc	aagatatcaa	gggacacttg	ttttgtttca	1500
tgccctgggtt	gagtgggcca	tgacatatgt	tctgggcctt	gttacatggc	tggattgggt	1560
ggacaagtgc	cagctctgat	cctgggactg	tggcatgtga	tgacatacac	cccctctcca	1620
cattctgcat	gtctctaggg	gggaaggggg	aagctcggta	tagaaccttt	attgtatttt	1680
ctgattgcct	cacttcttat	attgccccca	tgcccttctt	tgttcctcaa	gtaaccagag	1740
acagtgcctc	ccagaaccaa	ccctacaaga	aacaaagggc	taaacaaagc	caaattgggaa	1800
gcaggatcat	ggtttgaact	ctttctggcc	agagaacaat	acctgctatg	gactagatac	1860
tgggagaggg	aaaggaaaag	taggggtgaat	tatggaagga	agctggcagg	ctcagcgttt	1920

WO 02/02738

PCT/US01/20710

ctgtcttggc	atgaccagtc	tctcttcatt	ctcttcctag	atgtagggct	tggtaccaga	1980
gcccctgagg	ctttctgcat	gaatataaat	atatgaaact	gagtgatgct	tccatttcag	2040
gttcttgggg	gcgccgaatt	cgagctcggc	acccggggat	ctcgacggat	ccgattactt	2100
actggcaggt	gctgggggct	tccgagacaa	tcgcgaacat	ctacaccaca	caacaccgcc	2160
tcgaccaggg	tgagatatcg	gccggggacg	cggcgggtgg	aattacaagc	gagatccgat	2220
tacttactgg	caggtgctgg	gggcttccga	gacaatcgcg	aacatctaca	ccacacaaca	2280
ccgcctcgac	cagggtgaga	tatcgggcgg	ggacgcggcg	gtggtaatta	caagcgagat	2340
ctcgagttaa	cagatctagg	cctcctaggt	cgacggatcc	ccgggaattc	ggcgccgcca	2400
ccatgatgtc	ctttgtctct	ctgctcctgg	taggcatact	attccatgcc	accaggccc	2460
aggtccaact	gcagcagtct	gggcctgagc	tggtgaagcc	tgggacttca	gtgaggatat	2520
cctgcaaggc	ttctggctac	accttcacaa	gctactatct	acactgggtg	aagcagaggc	2580
ctggacaggg	acttgagtgg	attgcatgga	tttatcctgg	aaatgttatt	actacgtaca	2640
atgagaagtt	caagggcaag	gccacactga	ctgcagacaa	atcctccagc	acagcctaca	2700
tgcacctcaa	cagcctgacc	tctgaggact	ctgcgggtcta	tttctgtgca	aggggtgacc	2760
atgatcttga	ctactggggc	caaggcacca	ctctcacagt	ctcctcagcc	aaaacgacac	2820
ccccatctgt	ctatccactg	gcccctggat	ctgctgcca	aactaactcc	atggtgaccc	2880
tgggatgcct	ggtcaagggc	tatttccctg	agccagtgc	agtgacctgg	aactctggat	2940
ccctgtccag	cgggtgtcac	accttcccag	ctgtcctgca	gtctgacctc	tacactctga	3000
gcagctcagt	gactgtcccc	tccagcacct	ggcccagcga	gaccgtcacc	tgcaacgttg	3060
cccacccggc	cagcagcacc	aaggtggaca	agaaaattgt	gccagggat	tgtactagt	3120
gaggtggagg	tagctaaggg	agatctcgac	ggatccccgg	gaattcgccc	ctctccctcc	3180
ccccccccta	acgttactgg	ccgaagccgc	ttggaataag	gccggtgtgc	gtttgtctat	3240
atgttatctt	ccaccatatt	gccgtctttt	ggcaatgtga	gggcccggaa	acctggccct	3300
gtcttcttga	cgagcattcc	taggggtctt	tcccctctcg	ccaaaggaat	gcaaggtctg	3360
ttgaatgtcg	tgaaggaagc	agttcctctg	gaagcttctt	gaagacaaac	aacgtctgta	3420
gcgacccttt	gcaggcagcg	gaacccccca	cctggcgaca	ggtgcctctg	cggccaaaag	3480
ccacgtgtat	aagatacacc	tgcaaaggcg	gcacaacccc	agtgccacgt	tgtgagttgg	3540
atagttgtgg	aaagagtcaa	atggctctcc	tcaagcgtat	tcaacaaggg	gctgaaggat	3600
gcccagaagg	tacccatttg	tatgggatct	gatctggggc	ctcgggtgc	atgctttaca	3660
tgtgtttagt	cgagggttaa	aaaacgtcta	ggccccccga	accacgggga	cgtgggttttc	3720
ctttgaaaaa	cacgatgata	atatggcctc	ctttgtctct	ctgctcctgg	taggcatact	3780
attccatgcc	accagggccg	acattgtgct	gacacaatct	ccagcaatca	tgtctgcatc	3840
tccagggggag	aaggtcacca	tgacctgcag	tgccacctca	agtgtgaagt	acatacactg	3900
gtaccagcag	aagtcaggca	cctcccccaa	aagatggatt	tatgacacat	ccaaactggc	3960

WO 02/02738

PCT/US01/20710

```

ttctggagtc cctgctcgct tcagtggcag tgggtctggg acctctcact ctctcacact 4020
cagcagcatg gaggctgaag atgctgccac ttattactgc cagcagtggg gtagttacct 4080
cacgttcggt gcggggacca agctggagct gaaacgggct gatgctgcac caactgtatc 4140
catcttccca ccatccagtg agcagttaac atctggaggt gcctcagtcg tgtgcttctt 4200
gaacaacttc taccctaaag acatcaatgt caagtggaag attgatggca gtgaacgaca 4260
aatggcgctc ctgaacagtt ggactgatca ggacagcaaa gacagcacct acagcatgag 4320
cagcaccttc acgttgacca aggacgagta tgaacgacat aacagctata cctgtgaggc 4380
cactcacaag acatcaactt caccattgt caagagcttc aacaggaatg agtgtaata 4440
ggggagatct cgacatcgat aatcaacctc tggattacaa aatttgtgaa agattgactg 4500
gtattcttaa ctatgttgct ccttttacgc tatgtggata cgctgcttta atgcctttgt 4560
atcatgctat tgcttcccgt atggctttca tttctcctc cttgtataaa tcctgggttg 4620
tgtctcttta tgaggagttg tggcccgttg tcaggcaacg tggcgtggtg tgcactgtgt 4680
ttgctgacgc aacccccact ggttggggca ttgccaccac ctgtcagctc ctttccggga 4740
ctttcgcttt cccctccct attgccacgg cggaactcat cgccgcctgc cttgcccgct 4800
gctggacagg ggctcggctg ttgggcactg acaattccgt ggtgttgctg gggaaatcat 4860
cgtcctttcc ttggctgctc gcctgtgttg ccacctggat tctgcgcggg acgtccttct 4920
gctacgtccc ttcggccctc aatccagcgg accttccttc ccgcggcctg ctgccggctc 4980
tgccgcctct tccgcgtctt cgcttcgcc ctcagacgag tcggatctcc ctttgggccg 5040
cctccccgcc tgatcgataa aataaaagat tttatttagt ctccagaaaa aggggggaat 5100
gaaagacccc acctgtaggt ttggcaagct agcttaagta acgccatttt gcaaggcatg 5160
gaaaaataca taactgagaa tagagaagtt cagatcaagg tcaggaacag atggaacagc 5220
tgaatatggg ccaaacagga tatctgtggt aagcagttcc tgccccggct cagggccaaag 5280
aacagatgga acagctgaat atgggccaaa caggatatct gtggtaagca gttcctgccc 5340
cggctcaggg ccaagaacag atggtcccca gatgcggtcc agccctcagc agtttctaga 5400
gaaccatcag atgtttccag ggtgccccaa ggacctgaaa tgaccctgtg ccttatttga 5460
actaaccaat cagttcgctt ctgccttctg ttcgcgcgct tctgctcccc gagctcaata 5520
aaagagccca caaccctca ctcggggcgc cagtcctccg attgactgag tcgcccgggt 5580
accgtgtat ccaataaacc ctcttgagc tgcatccgac ttgtggctc gctgttcctt 5640
gggaggggtct cctctgagtg attgactacc cgtcagcggg ggtctttcat t 5691

```

<210> 12

<211> 668

<212> DNA

<213> Artificial Sequence

<220>

WO 02/02738

PCT/US01/20710

<223> Synthetic

<400> 12

```

ggaattcgcc cctctccctc cccccccct aacgttactg gccgaagccg cttggaataa      60
ggccggtgtg cgtttgtcta tatgttatTT tccaccatat tgccgtcttt tggcaatgtg     120
agggcccgga aacctggccc tgtcttcttg acgagcattc ctaggggtct tccccctctc     180
gccaaaggaa tgcaaggctc gttgaatgtc gtgaagggaag cagttcctct ggaagcttct     240
tgaagacaaa caacgtctgt agcgaccctt tgcaggcagc ggaaccccc acctggcgac      300
aggtgcctct gcggccaaaa gccacgtgta taagatacac ctgcaaaggc ggcacaaccc     360
cagtgccacg ttgtgagttg gatagttgtg gaaagagtca aatggctctc ctcaagcgta     420
ttcaacaagg ggctgaagga tgcccagaag gtacccatt gtatgggatc tgatctgggg     480
cctcgggtgca catgctttac atgtgtttag tcgagggtta aaaaacgtct agggcccccg     540
aaccacgggg acgtggtttt cctttgaaaa acacgatgat aatatggcct tgctcatcct     600
tacctgtctt gtggctgttg ctcttgccgg cgccatggga tatctagatc tcgagctcgc     660
gaaagctt                                     668

```

<210> 13

<211> 6255

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 13

```

tttgaaagac cccaccgta ggtggcaagc tagcttaagt aacgccactt tgcaaggcat      60
ggaaaaatac ataactgaga atagaaaagt tcagatcaag gtcaggaaca aagaaacagc     120
tgaataccaa acaggatatc tgtggtaagc ggttcctgcc ccggctcagg gccaagaaca     180
gatgagacag ctgagtgatg ggccaaacag gatatctgtg gtaagcagtt cctgccccgg     240
ctcggggcca agaacagatg gtccccagat gcggtccagc cctcagcagt ttctagttaa     300
tcatcagatg tttccagggt gccccaagga cctgaaaatg accctgtacc ttatttgaac     360
taaccaatca gttcgcttct cgcttctgtt cgcgcgcttc cgctctccga gctcaataaa     420
agagcccaca acccctcact cggcgcgcca gtcttccgat agactgcgtc gcccggttac     480
ccgtattccc aataaagcct cttgctgttt gcatccgaat cgtgggtctcg ctgttccttg     540
ggaggggtct ctctgagtga ttgactaccc acgacggggg tctttcattt gggggctcgt     600
ccgggatttg gagaccctg cccagggacc accgaccac caccgggagg taagctggcc     660
agcaacttat ctgtgtctgt ccgattgtct agtgtctatg tttgatgtta tgcgcctgcg     720
tctgtactag ttagctaact agctctgtat ctggcggacc cgtgggtggaa ctgacgagtt     780
ctgaacaccc ggccgcaacc ctgggagacg tcccagggac tttggggggc gtttttgtgg     840

```

WO 02/02738

PCT/US01/20710

cccgacctga	ggaagggagt	cgatgtggaa	tccgaccccg	tcaggatatg	tggttctggt	900
aggagacgag	aacctaaaac	agttccccgc	tccgtctgaa	tttttgcttt	cggtttggaa	960
ccgaagccgc	gcgtcttgtc	tgctgcagcg	ctgcagcatc	gttctgtggt	gtctctgtct	1020
gactgtgttt	ctgtatttgt	ctgaaaatta	gggccagact	gttaccactc	ccttaagttt	1080
gaccttaggt	cactggaaag	atgtcgagcg	gatcgctcac	aaccagtcgg	tagatgtcaa	1140
gaagagacgt	tgggttacct	tctgctctgc	agaatggcca	acctttaacg	tcggatggcc	1200
gcgagacggc	acctttaacc	gagacctcat	cacccagggt	aagatcaagg	tcttttcacc	1260
tggccccgat	ggacaccag	accagggtccc	ctacatcgtg	acctgggaag	ccttggcttt	1320
tgacccccct	ccctgggtca	agccctttgt	acaccctaag	cctccgcctc	ctcttcctcc	1380
atccgccccg	tctctcccc	ttgaacctcc	togttcgacc	ccgcctcgat	cctcccttta	1440
tccagccctc	actccttctc	taggcgcggg	aattccgatc	tgatcaagag	acaggatgag	1500
gatcgtttcg	catgattgaa	caagatggat	tgcacgcagg	ttctccggcc	gcttgggtgg	1560
agaggctatt	cggctatgac	tgggcacaa	agacaatcgg	ctgctctgat	gccgccgtgt	1620
tccggctgtc	agcgcagggg	cgcccggttc	tttttgctaa	gaccgacctg	tccggtgccc	1680
tgaatgaact	gcaggacgag	gcagcgcggc	tatcgtggct	ggccacgacg	ggcgttcctt	1740
gcgcagctgt	gctcgacgtt	gtcactgaag	cgggaaggga	ctggctgcta	ttggggcgaag	1800
tgccggggca	ggatctcctg	tcatctcacc	ttgctcctgc	cgagaaagta	tccatcatgg	1860
ctgatgcaat	gcggcggtg	catacgcttg	atccggctac	ctgcccattc	gaccaccaag	1920
cgaaacatcg	catcgagcga	gcacgtactc	ggatggaagc	cggtcttgtc	gatcaggatg	1980
atctggacga	agagcatcag	gggctcgcgc	cagccgaact	gttcgccagg	ctcaaggcgc	2040
gcatgcccga	cggcgaggat	ctcgtcgtga	cccatggcga	tgccctgcttg	ccgaatatca	2100
tggtggaaaa	tggccgcttt	tctggattca	tgcactgtgg	ccggctgggt	gtggcggacc	2160
gctatcagga	catagcgctg	gctacccgtg	atattgctga	agagcttggc	ggcgaatggg	2220
ctgaccgctt	cctcgtgctt	tacggtatcg	ccgctcccga	ttcgcagcgc	atcgccctct	2280
atcgccctct	tgacgagttc	ttctgagcgg	gactctgggg	ttcgaaatga	ccgaccaagc	2340
gacgcccac	ctgccatcac	gagatttcga	ttccaccgcc	gccttctatg	aaagggtggg	2400
cttcggaatc	gttttcgggg	acgccggctg	gatgatcctc	cagcgcgggg	atctcatgct	2460
ggagttcttc	gcccaccccg	ggctcgatcc	cctcgcgagt	tggttcagct	gctgcctgag	2520
gctggacgac	ctcgcggagt	tctaccggca	gtgcaaatcc	gtcggcatcc	aggaaaccag	2580
cagcggctat	ccgcgcatcc	atgccccga	actgcaggag	tggggaggca	cgatggccgc	2640
tttggtcgag	gcggatccgg	ccattagcca	tattattcat	tggttatata	gcataaatca	2700
atattggcta	ttggccattg	catacgttgt	atccatatca	taatatgtac	atttatattg	2760
gctcatgtcc	aacattaccg	ccatgttgac	attgattatt	gactagttat	taatagtaat	2820
caattacggg	gtcattagtt	catagcccat	atatggagtt	ccgcgttaca	taacttacgg	2880

WO 02/02738

PCT/US01/20710

taaatggccc	gcctggctga	ccgcccacg	acccccgccc	attgacgtca	ataatgacgt	2940
atgttcccat	agtaacgcca	atagggactt	tccattgacg	tcaatgggtg	gagtattttac	3000
ggtaaactgc	ccacttggca	gtacatcaag	tgtatcatat	gccaagtacg	ccccctattg	3060
acgtcaatga	cggtaaattg	cccgccctggc	attatgccc	gtacatgacc	ttatgggact	3120
ttcctacttg	gcagtacatc	tacgtattag	tcacgctat	taccatgggtg	atgcgggtttt	3180
ggcagtacat	caatgggctg	ggatagcggt	ttgactcacg	gggatttcca	agtctccacc	3240
ccattgacgt	caatgggagt	ttgttttggc	acaaaaatca	acgggacttt	ccaaaatgtc	3300
gtaacaactc	cgcctcattg	acgcaaatgg	gcggtaggca	tgtacgggtg	gaggtctata	3360
taagcagagc	tcgttttagtg	aaccgtcaga	tcgcctggag	acgccatcca	cgtgtttttg	3420
acctccatag	aagacaccgg	gaccgatcca	gcctccgctg	ccccaaagctt	ctcgacggat	3480
ccccgggaat	tcaggccatc	gatcccgccg	ccaccatgga	atggagctgg	gtctttctct	3540
tcttcctgtc	agtaactaca	ggtgtccact	ccgacatcca	gatgaccag	tctccagcct	3600
ccctatctgc	atctgtggga	gaaactgtca	ctatcacatg	tcgagcaagt	gggaatattc	3660
acaattattt	agcatgggtat	cagcagaaac	agggaaaatc	tcctcagctc	ctgggtctata	3720
atgcaaaaac	cttagcagat	ggtgtgccat	caagggttcag	tggcagtgg	tcaggaacac	3780
aatattctct	caagatcaac	agcctgcagc	ctgaagattt	tgggagttat	tactgtcaac	3840
atttttggag	tactccgtgg	acgttcggtg	gaggcaccaa	gctggaaatc	aaacgggctg	3900
atgctgcacc	aactgtatcc	atcttccac	catccagtga	gcagttaaca	tctggaggtg	3960
cctcagtcgt	gtgcttcttg	aacaacttct	accccaaaga	catcaatgtc	aagtggaaga	4020
ttgatggcag	tgaacgacaa	aatggcgtcc	tgaacagttg	gactgatcag	gacagcaaag	4080
acagcaccta	cagcatgagc	agcaccctca	cattgaccaa	ggacgagtat	gaacgacata	4140
acagctatac	ctgtgaggcc	actcacaaga	catcaacttc	acccattgtc	aagagcttca	4200
acaggaatga	gtgttgaaag	catcgatttc	ccctgaattc	gcccctctcc	ctcccccccc	4260
cctaacgtta	ctggccgaag	ccgcttggaa	taaggccggt	gtgcgtttgt	ctatatgtta	4320
ttttccacca	tattgccgtc	ttttggcaat	gtgaggggccc	ggaaacctgg	ccctgtcttc	4380
ttgacgagca	ttcctagggg	tctttcccct	ctcgccaaag	gaatgcaagg	tctgttgaat	4440
gtcgtgaagg	aagcagttcc	tctggaagct	tcttgaagac	aaacaacgtc	tgtagcgacc	4500
ctttgcaggc	agcggaaacc	cccacctggc	gacagggtgc	tctgcggcca	aaagccacgt	4560
gtataagata	cacctgcaaa	ggcggcacia	ccccagtgc	acgttgtgag	ttggatagtt	4620
gtggaaagag	tcaaatggct	ctcctcaagc	gtattcaaca	aggggctgaa	ggatgcccag	4680
aaggtacccc	attgtatggg	atctgatctg	gggcctcggt	gcacatgctt	tacatgtgtt	4740
tagtcgaggt	taaaaaaacg	tctaggcccc	ccgaaccacg	gggacgtggg	tttcctttga	4800
aaaacacgat	gataatatgg	cctcctttgt	ctctctgctc	ctggtaggca	tcctattcca	4860
tgccacccag	gccgagggtc	agcttcagca	gtctggggca	gagcttgtga	agccaggggc	4920

WO 02/02738

PCT/US01/20710

ctcagtcaag ttgtcctgca cagcttcttg cttcaacatt aaagacacct ttatgcactg 4980
 ggtgaagcag aggcctgaac agggcctgga gtggattgga aggattgatc ctgcgaatgg 5040
 gaatactgaa tatgacccga agttccaggg caaggccact ataacagcag acacatcctc 5100
 caacacagtc aacctgcagc tcagcagcct gacatctgag gacactgccg tctattactg 5160
 tgctagtgga ggggaactgg ggtttcctta ctggggccaa gggactcttg tcactgtctc 5220
 tgcagccaaa acgacacccc catctgtcta tccactggcc cctggatctg ctgccaaaac 5280
 taactccatg gtgaccctgg gatgcctggc caagggctat ttccctgagc cagtgcagct 5340
 gacctggaac tctggatccc tgtccagcgg tgtgcacacc ttcccagctg tcctgcagtc 5400
 tgacctctac actctgagca gctcagtgac tgtccccctc agcacctggc ccagcgagac 5460
 cgtcacctgc aacgttgccc acccggccag cagcaccaag gtggacaaga aaattgtgcc 5520
 cagggattgt actagtggag gtggaggtag ccaccatcac catcaccatt aatctagagt 5580
 taagcggccg tcgagatcta ggcctcctag gtgcacatcg ataaaataaa agatttttatt 5640
 tagtctccag aaaaaggggg gaatgaaaga cccacactgt aggtttggca agctagctta 5700
 agtaacgcca ttttgcaagg catggaaaaa tacataactg agaatagaga agttcagatc 5760
 aaggtcagga acagatggaa cagctgaata tgggccaac aggatatctg tggtaagcag 5820
 ttcttgcccc ggctcagggc caagaacaga tggaacagct gaatatgggc caaacaggat 5880
 atctgtggta agcagttcct gcccgggctc agggccaaga acagatggtc ccagatgctg 5940
 gtccagccct cagcagtttc tagagaacca tcagatgttt ccagggtgcc ccaaggacct 6000
 gaaatgacct tgtgccttat ttgaactaac caatcagttc gcttctcgct tctgttcgctg 6060
 cgcttctgct ccccgagctc aataaaagag cccacaaccc ctactcggg gcgccagtcc 6120
 tccgattgac tgagtcgccc gggtagccgt gtatccaata aaccctcttg cagttgcac 6180
 cgacttgtgg tctcgctgtt ccttgggagg gtctcctctg agtgattgac taccgctcag 6240
 cgggggtctt tcatt 6255

<210> 14

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 14

ctttgaaaaa cacgatgata atatggcctc ctttgtctct ctg 43

<210> 15

<211> 30

<212> DNA

<213> Artificial Sequence

WO 02/02738

PCT/US01/20710

<220>
<223> Synthetic
<400> 15
ttcgcgagct cgagatctag atatcccatg 30
<210> 16
<211> 35
<212> DNA
<213> Artificial Sequence
<220>
<223> Synthetic
<400> 16
ctacaggtgt ccacgtcgac atccagctga cccag 35
<210> 17
<211> 34
<212> DNA
<213> Artificial Sequence
<220>
<223> Synthetic
<400> 17
ctgcagaata gatctctaac actctcccct gttg 34
<210> 18
<211> 51
<212> DNA
<213> Artificial Sequence
<220>
<223> Synthetic
<400> 18
cagtgtgatc tcgagaattc aggacctcac catgggatgg agctgtatca t 51
<210> 19
<211> 23
<212> DNA
<213> Artificial Sequence
<220>
<223> Synthetic
<400> 19
aggctgtatt ggtggattcg tct 23

WO 02/02738

PCT/US01/20710

<210> 20
 <211> 41
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Synthetic
 <400> 20
 agcttctcga gttaacagat ctaggcctcc taggtcgaca t 41
 <210> 21
 <211> 39
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Synthetic
 <400> 21
 cgatgtcgac ctaggaggcc tagatctggt aactcgaga 39
 <210> 22
 <211> 64
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Synthetic
 <400> 22
 cgaggctctg cacaaccact acacgcagaa gagcctctcc ctgtctcccg ggaaatgaaa 60
 gccg 64
 <210> 23
 <211> 72
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Synthetic
 <400> 23
 aattcggctt tcatttcccg ggagacaggg agaggctctt ctgcgtgtag tggttgtgca 60
 gagcctcgtg ca 72
 <210> 24
 <211> 41

WO 02/02738

PCT/US01/20710

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 24

aaagcatatg ttctgggcct tgttacatgg ctggattggt t

41

<210> 25

<211> 54

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 25

tgaattcggc gcccacaaga acctgaaatg gaagcatcac tcagtttcat atat

54

<210> 26

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 26

ctacaggtgt ccacgtcgac atccagctga cccag

35

<210> 27

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 27

ctgcagaata gatctctaac actctcccct gttg

34

<210> 28

<211> 51

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

WO 02/02738

PCT/US01/20710

<400> 28
 cagtgtgatc tcgagaattc aggacctcac catgggatgg agctgtatca t 51
 <210> 29
 <211> 22
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Synthetic
 <400> 29
 gtgtcttcgg gtctcaggct gt 22
 <210> 30
 <211> 41
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Synthetic
 <400> 30
 agcttctcga gttaacagat ctaggcctcc taggtcgaca t 41
 <210> 31
 <211> 39
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Synthetic
 <400> 31
 cgatgtcgac ctaggaggcc tagatctggt aactcgaga 39
 <210> 32
 <211> 64
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Synthetic
 <400> 32
 cgaggctctg cacaaccact acacgcagaa gagcctctcc ctgtctcccg ggaaatgaaa 60
 gccg 64
 <210> 33

WO 02/02738

PCT/US01/20710

<211> 72

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 33

aattcggcctt tcatttcccg ggagacaggg agaggctcct ctgcgtgtag tggttgtgca 60

gagcctcgtg ca 72

<210> 34

<211> 9511

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 34

gaattaattc ataccagatc accgaaaact gtcctccaaa tgtgtccccc tcacactccc 60

aaattcgcgg gcttctgcct cttagaccac tctaccctat tccccacact caccggagcc 120

aaagccgcgg cccttccggtt tctttgcttt tgaaagaccc cacccgtagg tggcaagcta 180

gcttaagtaa cgccactttg caaggcatgg aaaaatacat aactgagaat agaaaagttc 240

agatcaaggt caggaacaaa gaaacagctg aataccaaac aggatatctg tggtaagcgg 300

ttcctgcccc ggctcagggc caagaacaga tgagacagct gagtgatggg ccaaacagga 360

tatctgtggg aagcagttcc tgccccgggt cggggccaag aacagatggg ccccagatgc 420

gggccagccc tcagcagttt ctagtgaatc atcagatggt tccaggggtgc cccaaggacc 480

tgaaaatgac cctgtacctt atttgaacta accaatcagt tcgcttctcg cttctgttcg 540

cgcgcttccg ctctccgagc tcaataaaag agcccacaac ccctcactcg gcgcgccagt 600

cttccgatag actgcgtcgc ccgggtaccc gtattcccaa taaagcctct tgctgtttgc 660

atccgaatcg tgggtctcgt gttccttggg agggctctct ctgagtgatt gactaccac 720

gacgggggtc tttcatttgg gggctcgtcc gggatttggg gaccctgcc cagggaaccac 780

cgaccacca ccgggaggta agctggccag caacttatct gtgtctgtcc gattgtctag 840

tgtctatggt tgatgttatg cgcctgcgtc tgtactagtt agctaactag ctctgtatct 900

ggcggaccog tgggtggaact gacgagttct gaacacccgg ccgcaaccct gggagacgtc 960

ccagggactt tgggggcccgt ttttgtggcc cgacctgagg aaggagtcg atgtggaatc 1020

cgaccccgtc aggatatgtg gttctggtag gagacgagaa cctaaaacag ttcccgctc 1080

cgtctgaatt tttgctttcg gtttggaaac gaagccgcgc gtcttgtctg ctgcagcgt 1140

gcagcatcgt tctgtgttgt ctctgtctga ctgtgtttct gtatttgtct gaaaattagg 1200

WO 02/02738

PCT/US01/20710

gccagactgt	taccactccc	ttaagtttga	ccttaggtca	ctggaaagat	gtcgagcgga	1260
tcgctcacia	ccagtcggta	gatgtcaaga	agagacgttg	ggttaccttc	tgctctgcag	1320
aatggccaac	ctttaacgtc	ggatggccgc	gagacggcac	ctttaaccga	gacctcatca	1380
cccagggttaa	gatcaaggtc	ttttcacctg	gcccgcacgg	acacccagac	cagggtcccct	1440
acatcggtgac	ctgggaagcc	ttggcttttg	acccccctcc	ctgggtcaag	ccctttgtac	1500
accctaagcc	tcgcctcct	cttcctccat	cgcgcccgtc	tctccccctt	gaacctcctc	1560
gttcgacccc	gcctcgatcc	tccctttatc	cagccctcac	tccttctcta	ggcgccggaa	1620
ttccgatctg	atcaagagac	aggatgaggg	agcttgtata	tccattttcg	gatctgatca	1680
gcacgtgttg	acaattaatc	atcggcatag	tatatcggca	tagtataata	cgacaagggtg	1740
aggaactaaa	ccatggccaa	gcctttgtct	caagaagaat	ccacctcat	tgaaagagca	1800
acggctacaa	tcaacagcat	ccccatctct	gaagactaca	gcgtcgccag	cgcagctctc	1860
tctagcgacg	gccgcacctt	cactgggtgc	aatgtatatc	attttactgg	gggaccttgt	1920
gcagaactcg	tggtgctggg	cactgctgct	gctgcggcag	ctggcaacct	gacttgtatc	1980
gtcgcgatcg	gaaatgagaa	cagggggcatc	ttgagcccct	gcggacgggtg	tcgacagggtg	2040
cttctcgatc	tgcatcctgg	gatcaaagcg	atagtgaagg	acagtgatgg	acagccgacg	2100
gcagttggga	ttcgtgaatt	gctgccctct	ggttatgtgt	gggagggcta	agcacttcgt	2160
ggccgaggag	caggactgac	acgtgctacg	agatttcgat	tccaccgccg	ccttctatga	2220
aaggttgggc	ttcggaatcg	ttttccggga	cgcgggctgg	atgatcctcc	agcgcgggga	2280
tctcatgctg	gagttcttcg	cccaccccaa	cttgtttatt	gcagcttata	atggttacaa	2340
ataaagcaat	agcatcacia	atctcacaaa	taaagcattt	ttttcactgc	attctagttg	2400
tggtttgtcc	aaactcatca	atgtatctta	tcatgtctgt	acgagttggg	tcagctgctg	2460
cctgaggctg	gacgacctcg	cggagttcta	ccggcagtcg	aaatccgtcg	gcatccagga	2520
aaccagcagc	ggctatccgc	gcatccatgc	ccccgaactg	caggagtggg	gaggcacgat	2580
ggccgctttg	gtcgaggcgg	atccggccat	tagccatatt	attcattggg	tatatagcat	2640
aatcaatat	tggtatttgg	ccattgcata	cgttgtatcc	atatcataat	atgtacattt	2700
atattggctc	atgtccaaca	ttaccgccat	gttgacattg	attattgact	agttattaat	2760
agtaatcaat	tacgggggtca	ttagttcata	gcccataatat	ggagttccgc	gttacataac	2820
ttacggtaaa	tggcccgctt	ggctgaccgc	ccaacgaccc	ccgcccattg	acgtcaataa	2880
tgacgtatgt	tcccatagta	acgccaatag	ggactttcca	ttgacgtcaa	tgggtggagt	2940
atttacggta	aactgcccac	ttggcagtac	atcaagtgtg	tcatatgccg	agtacgcccc	3000
ctattgacgt	caatgacggg	aaatggcccg	cctggcatta	tgcccagtac	atgaccttat	3060
gggactttcc	tacttggcag	tacatctacg	tattagtcac	cgctattacc	atgggtgatgc	3120
ggttttggca	gtacatcaat	gggcgtggat	agcggtttga	ctcacgggga	tttccaagtc	3180
tccaccccat	tgacgtcaat	gggagtttgt	tttggcacca	aatcaacggg	gactttccaa	3240

WO 02/02738

PCT/US01/20710

```

aatgtcgtaa caactccgcc ccattgacgc aaatgggcgg taggcatgta cgggtgggagg 3300
tctatataag cagagctcgt ttagtgaacc gtcagatcgc ctggagacgc catccacgct 3360
gttttgacct ccatagaaga caccgggacc gatccagcct ccgcggcccc aagcttctcg 3420
agttaacaga tctaggctgg cacgacaggt ttcccgactg gaaagcgggc agtgagcgca 3480
acgcaattaa tgtgagttag ctactcatt aggcacccca ggctttacac tttatgcttc 3540
cggctcgtat gttgtgtgga attgtgagcg gataacaatt tcacacagga aacagctatg 3600
accatgatta cgccaagctt ggctgcaggt cgacggatcc actagtaacg gccgccagtg 3660
tgctggaatt caccatgggg caaccggga acggcagcgc cttcttgctg gcacccaatg 3720
gaagccatgc gccggaccac gacgtcacgc agcaaaggga cgagggtgtg gtggtgggca 3780
tgggcatcgt catgtctctc atcgtcctgg ccacgtgtt tggcaatgtg ctggtcacat 3840
cagccattgc caagttcgag cgtctgcaga cggtcaccaa ctacttcac acaagcttgg 3900
cctgtgctga tctggtcacg gggctagcag tgggtgccct tggggccgcc catattctca 3960
tgaaaatgtg gacttttggc aacttctggg gcgagttctg gacttccatt gatgtgctgt 4020
gcgtcacggc atcgattgag accctgtgcg tgatcgcagt cgaccgctac tttgccatta 4080
ctagtccttt caagtaccag agcctgctga ccaagaataa ggcccgggtg atcattctga 4140
tggtgtggat tgtgtcaggc cttacctcct tcttgcccat tcagatgcac tggtagagg 4200
ccaccacca ggaagccatc aactgctatg ccaatgagac ctgctgtgac ttcttcacga 4260
accaagccta tgccattgcc tcttccatcg tgtccttcta cgttcccctg gtgatcatgg 4320
tcttcgtcta ctccagggtc tttcaggagg ccaaaaggca gctccagaag attgacaaat 4380
ctgagggccg cttccatgtc cagaacctta gccagggtgga gcaggatggg cggacggggc 4440
atggactccg cagatcttcc aagttctgct tgaaggagca caaagccctc aagacgttag 4500
gcatcatcat gggcactttc accctctgct ggctgccctt cttcatcgtt aacattgtgc 4560
atgtgatcca ggataacctc atccgtaagg aagtttacat cctcctaaat tggataggct 4620
atgtcaattc tggtttcaat ccccttatct actgccggag ccagatttc aggattgcct 4680
tccaggagct tctgtgcctg cgcaggctct ctttgaaggc ctatggcaat ggctactcca 4740
gcaacggcaa cacaggggag cagagtggat atcacgtgga acaggagaaa gaaaataaac 4800
tgctgtgtga agacctcca ggcacggaag actttgtggg ccatcaagggt actgtgccta 4860
gcgataacat tgattcaciaa gggaggaatt gtagtaciaa tgactcactg ctctcgagaa 4920
tcgagggggc gcaccaccat catcaccacg tcgaccccg ggactacaag gatgacgatg 4980
acaagtaagc tttatccatc aactggcgg ccgctcgagc atgcatctag cggccgctcg 5040
aggccggcaa ggccggatcc ccgggaattc gccctctcc ccccccccc cctaacgtta 5100
ctggccgaag ccgcttgga taaggccgg gtgcgtttgt ctatatgtta ttttccacca 5160
tattgccgtc ttttggcaat gtgagggccc ggaaacctgg ccctgtcttc ttgacgagca 5220
ttcctagggg tctttccct ctcgccaaag gaatgcaagg tctgttgaat gtcgtgaagg 5280

```

WO 02/02738

PCT/US01/20710

aagcagttcc	tctggaagct	tcttgaagac	aaacaacgtc	tgtagcgacc	ctttgcaggc	5340
agcggaaacc	cccacctggc	gacaggtgcc	tctgcggcca	aaagccacgt	gtataagata	5400
cacctgcaaa	ggcggcacaa	ccccagtgcc	acgttgtgag	ttggatagtt	gtggaaagag	5460
tcaaattggct	ctcctcaagc	gtattcaaca	aggggctgaa	ggatgcccag	aaggtacccc	5520
attgtatggg	atctgatctg	gggcctcggg	gcacatgctt	tacatgtgtt	tagtcgaggt	5580
taaaaaaacg	tctaggcccc	ccgaaccacg	gggacgtggg	tttcctttga	aaaacacgat	5640
gataatatgg	cctcctttgt	ctctctgctc	ctggtaggca	tcctattcca	tgccacccag	5700
gccgagctca	cccagtctcc	agactccctg	gctgtgtctc	tgggcgagag	ggccaccatc	5760
aactgcaagt	ccagccagag	tgttttgtac	agctccaaca	ataagaacta	tttagcttgg	5820
tatcagcaga	aaccaggaca	gcctcctaag	ctgctcattt	actgggcac	tacccgggaa	5880
tccgggggtcc	ctgaccgatt	cagtggcagc	gggtctggga	cagatttcac	tctcaccatc	5940
agcagcctgc	aggctgaaga	tgtggcagtt	tattactgtc	agcaatatta	tagtactcag	6000
acgttcggcc	aagggaccaa	ggtggaaatc	aaacgaactg	tggctgcacc	atctgtcttc	6060
atcttcccgc	catctgatga	gcagttgaaa	tctggaactg	cctctgttgt	gtgcctgctg	6120
aataacttct	atcccagaga	ggccaaagta	cagtggaagg	tggataacgc	cctccaatcg	6180
ggtaactccc	aggagagtgt	cacagagcag	gacagcaagg	acagcaccta	cagcctcagc	6240
agcaccctga	cgctgagcaa	agcagactac	gagaaacaca	aactctacgc	ctgcgaagtc	6300
acccatcagg	gcctgagatc	gcccgtcaca	aagagcttca	acaaggggag	agtgttagtt	6360
ctagataatt	aattaggagg	agatctcgag	ctcgcgaaag	cttggcactg	gccgtcgttt	6420
tacaacgtcg	tgactgggaa	aaccctggcg	ttacccaact	taatcgctt	gcagcacatc	6480
cccctttcgc	cagcctccta	ggtcgacatc	gataaaataa	aagattttat	ttagtctcca	6540
gaaaaagggg	ggaatgaaag	acccacctg	taggtttggc	aagctagctt	aagtaacgcc	6600
attttgcaag	gcatggaaaa	atacataact	gagaatagag	aagttcagat	caaggtcagg	6660
aacagatgga	acagctgaat	atgggccaac	caggatatct	gtggtaagca	gttcctgccc	6720
cggctcaggg	ccaagaacag	atggaacagc	tgaatatggg	ccaaacagga	tatctgtggg	6780
aagcagttcc	tgccccgggt	cagggccaaag	aacagatggg	ccccagatgc	ggccagccc	6840
tcagcagttt	ctagagaacc	atcagatggt	tccaggggtgc	ccaaggacc	tgaaatgacc	6900
ctgtgcctta	tttgaactaa	ccaatcagtt	cgcttctcgc	ttctgttcgc	gcgcttctgc	6960
tccccgagct	caataaaaga	gccacaacc	cctcactcgg	ggcgccagtc	ctccgattga	7020
ctgagtcgcc	cgggtacccg	tgtatccaat	aaaccctctt	gcagttgcat	ccgacttgtg	7080
gtctcgctgt	tccttgggag	ggtctcctct	gagtgattga	ctaccctca	gcgggggtct	7140
ttcatttggg	ggctcgtccg	ggatcgggag	acccctgccc	agggaccacc	gaaccaccac	7200
cgggaggtaa	gctggctgcc	tcgcgcgttt	cggtgatgac	ggtgaaaacc	tctgacacat	7260
gcagctcccg	gagacgggtca	cagcttgtct	gtaagcggat	gccgggagca	gacaagcccc	7320

WO 02/02738

PCT/US01/20710

tcagggcgcg	tcagcgggtg	ttggcgggtg	tcggggcgca	gccatgaccc	agtcacgtag	7380
cgatagcgga	gtgtatactg	gcttaactat	gcggcatcag	agcagattgt	actgagagtg	7440
caccatatgc	ggtgtgaaat	accgcacaga	tgcgtaagga	gaaaataccg	catcaggcgc	7500
tcttccgctt	cctcgctcac	tgactcgctg	cgctcggtcg	ttcgggtgcg	gcgagcggta	7560
tcagctcact	caaaggcggg	aatacgggtt	tccacagaat	caggggataa	cgcaggaaag	7620
aacatgtgag	caaaaggcca	gcaaaaggcc	aggaaccgta	aaaaggccgc	gttgctggcg	7680
tttttccata	ggctccgccc	ccctgacgag	catcacaaaa	atcgacgctc	aagtcagagg	7740
tggcgaaacc	cgacaggact	ataaagatac	caggcgtttc	cccctggaag	ctccctcgtg	7800
cgctctcctg	ttccgaccct	gccgcttacc	ggatacctgt	ccgcctttct	cccttcggga	7860
agcgtggcgc	tttctcatag	ctcacgctgt	aggtatctca	gttcgggtgta	ggtcgttcgc	7920
tccaagctgg	gctgtgtgca	cgaaccccc	gttcagccc	accgctgcgc	cttatccggt	7980
aactatcgtc	ttgagtccaa	cccggtaaga	cacgacttat	cgccactggc	agcagccact	8040
ggtaacagga	ttagcagagc	gaggtatgta	ggcgggtgcta	cagagttctt	gaagtgggtg	8100
cctaactacg	gctacactag	aaggacagta	tttggtatct	gcgctctgct	gaagccagtt	8160
accttcggaa	aaagagttgg	tagctcttga	tccggcaaac	aaaccaccgc	tggtagcggg	8220
ggtttttttg	tttgcaagca	gcagattacg	cgcagaaaaa	aaggatctca	agaagatcct	8280
ttgatctttt	ctacgggggtc	tgacgctcag	tggaacgaaa	actcacgtta	agggattttg	8340
gtcatgagat	tatcaaaaag	gatcttcacc	tagatccttt	taaattaaaa	atgaagtttt	8400
aatcaatct	aaagtatata	tgagtaaact	tggtctgaca	gttaccaatg	cttaatcagt	8460
gaggcaccta	tctcagcgat	ctgtctattt	cgttcatcca	tagttgcctg	actccccgtc	8520
gtgtagataa	ctacgatacg	ggagggctta	ccatctggcc	ccagtgcctg	aatgataccg	8580
cgagaccac	gctcaccggc	tccagattta	tcagcaataa	accagccagc	cgggaaggcc	8640
gagcgcagaa	gtggtcctgc	aactttatcc	gcctccatcc	agtctattaa	ttggttgccg	8700
gaagctagag	taagtagttc	gccagttaat	agtttgcgca	acgttggtgc	cattgctgca	8760
ggcatcgtgg	tgtcacgctc	gtcgtttggt	atggcttcat	tcagctccgg	ttcccaacga	8820
tcaaggcgag	ttacatgatc	ccccatgttg	tgcaaaaaag	cggttagctc	cttcggtcct	8880
ccgatcgttg	tcagaagtaa	gttggccgca	gtgttatcac	tcatgggttat	ggcagcactg	8940
cataattctc	ttactgtcat	gccatccgta	agatgctttt	ctgtgactgg	tgagtactca	9000
accaagtcac	tctgagaata	gtgtatgcgg	cgaccgagtt	gctcttgccc	ggcgtcaaca	9060
cgggataata	ccgcgccaca	tagcagaact	ttaaaagtgc	tcatcattgg	aaaacgttct	9120
tcggggcgaa	aactctcaag	gatcttaccg	ctgttgagat	ccagttcgat	gtaaccact	9180
cgtgcaccca	actgatcttc	agcatctttt	actttcacca	gcgtttctgg	gtgagcaaaa	9240
acaggaaggc	aaaatgccgc	aaaaaaggga	ataagggcga	cacggaaatg	ttgaatactc	9300
atactcttcc	tttttcaata	ttattgaagc	atztatcagg	gttattgtct	catgagcgga	9360

WO 02/02738

PCT/US01/20710

tacatatatttg aatgtatttta gaaaaataaaa caaatagggg ttccgcgcac atttccccga 9420
aaagtgccac ctgacgtcta agaaaccatt attatcatga cattaaccta taaaaatagg 9480
cgtatcacga ggcccttttcg tcttcaagaa t 9511

<210> 35

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 35

gatccactag taacggccgc cagaattcgc 30

<210> 36

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 36

cagagagaca aaggaggcca tattatcatc gtgtttttca aag 43

53116-13

CLAIMS:

1. A clonally selected host cell comprising a genome, said genome comprising from 20 to 100 copies of a first integrated retroviral vector, wherein said retroviral vector comprises at least one exogenous gene operably linked to a
5 promoter, said gene encoding a secreted protein, wherein said host cell does not produce infectious retroviral particles.
2. The host cell of Claim 1, wherein said retroviral vector further comprises a secretion signal sequence operably linked to said exogenous gene.
3. The host cell of Claim 1, wherein said retroviral vector further comprises
10 an RNA export element operably linked to said exogenous gene.
4. The host cell of Claim 1, wherein said retroviral vector comprises at least two exogenous genes.
5. The host cell of Claim 4, wherein said at least two exogenous genes are arranged in a polycistronic sequence.
- 15 6. The host cell of Claim 5, wherein said at least two exogenous genes are separated by at least one internal ribosome entry site.
7. The host cell of Claim 5, wherein two exogenous genes are arranged in said polycistronic sequence.
8. The host cell of Claim 7, wherein said two exogenous genes encode a
20 heavy chain of an immunoglobulin molecule and a light chain of an immunoglobulin molecule.
9. The host cell of Claim 4, wherein one of said at least two exogenous genes is a selectable marker.

53116-13

10. The host cell of Claim 1, wherein said retroviral vector comprises long terminal repeats selected from the group consisting of MoMLV, MoMuSV, and MMTV long terminal repeats.

11. The host cell of Claim 1, wherein said retroviral vector is a lentiviral
5 vector.

12. The host cell of Claim 11, wherein said lentiviral vector comprises long terminal repeats selected from the group consisting of HIV and equine infectious anemia virus long terminal repeats.

13. The host cell of Claim 1, wherein said host cell is selected from Chinese
10 hamster ovary cells, baby hamster kidney cells, and bovine mammary epithelial cells.

14. The host cell of Claim 1, wherein said genome is stable for greater than
10 passages.

15. The host cell of Claim 14, wherein said genome is stable for greater
than 50 passages.

15 16. The host cell of Claim 14, wherein said genome is stable for greater
than 100 passages.

17. The host cell of Claim 1, wherein said integrated exogenous gene is
stable in the absence of selection.

18. The host cell of Claim 1, wherein said promoter is selected from the
20 group consisting of alpha-lactalbumin promoter, cytomegalovirus promoter and the
long terminal repeat of Moloney murine leukemia virus.

19. The host cell of Claim 1, wherein said host cell expresses greater than
about 10 picograms of said secreted protein per day.

20. A method for transfecting host cells comprising:

53116-13

1) providing:

a) a host cell comprising a genome, and

b) a plurality of retroviral vectors encoding a first exogenous gene operably linked to a promoter; and

5 2) serially transducing said host cell with said plurality of retroviral vectors to provide said host cell with from 20 to 100 copies of the retroviral vector integrated into said genome of said host cell, said first exogenous gene encoding a secreted protein, wherein said host cell does not produce infectious retroviral particles; and

3) clonally selecting a transfected host cell.

10 21. The method of Claim 20, wherein said conditions comprise contacting said host cell at a multiplicity of infection of greater than 10.

22. The method of Claim 20, wherein said conditions comprise contacting said host cell at a multiplicity of infection of from about 10 to 1000.

15 23. The method of Claim 20, wherein said retroviral vectors further comprise a secretion signal sequence operably linked to said first exogenous gene.

24. The method of Claim 20, wherein said retroviral vectors further comprise an RNA export element operably linked to said first exogenous gene.

25. The method of Claim 20, wherein said retroviral vectors comprise at least two exogenous genes.

20 26. The method of Claim 25, wherein said at least two exogenous genes are arranged in a polycistronic sequence.

27. The method of Claim 20, wherein said retroviral vectors are pseudotyped retroviral vectors.

53116-13

28. The method of Claim 20, wherein said retroviral vectors are lentiviral vectors.

29. The method of Claim 28, wherein said lentiviral vectors are pseudotyped lentivirus vectors comprising a G glycoprotein.

5 30. The method of Claim 20, wherein said host cell is selected from Chinese hamster ovary cells, baby hamster kidney cells, and bovine mammary epithelial cells.

31. The method of Claim 20, comprising transfecting said host cell with a second retroviral vector encoding a second exogenous gene which is different from
10 the first exogenous gene.

32. A method of producing a protein of interest comprising:

1) providing a clonally selected host cell comprising a genome, said genome comprising from 20 to 100 integrated copies of a first retroviral integrating vector comprising an exogenous gene operably linked to a promoter, and a secretion signal
15 sequence operably linked to said exogenous gene wherein said exogenous gene encodes a secretable protein of interest, and

2) culturing said host cell under conditions such that said protein of interest is produced.

33. The method of Claim 32, further comprising step

20 3) isolating said protein of interest.

34. The method of Claim 32, wherein said conditions are selected from the group consisting of roller bottle cultures, perfusion cultures, batch fed cultures, and petri dish cultures.

35. The method of Claim 32, wherein said integrating vector is a retroviral
25 vector.

53116-13

36. The method of Claim 35, wherein said retroviral vector is a lentiviral vector.

37. The method of Claim 32, wherein said host cell is selected from Chinese hamster ovary cells, baby hamster kidney cells, and bovine mammary
5 epithelial cells.

38. The method of Claim 32, wherein said host cells synthesize greater than about 1 picograms per cell per day of said protein of interest.

39. The method of Claim 32, wherein said host cells synthesize greater than about 10 picograms per cell per day of said protein of interest.

10 40. The method of Claim 32, wherein said host cells synthesize greater than about 50 picograms per cell per day of said protein of interest.

41. A method for transfecting host cells comprising:

1) providing:

15 a) a clonally selected host cell comprising a genome comprising at least one integrated exogenous gene; and

b) a plurality of retroviral vectors comprising an exogenous gene identical to said exogenous gene in said clonally selected host cell, said genes encoding a secreted protein; and

20 2) serially transducing said host cell with said plurality of retroviral vectors to provide said host cell with from 20 to 100 copies of said retroviral vector integrated into said genome of said host cell, wherein said host cell does not produce infectious retroviral particles.

42. The method of Claim 41, wherein said integrated exogenous gene comprises an integrating vector.

53116-13

43. The method of Claim 41, wherein said host cell is non-clonally selected.

44. The method of Claim 41, wherein said conditions comprise contacting said host cell at a multiplicity of infection of greater than 10.

45. The method of Claim 41, wherein said conditions comprise contacting
5 said host cell at a multiplicity of infection of from about 10 to 1000.

46. The method of Claim 41, wherein said retroviral vector is a pseudotyped retroviral vector.

1/35

Figure 1

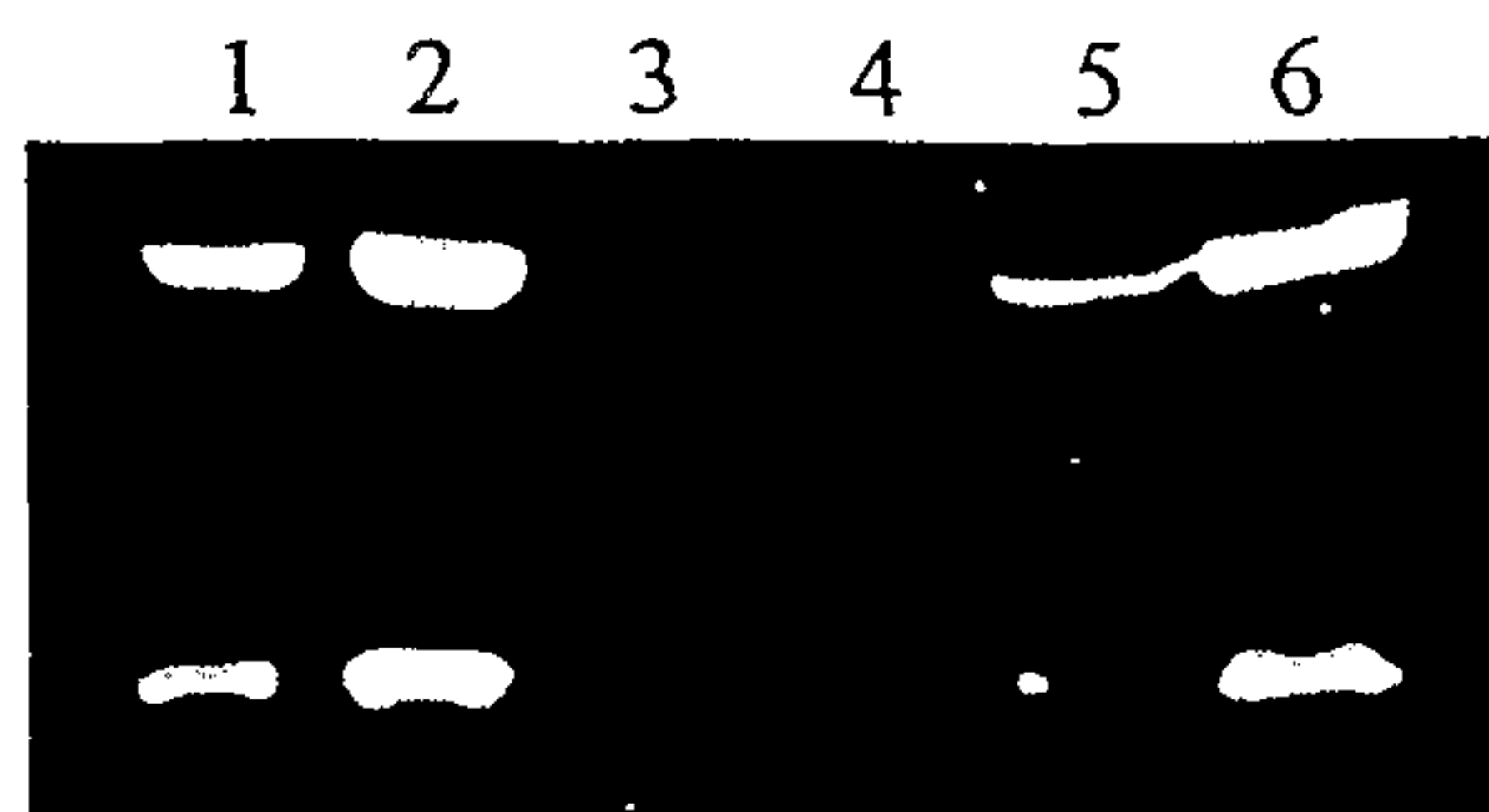


Figure 2

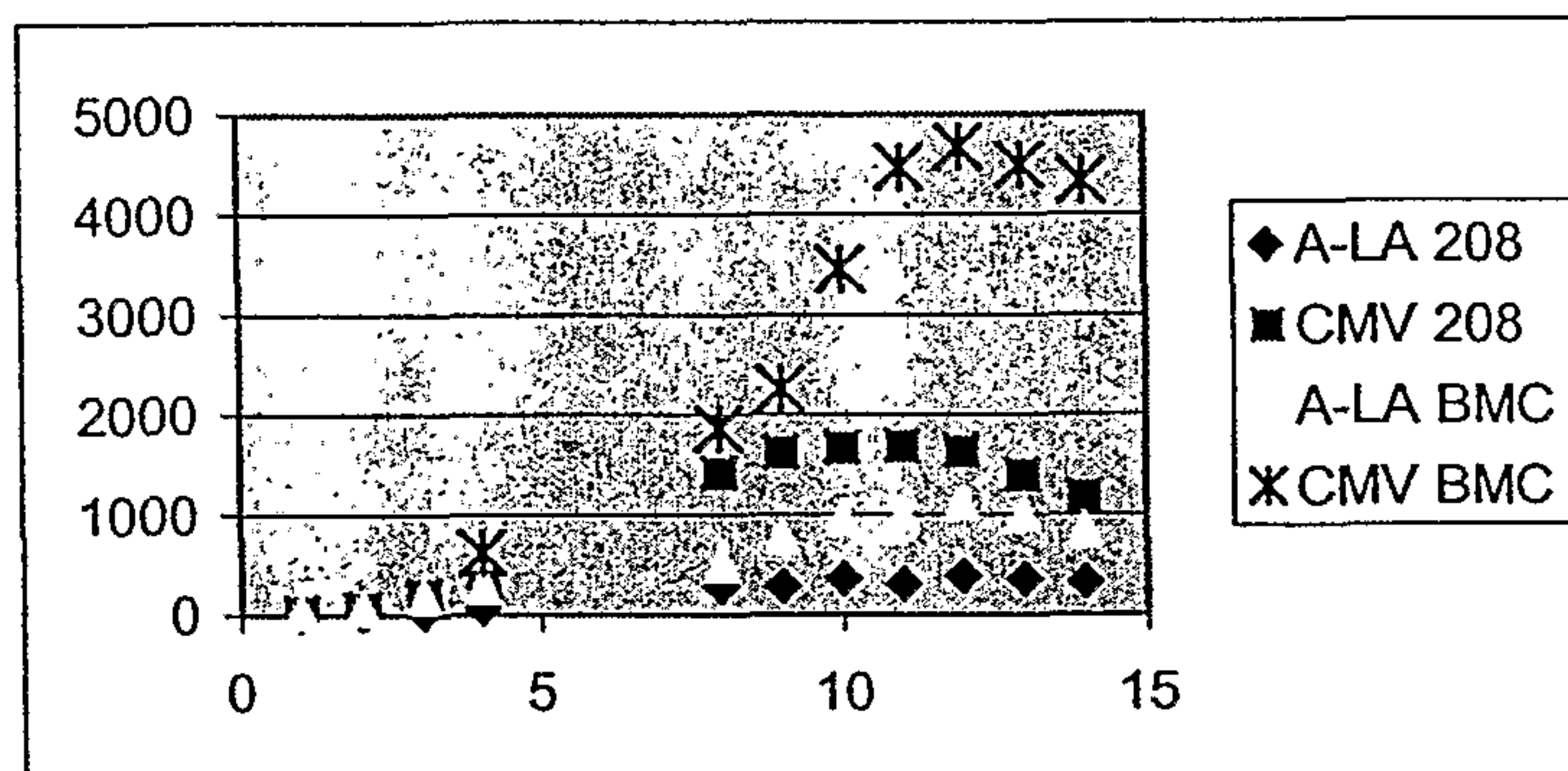
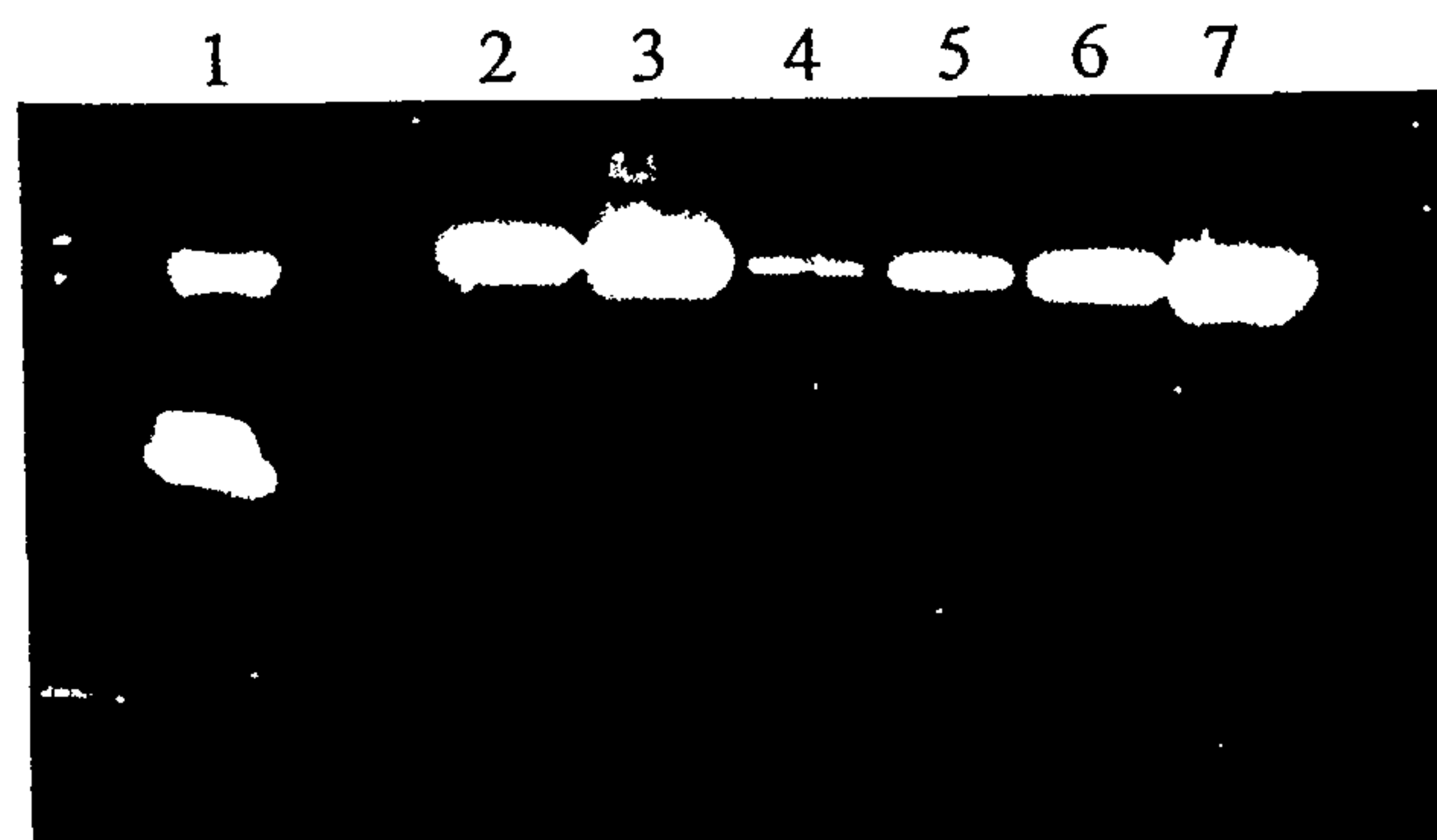


Figure 3



2/35

Figure 4
SEQ ID NO:1
Hybrid Human-Bovine Alpha-Lactalbumin Promoter

```

1   GATCAGTCCTGGGTGGTCATTGAAAGGACTGATGCTGAAGTTGAAGCTCC
51  AATACTTTGGCCACCTGATGCGAAGAACTGACTCATGTGATAAGACCCTG
101 ATACTGGGAAAGATTGAAGGCAGGAGGAGAAGGGATGACAGAGGATGGAA
151 GAGTTGGATGGAATCACCAACTCGATGGACATGAGTTTGAGCAAGCTTCC
201 AGGAGTTGGTAATGGGCAGGGAAGCCTGGCGTGCTGCAGTCCATGGGGTT
251 GCAAAGAGTTGGACACTACTGAGTGACTGAACTGAACTGATAGTGTAATC
301 CATGGTACAGAATATAGGATAAAAAAGAGGAAGAGTTTGCCCTGATTCTG
351 AAGAGTTGTAGGATATAAAAGTTTGAATAACCTTTAGTTTGGAAGTCTTA
401 AATTATTTACTTAGGATGGGTACCCACTGCAATATAAGAAATCAGGCTTT
451 AGAGACTGATGTAGAGAGAATGAGCCCTGGCATAACCAGAAGCTAACAGCT
501 ATTGGTTATAGCTGTTATAACCAATATATAACCAATATATTGGTTATATA
551 GCATGAAGCTTGATGCCAGCAATTTGAAGGAACCATTTAGAACTAGTATC
601 CTAAACTCTACATGTTCCAGGACACTGATCTTAAAGCTCAGGTTCAGAAT
651 CTTGTTTTATAGGCTCTAGGTGTATATTGTGGGGCTTCCCTGGTGGCTCA
701 GATGGTAAAGTGTCTGCCTGCAATGTGGGTGATCTGGGTTTCGATCCCTGG
751 CTTGGGAAGATCCCCTGGAGAAGGAAATGGCAACCCACTCTAGTACTCTT
801 ACCTGGAAAATTCCATGGACAGAGGAGCCTTGTAAGCTACAGTCCATGGG
851 ATTGCAAAGAGTTGAACACAACCTGAGCAACTAAGCACAGCACAGTACAGT
900 ATACACCTGTGAGGTGAAGTGAAGTGAAGGTTCAATGCAGGGTCTCCTGC
951 ATTGCAGAAAGATTCTTTACCATCTGAGCCACCAGGGAAGCCCAAGAATA
1001 CTGGAGTGGGTAGCCTATTCTTCTCCAGGGGATCTTCCCATCCCAGGAA
1051 TTGAACTGGAGTCTCCTGCATTTCAAGGTGGATTCTTCACCAGCTGAACTA
1101 CCAGGTGGATACTACTCCAATATTAAAGTGCTTAAAGTCCAGTTTTCCCA
1151 CCTTTCCCAAAAAGGTTGGGTCACTCTTTTTTAACCTTCTGTGGCCTACT
1201 CTGAGGCTGTCTACAAGCTTATATATTTATGAACACATTTATTGCAAGTT
1251 GTTAGTTTTAGATTTACAATGTGGTATCTGGCTATTTAGTGGTATTGGTG
1301 GTTGGGGATGGGGAGGCTGATAGCATCTCAGAGGGCAGCTAGATACTGTC
1351 ATACACACTTTTCAAGTTCTCCATTTTTGTGAAATAGAAAGTCTCTGGAT
1401 CTAAGTTATATGTGATTCTCAGTCTCTGTGGTCATATTCTATTCTACTCC
1451 TGACCACTCAACAAGGAACCAAGATATCAAGGGACACTTGTTTTGTTTCA
1501 TGCTTGGGTTGAGTGGGCCATGACATATGTTCTGGGCCTTGTTACATGGC
1551 TGGATTGGTTGGACAAGTGCCAGCTCTGATCCTGGGACTGTGGCATGTGA
1601 TGACATACACCCCCTCTCCACATTCTGCATGTCTCTAGGGGGGAAGGGGG
1651 AAGCTCGGTATAGAACCCTTTATTGTATTTTCTGATTGCCTCACTTCTTAT
1701 ATTGCCCCCATGCCCTTCTTTGTTCTCAAGTAACCAGAGACAGTGCTTC
1751 CCAGAACCAACCCTACAAGAAACAAAGGGCTAAACAAAGCCAAATGGGAA
1801 GCAGGATCATGGTTTGAACCTTTCTGGCCAGAGAACAAATACCTGCTATG
1851 GACTAGATACTGGGAGAGGGAAAGGAAAAGTAGGGTGAATTATGGAAGGA
1901 AGCTGGCAGGCTCAGCGTTTCTGTCTTGGCATGACCAGTCTCTCTTCATT
1951 CTCTTCCTAGATGTAGGGCTTGGTACCAGAGCCCCTGAGGCTTTCTGCAT
2001 GAATATAAATATATGAACTGAGTGATGCTTCCATTTCAAGGTTCTTGGGG
2051 GCGCCGAATTTCGAGCTCGGTACCCGGGGATCTCGAGGGGGGGCCCGGTAC
2101 C

```

1 - 1525	Bovine alpha lactalbumin 5' flanking region (-2000 to -550 from the bovine alpha-lactalbumin transcription start point)
1526 - 2056	Human alpha-lactalbumin 5' flanking region (-600 to +15 from the human alpha-lactalbumin transcription start point)
2057 - 2101	Multiple cloning site

3/35

Figure 5
SEQ ID NO:2
Mutated PPE Sequence

```
1      GATTACTTACTGGCAGGTGCTGGGGGCTTCCGAGACAATCGCGAACATCT
51     ACACCACACAACACCGCCTCGACCAGGGTGAGATATCGGCCGGGGACGCG
101    GCGGTGGTAATTACAAGCGAGGATCCGATTACTTACTGGCAGGTGCTGGG
151    GGCTTCCGAGACAATCGCGAACATCTACACCACACAACACCGCCTCGACC
201    AGGGTGAGATATCGGCCGGGGACGCGGCGGTGGTAATTACAAGCG
```

```
1 - 119      Mutated PPE
120 -126     Linker
127 - 245    Mutated PPE
```

4/35

Figure 6
SEQ ID NO:3
IRES-Signal Peptide Sequence

```
1   GGAATTCGCCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCG
51  CTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATAT
101 TGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTG
151 ACGAGCATTCCTAGGGGTCTTTCCTCTCGCCAAAGGAATGCAAGGTCT
201 GTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAA
251 CAACGTCTGTAGCGACCCTTTCAGGCAGCGGAACCCCCCACCTGGCGAC
301 AGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGC
351 GGCACAACCCCAAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCA
401 AATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAG
451 GTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTAC
501 ATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCGAACCACGGGG
551 ACGTGGTTTTCTTTGAAAAACACGATGATAATATGGCCTCCTTTGTCTC
601 TCTGCTCCTGGTAGGCATCCTATTCATGCCACCCAGGCCGGCGCCATGG
651 GATATCTAGATCTCGAGCTCGCGAAAGCTT
```

1 - 583	IRES
584 - 640	Modified bovine alpha-lactalbumin signal peptide coding region
641 - 680	Multiple cloning site

5/35

Figure 7a
SEQ ID NO:4
CMV MN14 Vector

```
1      CGGATCCGGCCATTAGCCATATTATTCATTGGTTATATAGCATAAATCAA
51     TATTGGCTATTGGCCATTGCATACGTTGTATCCATATCATAATATGTACA
101    TTTATATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATTG
151    ACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATA
201    TATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGAC
251    CGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATA
301    GTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACG
351    GTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGC
401    CCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAG
451    TACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGT
501    CATCGCTATTACCATGGTGTATGCGGTTTTGGCAGTACATCAATGGGCGTG
551    GATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATGACGTC
601    AATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCG
651    TAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCATGTACGGTGGG
701    AGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGA
751    CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAG
801    CCTCCGCGGCCCAAGCTTCTCGACGGATCCCCGGGAATTCAGGACCTCA
851    CCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGT
901    GTCCACTCCGAGGTCCAACCTGGTGGAGAGCGGTGGAGGTGTTGTGCAACC
951    TGGCCGGTCCCTGCGCCTGTCTGCTCCGCATCTGGCTTCGATTTACCA
1001   CATATTGGATGAGTTGGGTGAGACAGGCACCTGGAAAAGGTCTTGAGTGG
1051   ATTGGAGAAATTCATCCAGATAGCAGTACGATTAACATATGCGCCGTCTCT
1101   AAAGGATAGATTTACAATATCGCGAGACAACGCCAAGAACACATTGTTCC
1151   TGCAAATGGACAGCCTGAGACCCGAAGACACCGGGGTCTATTTTTGTGCA
1201   AGCCTTTACTTCGGCTTCCCCTGGTTTGCTTATTGGGGCCAAGGGACCCC
1251   GGTACCCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGG
1301   CACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG
1351   GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGC
1401   CCTGACCAGCGGCGTGACACCTTCCCGGTGTCTTACAGTCTCAGGAC
1451   TCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACC
1501   CAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA
1551   CAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGT
1601   GCCCAGCACCTGAACCTCTGGGGGGACCGTCAGTCTTCTCTTCCCCCA
1651   AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGT
1701   GGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACG
1751   TGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
1801   TACAACAGCACGTACCGTGTGGTCAGCGTCTCACCCTCCTGCACCAGGA
1851   CTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCC
1901   CAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA
1951   CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCA
2001   GGTACGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCG
2051   TGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCT
2101   CCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTATAGCAAGCTCACCGT
2151   GGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC
2201   ACGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCC
2251   GGGAAATGAAAGCCGAATTGCCCCCTCTCCCTCCCCCCCCCTAACGTTA
2301   CTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTA
2351   TTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGG
2401   CCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAG
2451   GAATGCAAGGTCTGTTGAATGTCTGTAAGGAAGCAGTTCCTCTGGAAGCT
2501   TCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCC
2551   CCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATA
2601   CACCTGCAAAGGCGGCACAACCCCAAGTGCCACGTTGTGAGTTGGATAGTT
2651   GTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAA
2701   GGATGCCCAGAAGGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGT
2751   GCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCC
2801   CCGAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATGATAATATGG
```

Figure 7b

2851 CCTCCTTTGTCTCTCTGCTCCTGGTAGGCATCCTATTCCATGCCACCCAG
2901 GCCGACATCCAGCTGACCCAGAGCCCAAGCAGCCTGAGCGCCAGCGTGGG
2951 TGACAGAGTGACCATCACCTGTAAGGCCAGTCAGGATGTGGGTACTTCTG
3001 TAGCCTGGTACCAGCAGAAGCCAGGTAAGGCTCCAAAGCTGCTGATCTAC
3051 TGGACATCCACCCGGCACACTGGTGTGCCAAGCAGATTGAGCGGTAGCGG
3101 TAGCGGTACCGACTTCACCTTCACCATCAGCAGCCTCCAGCCAGAGGACA
3151 TCGCCACCTACTACTGCCAGCAATATAGCCTCTATCGGTCTGTCGGCCAA
3201 GGGACCAAGGTGGAAATCAAACGAACTGTGGCTGCACCATCTGTCTTCAT
3251 CTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGT
3301 GCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTG
3351 GATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGA
3401 CAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAG
3451 CAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGC
3501 CTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGGAGAGTGTTAGAGATC
3551 TAGGCCTCCTAGGTCGACATCGATAAAATAAAAGATTTTATTTAGTCTCC
3601 AGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCT
3651 TAAGTAACGCCATTTTGCAAGGCATGGAATAACATAACTGAGAATAGA
3701 GAAGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAA
3751 ACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACA
3801 GATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTC
3851 CTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCC
3901 CTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGAC
3951 CTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCG
4001 CTTCTGTTGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAAC
4051 CCCTCACTCGGGGCGCCAGTCCCTCCGATTGACTGAGTCGCCCCGGGTACCC
4101 GTGTATCCAATAAACCTCTTGCAAGTTGCATCCGACTTGTGGTCTCGCTG
4151 TTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTC
4201 TTTCATT

1 - 812 CMV promoter/enhancer
853-855 MN14 antibody heavy chain gene signal peptide start codon
2257 - 2259 MN14 antibody heavy chain gene start codon
2271 - 2846 EMCV IRES
2847 - 2849 Bovine alpha-lactalbumin signal peptide start codon
2904 - 2906 First codon mature MN14 antibody light chain gene
3543 - 3544 MN14 antibody light chain gene stop codon
3614 - 4207 MoMuLV 3' LTR

7/35

Figure 8a
SEQ ID NO:5
CMV LL2 Vector

```
1      GGATCCGGCCATTAGCCATATTATTCATTGGTTATATAGCATAAATCAAT
51     ATTGGCTATTGGCCATTGCATACGTTGTATCCATATCATAATATGTACAT
101    TTATATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATTGA
151    CTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
201    ATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACC
251    GCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAG
301    TAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGG
351    TAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCC
401    CCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGT
451    ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTC
501    ATCGCTATTACCATGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGTGG
551    ATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATTTGACGTCA
601    ATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCGAAAATGTCGT
651    AACAACCTCCGCCCCATTGACGCAAATGGGCGGTAGGCATGTACGGTGGGA
701    GGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGAC
751    GCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGC
801    CTCCGCGGCCCAAGCTTCTCGACGGATCCCCGGGAATTCAGGACCTCAC
851    CATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTG
901    TCCACTCCCAGGTCCAGCTGGTCCAATCAGGGGCTGAAGTCAAGAAACCT
951    GGGTCATCAGTGAAGTCTCCTGCAAGGCTTCTGGCTACACCTTTACTAG
1001   CTACTGGCTGCACTGGGTGAGGCAGGCACCTGGACAGGGTCTGGAATGGA
1051   TTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTC
1101   AAGGACAAGGCCACAATAACTGCAGACGAATCCACCAATACAGCCTACAT
1151   GGAGCTGAGCAGCCTGAGGTCTGAGGACACGGCATTTTATTTTGTGCAA
1201   GAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACGGTCACCGTC
1251   TCCTCAGCCTCCACCAAGGGGCCATCGGTCTTCCCCCTGGCACCCCTCCTC
1301   CAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT
1351   ACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGCGCCCTGACCAGC
1401   GCGTGCACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCT
1451   CAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA
1501   TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTT
1551   GAGCCCAAATCTTGTGACAAACTCACACATGCCACCGTGCCAGCACC
1601   TGAAGTCTTGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGG
1651   ACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGAC
1701   GTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
1751   GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA
1801   CGTACCGTGTGGTCAGCGTCTCACCCTGCTGCACCAGGACTGGCTGAAT
1851   GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCAT
1901   CGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGT
1951   ACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTG
2001   ACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGA
2051   GAGCAATGGGCAGCCGGAGAACAATAAGACACGCTCCCGTGCTGG
2101   ACTCCGACGGCTCCTTCTTCTCTATAGCAAGCTCACCGTGGACAAGAGC
2151   AGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCT
2201   GCACAACCACTACACGAGAAAGAGCCTCTCCCTGTCTCCCGGGAATGAA
2251   AGCCGAATTGCCCCCTCTCCCTCCCCCCCCCTAACGTTACTGGCCGAAG
2301   CCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTCCACCA
2351   TATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTC
2401   TTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGG
2451   TCTGTTGAATGTCGTGAAGGAAGCAGTTCTCTGGAAGCTTCTTGAAGAC
2501   AAACAACGTCTGTAGCGACCTTTGCAGGCAGCGGAACCCCCACCTGGC
2551   GACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAA
2601   GGCGGCACAACCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAG
2651   TCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCAG
2701   AAGGTACCCCATTTGATGGGATCTGATCTGGGGCCTCGGTGCACATGCTT
2751   TACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCGAACCAG
2801   GGGACGTGGTTTTCTTTGAAAAACACGATGATAATATGGCCTCCTTTGT
```

Figure 8b

2851 CTCTCTGCTCCTGGTAGGCATCCTATTCCATGCCACCCAGGCCGACATCC
2901 AGCTGACCCAGTCTCCATCATCTCTGAGCGCATCTGTTGGAGATAGGGTC
2951 ACTATGAGCTGTAAGTCCAGTCAAAGTGTTTTATACAGTGCAAATCACAA
3001 GAACTACTTGGCCTGGTACCAGCAGAAACCAGGGAAAGCACCTAAACTGC
3051 TGATCTACTGGGCATCCACTAGGGAATCTGGTGTCCCTTCGCGATTCTCT
3101 GGCAGCGGATCTGGGACAGATTTTACTTTCACCATCAGCTCTCTTCAACC
3151 AGAAGACATTGCAACATATTATTGTCAACCAATACCTCTCCTCGTGGACGT
3201 TCGGTGGAGGGACCAAGGTGCAGATCAAACGAACTGTGGCTGCACCATCT
3251 GTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTC
3301 TGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGT
3351 GGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
3401 GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTTGACGCT
3451 GAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCC
3501 ATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
3551 TAGAGATCTAGGCCTCCTAGGTGACATCGATAAAATAAAAGATTTTATT
3601 TAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGCA
3651 AGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATACATAACTG
3701 AGAATAGAGAAGTTCAGATCAAGGTGAGGAACAGATGGAACAGCTGAATA
3751 TGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGC
3801 CAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTA
3851 AGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCG
3901 GTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCC
3951 CCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTC
4001 GCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAG
4051 CCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCC
4101 GGGTACCCGTGTATCCAATAAACCTCTTGACAGTTGCATCCGACTTGTGG
4151 TCTCGCTGTTCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAG
4201 GTCTTTCATT

1 - 812	CMV promoter/enhancer
852 - 854	LL2 antibody heavy chain signal peptide start codon
2247 - 2249	LL2 antibody heavy chain stop codon
2261 - 2836	EMCV IRES
2837 - 2839	Bovine alpha-lactalbumin signal peptide start codon
2894-2896	First codon of mature LL2 antibody light chain gene
3551 - 3553	LL2 antibody light chain gene stop codon
3622 - 4210	MoMuLV 3' LTR

9/35

Figure 9a
SEQ ID NO:6
MMTV MN14 Vector

```
1    CGAGCTTGGCAGAAATGGTTGAACTCCCGAGAGTGTCTACACCTAGGGG
51   AGAAGCAGCCAAGGGGTGTTTCCCACCAAGGACGACCCGTCTGCGCACA
101  AACGGATGAGCCCATCAGACAAAGACATATTCATTCTCTGCTGCAAACCTT
151  GGCATAGCTCTGCTTTGCCTGGGGCTATTGGGGGAAGTTGCGGTTCTGTGC
201  TCGCAGGGCTCTCACCCCTTGACTCTTCAATAATAACTCTTCTGTGCAAG
251  ATTACAATCTAAACAATTCGGAGAAGCTCGACCTTCCTCCTGAGGCAAGGA
301  CCACAGCCAACCTTCCTCTTACAAGCCGCATCGATTTTGTCTTCAGAAAT
351  AGAAATAAGAATGCTTGCTAAAAATTATATTTTACCAATAAGACCAATC
401  CAATAGGTAGATTATTAGTTACTATGTTAAGAAATGAATCATTATCTTTT
451  AGTACTATTTTTACTCAAATTCAGAAAGTTAGAAATGGGAATAGAAAATAG
501  AAAGAGACGCTCAACCTCAATTGAAGAACAGGTGCAAGGACTATTGACCA
551  CAGGCCTAGAAGTAAAAAAGGGAAAAAAGAGTGTTTTTGTCAAAATAGGA
601  GACAGGTGGTGGCAACCAGGGACTTATAGGGGACCTTACATCTACAGACC
651  AACAGATGCCCCCTTACCATATACAGGAAGATATGACTTAAATTGGGATA
701  GGTGGGTTACAGTCAATGGCTATAAAGTGTTATATAGATCCCTCCCCTTT
751  CGTGAAAGACTCGCCAGAGCTAGACCTCCTTGGTGTATGTTGTCTCAAGA
801  AAAGAAAGACGACATGAAACAACAGGTACATGATTATATTTATCTAGGAA
851  CAGGAATGCACTTTTGGGGAAAGATTTTCCATACCAAGGAGGGGACAGTG
901  GCTGGACTAATAGAACATTATTCTGCAAAAACCTTATGGCATGAGTTATTA
951  TGATTAGCCTTGATTTGCCCAACCTTGCGGTTCCCAAGGCTTAAGTAAGT
1001 TTTTGGTTACAAACTGTTCTTAAAACAAGGATGTGAGACAAGTGGTTTCC
1051 TGACTTGGTTTGGTATCAAAGGTTCTGATCTGAGCTCTGAGTGTTCTATT
1101 TTCCTATGTTCTTTTGAATTTATCCAAATCTTATGTAAATGCTTATGTA
1151 AACCAAGATATAAAAGAGTGCTGATTTTTTGTAGTAAACTTGCAACAGTCC
1201 TAACATTCACCTCTTGTGTGTTTGTGTCTGTTTCGCCATCCCGTCTCCGCT
1251 CGTCACCTTATCCTTCACTTTCAGAGGGTCCCCCGCAGACCCCGGCGAC
1301 CCTCAGGTCGGCCGACTGCGGCAGCTGGCGCCCGAACAGGGACCCCTCGGA
1351 TAAGTGACCCTTGTCTTTATTTCTACTATTTTGTGTTCGTCTTGTTTTGT
1401 CTCTATCTTGTCTGGCTATCATCACAAGAGCGGAACGGAATCACCTCAGG
1451 GAACCAAGCTAGCCCGGGGTCGACGGATCCGATTACTTACTGGCAGGTGC
1501 TGGGGGCTTCCGAGACAATCGCGAACATCTACACCACACAACACCGCCTC
1551 GACCAGGGTGAGATATCGGCGGGGACGCGGCGGTGGTAATTACAAGCGA
1601 GATCCGATTACTTACTGGCAGGTGCTGGGGGCTTCCGAGACAATCGCGAA
1651 CATCTACACCACACAACACCGCCTCGACCAGGGTGAGATATCGGCCGGGG
1701 ACGCGGCGGTGGTAATTACAAGCGAGATCCCCGGGAATTCAGGACCTCAC
1751 CATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTG
1801 TCCACTCCGAGGTCCAACCTGGTGGAGAGCGGTGGAGGTGTTGTGCAACCT
1851 GGCCGGTCCCTGCGCCTGTCTGCTCCGCATCTGGCTTCGATTTACCAC
1901 ATATTGGATGAGTTGGGTGAGACAGGCACCTGGAAAAGGTCTTGAGTGGA
1951 TTGGAGAAATTCATCCAGATAGCAGTACGATTAATATGCGCCGTCTCTA
2001 AAGGATAGATTTACAATATCGCGAGACAACGCCAAGAACACATTGTTCTT
2051 GCAAATGGACAGCCTGAGACCCGAAGACACCGGGGTCTATTTTTGTGCAA
2101 GCCTTTACTTCGGCTTCCCCTGGTTTGCTTATTGGGGCCAAGGGACCCCG
2151 GTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGC
2201 ACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG
2251 TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCC
2301 CTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGTCTCAGGACT
2351 CTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCAGCAGCTTGGGCACCC
2401 AGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC
2451 AAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTG
2501 CCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCCAA
2551 AACCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTG
2601 GTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGT
2651 GGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT
2701 ACAACAGCACGTACCGTGTGGTCAGCGTCTCACCCTCCTGCACCAGGAC
2751 TGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCC
2801 AGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAAC
```

10/35

Figure 9b

2851 CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAG
2901 GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGT
2951 GGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTC
3001 CCGTGCTGGACTCCGACGGCTCCTTCTTCTCTATAGCAAGCTCACCGTG
3051 GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
3101 CGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCCG
3151 GGAAATGAAAGCCGAATTCGCCCCCTCTCCCTCCCCCCCCCCTAACGTTAC
3201 TGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTAT
3251 TTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGC
3301 CCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCTCTCGCCAAAGG
3351 AATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTT
3401 CTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCC
3451 CCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATAC
3501 ACCTGCAAAGGCGGCACAACCCCAAGTGCCACGTTGTGAGTTGGATAGTTG
3551 TGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAG
3601 GATGCCCAGAAGGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTG
3651 CACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCC
3701 CGAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATGATAATATGGC
3751 CTCCTTTGTCTCTCTGCTCCTGGTAGGCATCCTATTCCATGCCACCCAGG
3801 CCGACATCCAGCTGACCCAGAGCCCAAGCAGCCTGAGCGCCAGCGTGGGT
3851 GACAGAGTGACCATCACCTGTAAGGCCAGTCAGGATGTGGGTACTTCTGT
3901 AGCCTGGTACCAGCAGAAGCCAGGTAAGGCTCCAAAGCTGCTGATCTACT
3951 GGACATCCACCCGGCACAACCTGGTGTGCCAAGCAGATTTCAGCGGTAGCGGT
4001 AGCGGTACCGACTTCACCTTCACCATCAGCAGCCTCCAGCCAGAGGACAT
4051 CGCCACCTACTACTGCCAGCAATATAGCCTCTATCGGTCTGTCGGCCAAG
4101 GGACCAAGGTGGAAATCAAACGAACTGTGGCTGCACCATCTGTCTTCATC
4151 TTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTCTGTTGTGTG
4201 CCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGG
4251 ATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGAC
4301 AGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGC
4351 AGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC
4401 TGAGCTCGCCCGTCAAAAGAGCTTCAACAGGGGAGAGTGTTAGAGATCC
4451 CCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATAATCAACCTCTGGA
4501 TTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACCTATGTTGCTCCTT
4551 TTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCT
4601 TCCCGTATGGCTTTCATTTTCTCCTCCTTGATAAATCCTGGTTGCTGTC
4651 TCTTTATGAGGAGTTGTGGCCCGTTGTGTCAGGCAACGTGGCGTGGTGTGCA
4701 CTGTGTTTGTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGT
4751 CAGCTCCTTTCCGGGACTTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGA
4801 ACTCATCGCCGCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGG
4851 GCACTGACAATTCCGTGGTGTGTGCGGGGAAATCATCGTCTTTTCTTGG
4901 CTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCTTCTGCTA
4951 CGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGC
5001 CGGCTCTGCGGCCTCTTCCGCGTCTTCGCTTTCGCCCTCAGACGAGTCGG
5051 ATCTCCCTTTGGGCCGCTCCCCGCTGATCGATACCGTCAACATCGATA
5101 AAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCC
5151 CACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCAT
5201 GGAAAAATACATAACTGAGAATAGAGAAGTTCAGATCAAGGTCAGGAACA
5251 GATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTC
5301 CTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAA
5351 ACAGGATATCTGTGGTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAAC
5401 GATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCA
5451 GATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTG
5501 AACTAACCAATCAGTTCGCTTCTCGCTTCTGTTGCGCGCTTCTGCTCCC
5551 CGAGCTCAATAAAAGAGCCCAACCCCTCACTCGGGGCGCCAGTCCTCC
5601 GATTGACTGAGTCGCCCCGGTACCCGTGTATCCAATAAACCCCTCTTGCA
5651 TTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGT
5701 GATTGACTACCCGTCAGCGGGGGTCTTTCATT

1 - 1457 Mouse mammary tumor virus LTR

1475 - 1726 Double mutated PPE sequence

Figure 9c

1752 - 1754	MN14 heavy chain signal peptide start codon
3156 - 3158	MN14 heavy chain stop codon
3170 - 3745	EMCV IRES
3746 - 3748	Bovine alpha-lactalbumin signal peptide start codon
3803 - 3805	First codon of mature MN14 light chain gene
4442 - 4444	MN14 antibody light chain gene stop codon
4487 - 5078	WPRES sequence
5133 - 5372	MoMuLV 3' LTR

12/35

Figure 10a
SEQ ID NO:7

Alpha-Lactalbumin MN14 Vector

```
1 AAAGACCCACCCGTAGGTGGCAAGCTAGCTTAAGTAACGCCACTTTGCA
51 AGGCATGGAAAAATACATAACTGAGAATAGAAAAGTTCAGATCAAGGTCA
101 GGAACAAAGAAACAGCTGAATACCAAACAGGATATCTGTGGTAAGCGGTT
151 CCTGCCCCGGCTCAGGGCCAAGAACAGATGAGACAGCTGAGTGATGGGCC
201 AAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCGGGGCCAAGAA
251 CAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGTGAATCAT
301 CAGATGTTTCCAGGGTGCCCCAAGGACCTGAAAATGACCCTGTACCTTAT
351 TTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCCGCT
401 CTCCGAGCTCAATAAAAGAGCCCAACCCCTCACTCGGCGCGCCAGTCT
451 TCCGATAGACTGCGTCGCCCCGGGTACCCGTATTCCCAATAAAGCCTCTTG
501 CTGTTTGCATCCGAATCGTGGTCTCGCTGTTCCCTTGGGAGGGTCTCCTCT
551 GAGTGATTGACTACCCACGACGGGGGTCTTTCATTTGGGGGCTCGTCCGG
601 GATTTGGAGACCCCTGCCCAGGGACCACCGACCCACCACCGGGAGGTAAAG
651 CTGGCCAGCAACTTATCTGTGTCTGTCCGATTGTCTAGTGTCTATGTTTG
701 ATGTTATGCGCCTGCGTCTGTACTAGTTAGCTAACTAGCTCTGTATCTGG
751 CGGACCCGTGGTGGAAGTACGAGTTCTGAACACCCGGCCGCAACCCTGG
801 GAGACGTCCCAGGGACTTTGGGGGCGGTTTTTGTGGCCCGACCTGAGGAA
851 GGGAGTCGATGTGGAATCCGACCCCGTCAGGATATGTGGTTCTGGTAGGA
901 GACGAGAACCTAAAACAGTTCCCGCGCTCCGTCTGAATTTTTGCTTTCGGT
951 TTGGAACCGAAGCCGCGCGTCTTGTCTGCTGCAGCGCTGCAGCATCGTTC
1001 TGTGTTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATTAGGGC
1051 CAGACTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGATGT
1101 CGAGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAGAGACGTTGGG
1151 TTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCCGATGGCCGCGA
1201 GACGGCACCTTTAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTCTT
1251 TTCACCTGGCCCGCATGGACACCCAGACCAGGTCCCCTACATCGTGACCT
1301 GGGAAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTTTGTACAC
1351 CCTAAGCCTCCGCGCTCCTCTTCCCTCCATCCGCCCCGTCTCTCCCCCTTGA
1401 ACCTCCTCGTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCCTCACTC
1451 CTTCTCTAGGCGCCGGAATTCGATCTGATCAAGAGACAGGATGAGGATC
1501 GTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTT
1551 GGGTGGAGAGGCTATTCCGGCTATGACTGGGCACAACAGACAATCGGCTGC
1601 TCTGATGCCGCCGTGTTCCGGCTGTGACGCGAGGGGCGCCCGGTTCTTTT
1651 TGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAG
1701 CGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTC
1751 GACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCC
1801 GGGGCAGGATCTCCTGTCTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCA
1851 TCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGC
1901 CCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTAATCGGAT
1951 GGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGC
2001 TCGCGCCAGCCGAAGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGC
2051 GAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGT
2101 GGAAAATGGCCGCTTTTCTGGATTTCATCGACTGTGGCCGGCTGGGTGTGG
2151 CGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAG
2201 CTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGC
2251 TCCCGATTTCGAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCT
2301 GAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCCAACCTGC
2351 CATCACGAGATTTTCGATTCACCGCCGCTTCTATGAAAGGTGGGCTTC
2401 GGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCT
2451 CATGCTGGAGTTCTTCGCCACCCCGGGCTCGATCCCCTCGCGAGTTGGT
2501 TCAGCTGCTGCCTGAGGCTGGACGACCTCGCGGAGTTCTACCGGCAGTGC
2551 AAATCCGTCGGCATCCAGGAAACCAGCAGCGGCTATCCGCGCATCCATGC
2601 CCCCGAAGTGCAGGAGTGGGGAGGCACGATGGCCGCTTTGGTCGAGGCGG
2651 ATCCTAGAAGTAGCGAAAATGCAAGAGCAAAGACGAAAACATGCCACACA
2701 TGAGGAATACCGATTCTCTCATTAAACATATTCAGGCCAGTTATCTGGGCT
2751 TAAAAGCAGAAGTCCAACCCAGATAACGATCATATACATGGTTCTCTCCA
2801 GAGGTTCACTACTGAACACTCGTCCGAGAATAACGAGTGGATCAGTCCTG
```

Figure 10b

2851 GGTGGTCATTGAAAGGACTGATGCTGAAGTTGAAGCTCCAATACTTTGGC
2901 CACCTGATGCGAAGAAGTACTCATGTGATAAGACCCTGATACTGGGAAA
2951 GATTGAAGGCAGGAGGAGAAGGGATGACAGAGGATGGAAGAGTTGGATGG
3001 AATCACCAACTCGATGGACATGAGTTTGAGCAAGCTTCCAGGAGTTGGTA
3051 ATGGGCAGGGAAGCCTGGCGTGCTGCAGTCCATGGGGTTGCAAAGAGTTG
3101 GACACTACTGAGTGACTGAACTGAACTGATAGTGTAATCCATGGTACAGA
3151 ATATAGGATAAAAAAGAGGAAGAGTTTGCCCTGATTCTGAAGAGTTGTAG
3201 GATATAAAAGTTTAGAATACCTTTAGTTTGGAAGTCTTAAATTATTTACT
3251 TAGGATGGGTACCCACTGCAATATAAGAAATCAGGCTTTAGAGACTGATG
3301 TAGAGAGAATGAGCCCTGGCATAACCAGAAGCTAACAGCTATTGGTTATAG
3351 CTGTTATAACCAATATATAACCAATATATTGGTTATATAGCATGAAGCTT
3401 GATGCCAGCAATTTGAAGGAACCATTTAGAAGTAGTATCCTAAACTCTAC
3451 ATGTTCCAGGACACTGATCTTAAAGCTCAGGTTTCAAGATCTTGTTTTATA
3501 GGCTCTAGGTGTATATTGTGGGGCTTCCCTGGTGGCTCAGATGGTAAAGT
3551 GTCTGCCTGCAATGTGGGTGATCTGGGTTTCGATCCCTGGCTTGGGAAGAT
3601 CCCCTGGAGAAGGAAATGGCAACCCACTCTAGTACTCTTACCTGGAAAAT
3651 TCCATGGACAGAGGAGCCTTGTAAAGCTACAGTCCATGGGATTGCAAAGAG
3701 TTGAACACAAGTGAAGGTTCAATGCAGGGTCTCCTGCATTGCAGAAAG
3751 AGGTGAAGTGAAGTGAAGGTTCAATGCAGGGTCTCCTGCATTGCAGAAAG
3801 ATTCTTTACCATCTGAGCCACCAGGGAAGCCCAAGAATACTGGAGTGGGT
3851 AGCCTATTCTTCTCCAGGGGATCTTCCCATCCCAGGAATTGAACTGGAG
3901 TCTCCTGCATTTTCAAGGTGATTCTTACCAGCTGAACTACCAGGTGGATA
3951 CTACTCCAATATTAAAGTGCTTAAAGTCCAGTTTTCCACCTTTCCCAA
4001 AAGGTTGGGTCACTCTTTTTTAACCTTCTGTGGCCTACTCTGAGGCTGTC
4051 TACAAGCTTATATATTTATGAACACATTTATTGCAAGTTGTTAGTTTTAG
4101 ATTTACAATGTGGTATCTGGCTATTTAGTGGTATTGGTGGTTGGGGATGG
4151 GGAGGCTGATAGCATCTCAGAGGGCAGCTAGATACTGTCATACACACTTT
4201 TCAAGTTCTCCATTTTTGTGAAATAGAAAGTCTCTGGATCTAAGTTATAT
4251 GTGATTCTCAGTCTCTGTGGTCAATTTCTATTCTACTCCTGACCACTCAA
4301 CAAGGAACCAAGATATCAAGGGACACTTGTGTTTGTTCATGCCTGGGTTG
4351 AGTGGGCCATGACATATGTTCTGGGCCTTGTACATGGCTGGATTGGTTG
4401 GACAAGTGCCAGCTCTGATCCTGGGACTGTGGCATGTGATGACATACACC
4451 CCCTCTCCACATTTCTGCATGTCTCTAGGGGGGAAGGGGAAGCTCGGTAT
4501 AGAACCTTTATTGTATTTTCTGATTGCCTCACTTCTTATATTGCCCCCAT
4551 GCCCTTCTTTGTTCTCAAGTAACCAGAGACAGTGCTTCCAGAACCAAC
4601 CCTACAAGAAACAAAGGGCTAAACAAAGCCAAATGGGAAGCAGGATCATG
4651 GTTTGAACTCTTTCTGGCCAGAGAACAAATACCTGCTATGGACTAGATACT
4701 GGGAGAGGGAAAGGAAAAGTAGGGTGAATTATGGAAGGAAGCTGGCAGGC
4751 TCAGCGTTTCTGTCTTGGCATGACCAGTCTCTCTTCATTCTCTTCTCTAGA
4801 TGTAGGGCTTGGTACCAGAGCCCCTGAGGCTTTCTGCATGAATATAAATA
4851 TATGAAACTGAGTGATGCTTCCATTTCAAGGTTCTTGGGGGCGCCGAATTC
4901 GAGCTCGGTACCCGGGGATCTCGACGGATCCGATTACTTACTGGCAGGTG
4951 CTGGGGGCTTCCGAGACAATCGCGAACATCTACACCACACAACACCGCCT
5001 CGACCAGGGTGAGATATCGGCCGGGGACGCGGCGGTGGTAATTACAAGCG
5051 AGATCCGATTACTTACTGGCAGGTGCTGGGGGCTTCCGAGACAATCGCGA
5101 ACATCTACACCACACAACACCGCCTCGACCAGGGTGAGATATCGGCCGGG
5151 GACGCGGCGGTGGTAATTACAAGCGAGATCCCCGGGAATTCAGGACCTCA
5201 CCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGT
5251 GTCCACTCCGAGGTCCAAGTGGTGGAGAGCGGTGGAGGTGTTGTGCAACC
5301 TGGCCGGTCCCTGCGCCTGTCTGCTCCGCATCTGGCTTCGATTTACCA
5351 CATATTGGATGAGTTGGGTGAGACAGGCACCTGGAAAAGGTCTTGAGTGG
5401 ATTGGAGAAATTCATCCAGATAGCAGTACGATTAACTATGCGCCGTCTCT
5451 AAAGGATAGATTTACAATATCGCGAGACAACGCCAAGAACACATTGTTCC
5501 TGCAAATGGACAGCCTGAGACCCGAAGACACCGGGGTCTATTTTTGTGCA
5551 AGCCTTTACTTCGGCTTCCCCTGGTTTGCTTATTGGGGCCAAGGGACCCC
5601 GGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGG
5651 CACCCTCCTCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG
5701 GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGGCGC
5751 CCTGACCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCTCAGGAC
5801 TCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACC
5851 CAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA
5901 CAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCCACCGT

14/35

Figure 10c

5951 GCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCA
6001 AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGT
6051 GGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACG
6101 TGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
6151 TACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGA
6201 CTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCC
6251 CAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA
6301 CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCA
6351 GGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCG
6401 TGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCT
6451 CCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTATAGCAAGCTCACCGT
6501 GGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC
6551 ACGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCC
6601 GGGAAATGAAAGCCGAATTCGCCCCCTCTCCCTCCCCCCCCCCTAACGTTA
6651 CTGGCCGAAGCCGCTTGGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTA
6701 TTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGG
6751 CCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCTCTCGCCAAAG
6801 GAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCT
6851 TCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCC
6901 CCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATA
6951 CACCTGCAAAGGCGGCACAACCCCAAGTGCACAGTTGTGAGTTGGATAGTT
7001 GTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAA
7051 GGATGCCCAGAAGGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGT
7101 GCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCC
7151 CCGAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATGATAATATGG
7201 CCTCCTTTGTCTCTCTGCTCCTGGTAGGCATCCTATTCCATGCCACCCAG
7251 GCCGACATCCAGCTGACCCAGAGCCCAAGCAGCCTGAGCGCCAGCGTGGG
7301 TGACAGAGTGACCATCACCTGTAAGGCCAGTCAGGATGTGGGTACTTCTG
7351 TAGCCTGGTACCAGCAGAAGCCAGGTAAGGCTCCAAAGCTGCTGATCTAC
7401 TGGACATCCACCCGGCACACTGGTGTGCCAAGCAGATTGAGCGGTAGCGG
7451 TAGCGGTACCGACTTCACCTTCACCATCAGCAGCCTCCAGCCAGAGGACA
7501 TCGCCACCTACTACTGCCAGCAATATAGCCTCTATCGGTGCTTCGGCCAA
7551 GGGACCAAGGTGGAAATCAAACGAACTGTGGCTGCACCATCTGTCTTCAT
7601 CTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTCTGTTGTGT
7651 GCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTG
7701 GATAACGCCCTCCAATCGGGTAACCTCCAGGAGAGTGTACAGAGCAGGA
7751 CAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAG
7801 CAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGC
7851 CTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAGAGATC
7901 CCCC GGCTGCAGGAATTGATATCAAGCTTATCGATAATCAACCTCTGG
7951 ATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCT
8001 TTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGC
8051 TTCCCGTATGGCTTTCATTTTCTCCTCCTTGATATAAATCCTGGTTGCTGT
8101 CTCTTTATGAGGAGTTGTGGCCCGTTGTGAGGCAACGTGGCGTGGTGTGC
8151 ACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTG
8201 TCAGCTCCTTTCCGGGACTTTGCTTTCCCCCTCCCTATTGCCACGGCGG
8251 AACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTG
8301 GGCATGACAATTCCGTGGTGTGTGCGGGGAAATCATCGTCCTTTCCTTG
8351 GCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCTTCTGCT
8401 ACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTG
8451 CCGGCTCTGCGGCCTCTTCCGCGTCTTCGCTTCCGCTCAGACGAGTCG
8501 GATCTCCCTTTGGGCGCCTCCCCGCTGATCGATACCGTCAACATCGAT
8551 AAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACC
8601 CCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCA
8651 TGGAAAAATACATAACTGAGAATAGAGAAGTTCAGATCAAGGTCAGGAAC
8701 AGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTT
8751 CCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCA
8801 AACAGGATATCTGTGGTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAAC
8851 AGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATC
8901 AGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTT
8951 GAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTGCGCGCTTCTGCTCC
9001 CCGAGCTCAATAAAAGAGCCCAACCCCTCACTCGGGGCGCCAGTCCTC

Figure 10d

9051 CGATTGACTGAGTCGCCCCGGGTACCCGTGTATCCAATAAACCTCTTGCA
9101 GTTGCATCCGACTTGTGGTCTCGCTGTTTCCTTGGGAGGGTCTCCTCTGAG
9151 TGATTGACTACCCGTCAGCGGGGGTCTTTCATT

1 - 658	MoMuSV 5' LTR
659 - 1468	Extended packaging region
1512 - 2306	Neomycin resistance gene
2661 - 4896	Bovine/human alpha-lactalbumin 5' flanking region
5084 - 5327	Double mutated PPE sequence
6207 - 6209	MN14 antibody heavy chain gene signal peptide start codon
6611-6613	MN14 antibody heavy chain stop codon
6625 - 7200	EMCV IRES
7201 - 7203	Bovine alpha-lactalbumin signal peptide start codon
7258 - 7260	First codon of mature MN14 antibody light chain gene
7897 - 7899	MN14 antibody light gene stop codon
7938 - 8529	WPRE sequence
8600 - 9138	Moloney murine leukemia virus 3' LTR

16/35

Figure 11a
SEQ ID NO:8
Alpha-Lactalbumin Bot Vector

```
1   GATCAGTCCTGGGTGGTCATTGAAAGGACTGATGCTGAAGTTGAAGCTCC
51  AATACTTTGGCCACCTGATGCGAAGAACTGACTCATGTGATAAGACCCTG
101 ATACTGGGAAAGATTGAAGGCAGGAGGAGAAGGGATGACAGAGGATGGAA
151 GAGTTGGATGGAATCACCAACTCGATGGACATGAGTTTGAGCAAGCTTCC
201 AGGAGTTGGTAATGGGCAGGGAAGCCTGGCGTGCTGCAGTCCATGGGGTT
251 GCAAAGAGTTGGACACTACTGAGTGACTGAACTGAACTGATAGTGTAATC
301 CATGGTACAGAATATAGGATAAAAAAGAGGAAGAGTTTGCCCTGATTCTG
351 AAGAGTTGTAGGATATAAAAGTTTAGAATACCTTTAGTTTGGAAGTCTTA
401 AATTATTTACTTAGGATGGGTACCCACTGCAATATAAGAAATCAGGCTTT
451 AGAGACTGATGTAGAGAGAATGAGCCCTGGCATAACCAGAAGCTAACAGCT
501 ATTGGTTATAGCTGTTATAACCAATATATAACCAATATATTGGTTATATA
551 GCATGAAGCTTGATGCCAGCAATTTGAAGGAACCATTTAGAACTAGTATC
601 CTAAACTCTACATGTTCCAGGACACTGATCTTAAAGCTCAGGTTTCAGAAAT
651 CTTGTTTTATAGGCTCTAGGTGTATATTGTGGGGCTTCCCTGGTGGCTCA
701 GATGGTAAAGTGTCTGCCTGCAATGTGGGTGATCTGGGTTTCGATCCCTGG
751 CTTGGGAAGATCCCCTGGAGAAGGAAATGGCAACCCACTCTAGTACTCTT
801 ACCTGGAAAATTCATGGACAGAGGAGCCTTGTAAGCTACAGTCCATGGG
851 ATTGCAAAGAGTTGAACACAACCTGAGCAACTAAGCACAGCACAGTACAGT
901 ATACACCTGTGAGGTGAAGTGAAGTGAAGGTTCAATGCAGGGTCTCCTGC
951 ATTGCAGAAAGATTCTTTACCATCTGAGCCACCAGGGAAGCCCAAGAATA
1001 CTGGAGTGGGTAGCCTATTCTTCTCCAGGGGATCTTCCCATCCCAGGAA
1051 TTGAACTGGAGTCTCCTGCATTTTCAGGTGGATTCTTCACCAGCTGAACTA
1101 CCAGGTGGATACTACTCCAATATTAAAGTGCTTAAAGTCCAGTTTTCCCA
1151 CCTTCCCAAAAAGGTTGGGTCACCTTTTTTTAACTTCTGTGGCCTACT
1201 CTGAGGCTGTCTACAAGCTTATATATTTATGAACACATTTATTGCAAGTT
1251 GTTAGTTTTAGATTTACAATGTGGTATCTGGCTATTTAGTGGTATTGGTG
1301 GTTGGGGATGGGGAGGCTGATAGCATCTCAGAGGGCAGCTAGATACTGTC
1351 ATACACACTTTTCAAGTTCTCCATTTTTGTGAAATAGAAAGTCTCTGGAT
1401 CTAAGTTATATGTGATTCTCAGTCTCTGTGGTCATATTCTATTCTACTCC
1451 TGACCACTCAACAAGGAACCAAGATATCAAGGGACACTTGTTTTGTTTCA
1501 TGCCTGGGTTGAGTGGGCCATGACATATGTTCTGGGCCTTGTTACATGGC
1551 TGGATTGGTTGGACAAGTGCCAGCTCTGATCCTGGGACTGTGGCATGTGA
1601 TGACATACACCCCCTCTCCACATTCTGCATGTCTCTAGGGGGGAAGGGGG
1651 AAGCTCGGTATAGAACCTTTATTGTATTTTCTGATTGCCTCACTTCTTAT
1701 ATTGCCCCCATGCCCTTCTTTGTTCCCTCAAGTAACCAGAGACAGTGCTTC
1751 CCAGAACCAACCCTACAAGAAACAAAGGGCTAAACAAAGCCAAATGGGAA
1801 GCAGGATCATGGTTTGAACCTTTCTGGCCAGAGAACAAATACCTGCTATG
1851 GACTAGATACTGGGAGAGGGAAAGGAAAAGTAGGGTGAATTATGGAAGGA
1901 AGCTGGCAGGCTCAGCGTTTCTGTCTTGGCATGACCAGTCTCTCTTCATT
1951 CTCTTCCTAGATGTAGGGCTTGGTACCAGAGCCCCTGAGGCTTTCTGCAT
2001 GAATATAAATATATGAACTGAGTGATGCTTCCATTTTCAGGTTCTTGGGG
2051 GCGCCGAATTGAGCTCGGTACCCGGGGATCTCGACGGATCCGATTACTT
2101 ACTGGCAGGTGCTGGGGGCTTCCGAGACAATCGCGAACATCTACACCACA
2151 CAACACCGCCTCGACCAGGGTGAGATATCGGCCGGGGACGCGGCGGTGGT
2201 AATTACAAGCGAGATCCGATTACTTACTGGCAGGTGCTGGGGGCTTCCGA
2251 GACAATCGCGAACATCTACACCACACAACACCGCCTCGACCAGGGTGAGA
2301 TATCGGCCGGGGACGCGGCGGTGGTAATTACAAGCGAGATCTCGAGAAGC
2351 TTGTTGGGAATTCAGGCCATCGATCCCGCCGCCACCATGGAATGGAGCTG
2401 GGTCTTTCTCTTCTTCTGTCAGTAACTACAGGTGTCCACTCCGACATCC
2451 AGATGACCCAGTCTCCAGCCTCCCTATCTGCATCTGTGGGAGAACTGTC
2501 ACTATCACATGTGAGCAAGTGGGAATATTCACAATTATTTAGCATGGTA
2551 TCAGCAGAAACAGGGAAATCTCCTCAGCTCCTGGTCTATAATGCAAAAA
2601 CCTTAGCAGATGGTGTGCCATCAAGGTTCAAGTGGCAGTGGATCAGGAACA
2651 CAATATTCTCTCAAGATCAACAGCCTGCAGCCTGAAGATTTTGGGAGTTA
2701 TTAAGTGTCAACATTTTTTGGAGTACTCCGTGGACGTTCCGTGGAGGCACCA
2751 AGCTGGAAATCAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCA
2801 CCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTT
```

17/35

Figure 11b

2851 GAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCA
2901 GTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAA
2951 GACAGCACCTACAGCATGAGCAGCACCCCTCACATTGACCAAGGACGAGTA
3001 TGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTT
3051 CACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTGAAAGCATCGATTT
3101 CCCCTGAATTCGCCCCCTCTCCCTCCCCCCCCCTAACGTTACTGGCCGAA
3151 GCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACC
3201 ATATTGCCGTCTTTTGGCAATGTGAGGGCCCCGAAACCTGGCCCTGTCTT
3251 CTTGACGAGCATTCTAGGGGTCTTTCCCTCTCGCCAAAGGAATGCAAG
3301 GTCTGTTGAATGTCGTGAAGGAAGCAGTTCTCTGGAAGCTTCTTGAAGA
3351 CAAACAACGTCTGTAGCGACCCTTTCGAGGCAGCGGAACCCCCCACCTGG
3401 CGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAA
3451 AGGCGGCACAACCCCAAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGA
3501 GTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCA
3551 GAAGGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCT
3601 TTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCGAACCAC
3651 GGGGACGTGGTTTTCTTTGAAAAACACGATGATAATATGGCCTCCTTTG
3701 TCTCTCTGCTCCTGGTAGGCATCCTATTCCATGCCACCCAGGCCGAGGTT
3751 CAGCTTCAGCAGTCTGGGGCAGAGCTTGTGAAGCCAGGGGCCTCAGTCAA
3801 GTTGTCTTGCACAGCTTCTGGCTTCAACATTAAAGACACCTTTATGCACT
3851 GGGTGAAGCAGAGGCCTGAACAGGGCCTGGAGTGGATTGGAAGGATTGAT
3901 CCTGCGAATGGGAATACTGAATATGACCCGAAGTTCCAGGGCAAGGCCAC
3951 TATAACAGCAGACACATCCTCCAACACAGTCAACCTGCAGCTCAGCAGCC
4001 TGACATCTGAGGACACTGCCGTCTATTACTGTGCTAGTGGAGGGGAACTG
4051 GGGTTTCCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCCAA
4101 AACGACACCCCCATCTGTCTATCCACTGGCCCCCTGGATCTGCTGCCCAA
4151 CTAACCTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAG
4201 CCAGTGACAGTGACCTGGAACCTCTGGATCCCTGTCCAGCGGTGTGCACAC
4251 CTTCCAGCTGTCCTGCAGTTTGACCTCTACACTCTGAGCAGCTCAGTGA
4301 CTGTCCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCC
4351 CACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTG
4401 TACTAGTGGAGGTGGAGGTAGCCACCATCACCATCACCATTAATCTAGAG
4451 TTAAGCGGCCGTCGAGATCTCGACATCGATAATCAACCTCTGGATTACAA
4501 AATTTGTGAAAGATTGACTGGTATTCTTAACCTATGTTGCTCCTTTTACGC
4551 TATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGT
4601 ATGGCTTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTA
4651 TGAGGAGTTGTGGCCCGTTGTGAGGCAACGTGGCGTGGTGTGCACTGTGT
4701 TTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTC
4751 CTTTCCGGGACTTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACCTCAT
4801 CGCCGCTGCTTGGCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTG
4851 ACAATTCCGTGGTGTGTCGGGGAAATCATCGTCCTTTCCTTGGCTGCTC
4901 GCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCCTTCTGCTACGTCCC
4951 TTCGGCCCTCAATCCAGCGGACCTTCCCTTCCCGCGGCTGCTGCCGGCTC
5001 TGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCC
5051 CTTTGGGCGCCTCCCCGCTGATCGATAAAATAAAAGATTTTATTTAGT
5101 CTCCAGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGCAAGCT
5151 AGCTTAAGTAACGCCATTTTGAAGGCATGGAAAAATACATAACTGAGAA
5201 TAGAGAAGTTCAAGGTCAGGAACAGATGGAACAGCTGAATATGGG
5251 CCAAACAGGATATCTGTGGTAAGCAGTTCTTCCCCGGCTCAGGGCCAAG
5301 AACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCA
5351 GTTCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCC
5401 AGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAA
5451 GGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTT
5501 CTCGCTTCTGTTGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCA
5551 CAACCCCTCACTCGGGGCGCCAGTCTCCGATTGACTGAGTCGCCCCGGGT
5601 ACCCGTGTATCCAATAAACCCCTCTTGCAGTTGCATCCGACTTGTGGTCTC

Figure 11c

5651 GCTGTTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGG
5701 GGTCTTTCATT

1 - 2053	Bovine/human alpha-lactalbumin 5' flanking region
2093 - 2336	Double mtated PPE sequence
2387 - 2443	cc49 signal peptide coding region
2444 - 3088	Bot antibody light chain Fab coding region
3112 - 3686	EMCV IRES
3687 - 3745	Bovine alpha-lactalbumin signal peptide coding region
3746 - 4443	Bot antibody heavy chain Fab coding region
4481 - 5072	WPRE sequence
5118 - 5711	Moloney murine leukemia virus 3' LTR

19/35

Figure 12a
SEQ ID NO:9
LSNRL Vector

```
1   TTTGAAAGACCCACCCGCTAGGTGGCAAGCTAGCTTAAGTAACGCCACTT
51  TGCAAGGCATGGAAAAATACATAACTGAGAATAGAAAAGTTCAGATCAAG
101 GTCAGGAACAAAGAAACAGCTGAATACCAAACAGGATATCTGTGGTAAGC
151 GGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGAGACAGCTGAGTGATG
201 GGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCGGGGCCA
251 AGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGTGAA
301 TCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAAATGACCCTGTACC
351 TTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTC
401 CGCTCTCCGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGCGCGCCA
451 GTCTTCCGATAGACTGCGTCGCGGGGTACCCGTATTCCCAATAAAGCCT
501 CTTGCTGTTTGCATCCGAATCGTGGTCTCGCTGTTCCCTGGGAGGGTCTC
551 CTCTGAGTGATTGACTACCCACGACGGGGGTCTTTCATTTGGGGGCTCGT
601 CCGGGATTTGGAGACCCCTGCCAGGGACCACCGACCCACCACCGGGAGG
651 TAAGCTGGCCAGCAACTTATCTGTGTCTGTCCGATTGTCTAGTGTCTATG
701 TTTGATGTTATGCGCCTGCGTCTGTACTAGTTAGCTAACTAGCTCTGTAT
751 CTGGCGGACCCGTGGTGGAACTGACGAGTTCTGAACACCCGGCCGCAACC
801 CTGGGAGACGTCCAGGGACTTTGGGGGCCGTTTTTGTGGCCCGACCTGA
851 GGAAGGGAGTCGATGTGGAATCCGACCCCGTCAGGATATGTGGTTCTGGT
901 AGGAGACGAGAACCTAAACAGTTCCCGCCTCCGTCTGAATTTTTGCTTT
951 CGGTTTGAACCGAAGCCGCGCGTCTTGTCTGCTGCAGCCAAGCTTGGGC
1001 TGCAGGTTCGAGGACTGGGGACCCTGCACCGAACATGGAGAACACAACATC
1051 AGGATTCCTAGGACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGA
1101 CAAGAATCCTCACAATACCACAGAGTCTAGACTCGTGGTGGACTTCTCTC
1151 AATTTTCTAGGGGGAGCACCCACGTGTCCTGGCCAAAATTCGCAGTCCCC
1201 AACCTCCAATCACTCACCAACCTCTTGTCTCTCCAATTTGTCTTGCTATC
1251 GCTGGATGTGTCTGCGGCGTTTTATCATATTCTCTTCATCCTGCTGCTA
1301 TGCCTCATCTTCTTGTGTTGGTTCTTCTGGACTACCAAGGTATGTTGCCCGT
1351 TTGTCTCTACTTCCAGGAACATCAACTACCAGCACGGGACCATGCAAGA
1401 CCTGCACGATTCCTGCTCAAGGAACCTCTATGTTTCCCTCTTGTTGCTGT
1451 ACAAACCTTCGGACGGAACTGCACTTGTATTCCCATCCCATCATCCTG
1501 GGCTTTCGCAAGATTCCATATGGGAGTGGGCCTCAGTCCGTTTCTCCTGGC
1551 TCAGTTTACTAGTGCCATTTGTTCAAGTGGTTCGTAGGGCTTTCCCCCACT
1601 GTTTGGCTTTTCAAGTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTA
1651 CAACATCTTGAGTCCCTTTTTTACCTCTATTACCAATTTTCTTTTGTCTTT
1701 GGGTATACATTTAAACCCTAATAAAACCAAACGTTGGGGCTACTCCCTTA
1751 ACTTCATGGGATATGTAATTGGATGTTGGGGTACTTTACCGCAAGAACAT
1801 ATTGTAATAAAATCAAGCAATGTTTTCGAAACTGCCTGTAAATAGACC
1851 TATTGATTGGAAAGTATGTCAGAGACTTGTGGGTCTTTTGGGCTTTGCTG
1901 CCCCTTTTACACAATGTGGCTATCCTGCCTTAATGCCTTTATATGCATGT
1951 ATACAATCTAAGCAGGCTTTCACCTTCTCGCCAACTTACAAGGCCTTTCT
2001 GTGTAAACAATATCTGAACCTTTACCCCGTTGCCCGGCAACGGTCAGGTC
2051 TCTGCCAAGTGTTTGCTGACGCAACCCCCACTGGATGGGGCTTGGCTATC
2101 GGCCATAGCCGCATGCGCGGACCTTTGTGGCTCCTCTGCCGATCCATACT
2151 GCGGAACTCCTAGCAGCTTGTTTTGTCTCGCAGGCGGTCTGGAGCGAACT
2201 TATCGGCACCGACAACCTCTGTTGTCCTCTCTCGGAAATACACCTCCTTTC
2251 CATGGCTGCTAGGGTGTGCTGCCAACTGGATCCCCTCAGGATATAGTAGT
2301 TTCGCTTTTGCATAGGGAGGGGGAAATGTAGTCTTATGCAATACACTTGT
2351 AGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGAGAG
2401 AAAAAGCACCGTGCATGCCGATTGGTGGAAAGTAAGGTGGTACGATCGTGC
2451 CTTATTAGGAAGGCAACAGACAGGTCTGACATGGATTGGACGAACCACTG
2501 AATTCCGCATTGCAGAGATAATTGTATTTAAGTGCCTAGCTCGATACAGC
2551 AAACGCCATTTTTGACCATTACCAACATTGGTGTGCACCTTCCAAAGCTT
2601 CACGCTGCCGCAAGCACTCAGGGCGCAAGGGCTGCTAAAGGAAGCGGAAC
2651 ACGTAGAAAGCCAGTCCGCAGAAACGGTGCTGACCCCGGATGAATGTCAG
2701 CTACTGGGCTATCTGGACAAGGGAAAACGCAAGCGCAAAGAGAAAGCAGG
2751 TAGCTTGCAAGTGGGCTTACATGGCGATAGCTAGACTGGGCGGTTTTATGG
2801 ACAGCAAGCGAACCAGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGG
```

20/35

Figure 12b

2851 GAAGCCCTGCAAAGTAAACTGGATGGCTTTCTTGCCGCCAAGGATCTGAT
 2901 GGCGCAGGGGATCAAGATCTGATCAAGAGACAGGATGAGGATCGTTTCGC
 2951 ATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGA
 3001 GAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATG
 3051 CCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAG
 3101 ACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCT
 3151 ATCGTGGCTGGCCACGACGGGCGTTCTTGCGCAGCTGTGCTCGACGTTG
 3201 TCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAG
 3251 GATCTCCTGTCTATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGC
 3301 TGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCTG
 3351 ACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCC
 3401 GGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCC
 3451 AGCCGAACGTGTTCCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATC
 3501 TCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAAT
 3551 GGCCGCTTTTCTGGATTCTATCGACTGTGGCCGGCTGGGTGTGGCGGACCG
 3601 CTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCG
 3651 GCGAATGGGCTGACCGCTTCTCGTGCTTTACGGTATCGCCGCTCCCGAT
 3701 TCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGG
 3751 ACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACG
 3801 AGATTTTCGATTCCACCGCCGCTTCTATGAAAGGTTGGGCTTCGGAATCG
 3851 TTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTG
 3901 GAGTCTTCGCCCACCCCAACCCTGGCCCTATTATTGGGTGGACTAACCA
 3951 TGGGGGGAATTGCCGCTGGAATAGGAACAGGGACTACTGCTCTAATGGCC
 4001 ACTCAGCAATTCCAGCAGCTCCAAGCCGAGTACAGGATGATCTCAGGGA
 4051 GGTGAAAAATCAATCTCTAACCTAGAAAAGTCTCTCACTTCCCTGTCTG
 4101 AAGTTGTCTACAGAATCGAAGGGGCCTAGACTTGTTATTTCTAAAAGAA
 4151 GGAGGGCTGTGTGCTGCTCTAAAAGAAGAATGTTGCTTCTATGCGGACCA
 4201 CACAGGACTAGTGAGAGACAGCATGGCCAAATTGAGAGAGAGGCTTAATC
 4251 AGAGACAGAACTGTTTGAGTCAACTCAAGGATGGTTTGAGGGACTGTTT
 4301 AACAGATCCCCTTGGTTTACCACCTTGATATCTACCATTATGGGACCCCT
 4351 CATTGTACTCCTAATGATTTTGCTCTTCGGACCCTGCATTCTTAATCGAT
 4401 TAGTCCAATTTGTTAAAGACAGGATATCAGTGGTCCAGGCTCTAGTTTTG
 4451 ACTCAACAATATCACCAGCTGAAGCCTATAGAGTACGAGCCATAGATAAA
 4501 ATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCCA
 4551 CCTGTAGGTTTGGCAAGCTAGCTTAAAGTAACGCCATTTTGCAAGGCATGG
 4601 AAAAATACATAACTGAGAATAGAGAAGTTTCAAGATCAAGGTCAGGAACAGA
 4651 TGGAACAGCTGAATATGGGGCAAACAGGATATCTGTGGTAAGCAGTTCCT
 4701 GCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGGCAAAC
 4751 AGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGA
 4801 TGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGA
 4851 TGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAA
 4901 CTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCG
 4951 AGCTCAATAAAAGAGCCCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGA
 5001 TTGACTGAGTCGCCCCGGGTACCCGTGTATCCAATAAACCCCTCTTGCAATT
 5051 GCATCCGACTTGTGGTCTCGCTGTTCTTGGGAGGGTCTCCTCTGAGTGA
 5101 TTGACTACCCGTCAGCGGGGGTCTTTCATT

1 - 589	MoMuSV 5' LTR
659 - 897	Retroviral packaging region
1034 - 1714	Hepatitis B surface antigen
2279 - 2595	RSV promoter
2951 - 3745	Neomycin phosphotransferase gene
4537 - 5130	MoMuLV 3' LTR

21/35

Figure 13a
SEQ ID NO:10
Alpha-Lactalbumin cc49IL2 Vector

```
1   GATCAGTCCTGGGTGGTCATTGAAAGGACTGATGCTGAAGTTGAAGCTCC
51  AATACTTTGGCCACCTGATGCGAAGAACTGACTCATGTGATAAGACCCTG
101 ATACTGGGAAAGATTGAAGGCAGGAGGAGAAGGGATGACAGAGGATGGAA
151 GAGTTGGATGGAATCACCAACTCGATGGACATGAGTTTGAGCAAGCTTCC
201 AGGAGTTGGTAATGGGCAGGGAAGCCTGGCGTGCTGCAGTCCATGGGGTT
251 GCAAAGAGTTGGACACTACTGAGTGACTGAACTGAACTGATAGTGTAATC
301 CATGGTACAGAATATAGGATAAAAAAGAGGAAGAGTTTGCCCTGATTCTG
351 AAGAGTTGTAGGATATAAAAGTTTAGAATACCTTTAGTTTGGAAGTCTTA
401 AATTATTTACTTAGGATGGGTACCCACTGCAATATAAGAAATCAGGCTTT
451 AGAGACTGATGTAGAGAGAATGAGCCCTGGCATAACCAGAAGCTAACAGCT
501 ATTGGTTATAGCTGTTATAACCAATATATAACCAATATATTGGTTATATA
551 GCATGAAGCTTGATGCCAGCAATTTGAAGGAACCATTTAGAAGTAGTATC
601 CTAAACTCTACATGTTCCAGGACACTGATCTTAAAGCTCAGGTTTCAAGAT
651 CTTGTTTTATAGGCTCTAGGTGTATATTGTGGGGCTTCCCTGGTGGCTCA
701 GATGGTAAAGTGTCTGCCTGCAATGTGGGTGATCTGGGTTCGATCCCTGG
751 CTTGGGAAGATCCCCTGGAGAAGGAAATGGCAACCCACTCTAGTACTCTT
801 ACCTGGAAAATTCCATGGACAGAGGAGCCTTGTAAGCTACAGTCCATGGG
851 ATTGCAAAGAGTTGAACACAAGTGAAGCAACTAAGCACAGCACAGTACAGT
901 ATACACCTGTGAGGTGAAGTGAAGTGAAGGTTCAATGCAGGGTCTCCTGC
951 ATTGCAGAAAGATTCTTTACCATCTGAGCCACCAGGGAAGCCCAAGAATA
1001 CTGGAGTGGGTAGCCTATTCCTTCTCCAGGGGATCTTCCCATCCCAGGAA
1051 TTGAACTGGAGTCTCCTGCATTTTCAGGTGGATTCTTCACCAGCTGAACTA
1101 CCAGGTGGATACTACTCCAATATTAAAGTGCTTAAAGTCCAGTTTTCCTCA
1151 CCTTTCCCAAAAAGGTTGGGTCACTCTTTTTTAAACCTTCTGTGGCCTACT
1201 CTGAGGCTGTCTACAAGCTTATATATTTATGAACACATTTATTGCAAGTT
1251 GTTAGTTTTAGATTTACAATGTGGTATCTGGCTATTTAGTGGTATTGGTG
1301 GTTGGGGATGGGGAGGCTGATAGCATCTCAGAGGGCAGCTAGATACTGTC
1351 ATACACACTTTTCAAGTTCTCCATTTTTGTGAAATAGAAAGTCTCTGGAT
1401 CTAAGTTATATGTGATTCTCAGTCTCTGTGGTCATATTCTATTCTACTCC
1451 TGACCACTCAACAAGGAACCAAGATATCAAGGGACACTTGTTTTGTTTCA
1501 TGCTTGGGTGAGTGGGCCATGACATATGTTCTGGGCCTTGTTACATGGC
1551 TGGATTGGTTGGACAAGTGCCAGCTCTGATCCTGGGACTGTGGCATGTGA
1601 TGACATACACCCCTCTCCACATTCTGCATGTCTCTAGGGGGGAAGGGGG
1651 AAGCTCGGTATAGAACCTTTATTGTATTTTCTGATTGCCTCACTTCTTAT
1701 ATTGCCCCCATGCCCTTCTTTGTTTCTCAAGTAACCAGAGACAGTGCTTC
1751 CCAGAACCAACCCTACAAGAAACAAAGGGCTAAACAAAGCCAAATGGGAA
1801 GCAGGATCATGGTTTGAAGTCTTTCTGGCCAGAGAACAAATACCTGCTATG
1851 GACTAGATACTGGGAGAGGGAAAGGAAAAGTAGGGTGAATTATGGAAGGA
1901 AGCTGGCAGGCTCAGCGTTTCTGTCTTGGCATGACCAGTCTCTCTTCATT
1951 CTCTTCCTAGATGTAGGGCTTGGTACCAGAGCCCCTGAGGCTTTCTGCAT
2001 GAATATAAATATATGAACTGAGTGATGCTTCCATTTTCAGGTTCTTGGGG
2051 GCGCCGAATTCGAGCTCGGTACCCGGGGATCTCGAGAAGCTTTAACCATG
2101 GAATGGAGCTGGGTCTTTCTCTTCTTCTGTCAGTAACTACAGGTGTCCA
2151 CTCCCAGGTTTCAGTTGCAGCAGTCTGACGCTGAGTTGGTGAAACCTGGGG
2201 CTTCAAGTGAAGATTTCTGCAAGGCTTCTGGCTACACCTTCACTGACCAT
2251 GCAATTCAGTGGGTGAAACAGAACCCTGAACAGGGCCTGGAATGGATTGG
2301 ATATTTTTCTCCCGGAAATGATGATTTTAAATACAATGAGAGGTTCAAGG
2351 GCAAGGCCACACTGACTGCAGACAAATCCTCCAGCACTGCCTACGTGCAG
2401 CTCAACAGCCTGACATCTGAGGATTCTGCAGTGTATTTCTGTACAAGATC
2451 CCTGAATATGGCCTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAG
2501 GAGGCGGAGGCAGCGGAGGCGGTGGCTCGGGAGGCGGAGGCTCGGACATT
2551 GTGATGTCACAGTCTCCATCCTCCCTACCTGTGTGTCAGTTGGCGAGAAGGT
2601 TACTTTGAGCTGCAAGTCCAGTCAGAGCCTTTTATATAGTGGTAATCAAA
2651 AGAACTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTG
2701 CTGATTTACTGGGCATCCGCTAGGGAATCTGGGGTCCCTGATCGCTTCAC
2751 AGGCAGTGGATCTGGGACAGATTTCACTCTCTCCATCAGCAGTGTGAAGA
2801 CTGAAGACCTGGCAGTTTATTACTGTCAGCAGTATTATAGCTATCCCCTC
```

22/35

Figure 13b

2851 ACGTTCGGTGCTGGGACCAAGCTGGTGCTGAAACGGGGCCGCGAGCCCCAA
2901 ATCTCCTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCC
2951 TGGGGGGACCGTCAGTCTTCCTCTTCCCCCCTAAACCCAAGGACACCCTC
3001 ATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCA
3051 CGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGC
3101 ATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGT
3151 GTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGA
3201 GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAA
3251 CCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG
3301 CCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTGAGCCTGACCTGCCT
3351 GGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATG
3401 GGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGGACTCCGAC
3451 GGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCA
3501 GCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACC
3551 ACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGGAGGCGGATCA
3601 GGAGGTGGCGCACCTACTTCAAGTTCTACAAAGAAAACACAGCTACAACCT
3651 GGAGCATTTACTGCTGGATTTACAGATGATTTTGAATGGAATTAATAATT
3701 ACAAGAATCCCAAACCTCACCAGGATGCTCACATTTAAGTTTTACATGCCC
3751 AAGAAGGCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAA
3801 ACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACCTTCACTTAA
3851 GACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAATAAAG
3901 GGATCTGAAACAACATTCTGTGTGAATATGCTGATGAGACAGCAACCAT
3951 TGTAGAATTTCTGAACAGATGGATTACCTTTTGTCAAAGCATCATCTCAA
4001 CACTAACTTGAAGCTTGTTAACATCGATAAAATAAAAGATTTTATTTAGT
4051 CTCCAGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGCAAGCT
4101 AGCTTAAGTAACGCCATTTTGAAGGCATGGAAAAATACATAACTGAGAA
4151 TAGAGAAGTTCAGATCAAGGTGAGGAACAGATGGAAACAGCTGAATATGGG
4201 CCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAG
4251 AACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCA
4301 GTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCC
4351 AGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAA
4401 GGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTT
4451 CTCGCTTCTGTTGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCA
4501 CAACCCCTCACTCGGGGCGCCAGTCTCCGATTGACTGAGTCGCCCCGGT
4551 ACCCGTGTATCCAATAAACCTCTTGCAGTTGCATCCGACTTGTGGTCTC
4601 GCTGTTCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGG
4651 GGTCTTTCATT

1 - 2055 Bovine/human alpha-lactalbumin 5' flanking region
2098 - 4011 cc49-IL2 coding region
4068 - 4661 MoMuLV 3' LTR

Figure 14a
SEQ ID NO:11
Alpha-Lactalbumin YP Vector

```
1      GATCAGTCCTGGGTGGTCATTGAAAGGACTGATGCTGAAGTTGAAGCTCC
51     AATACTTTGGCCACCTGATGCGAAGAACTGACTCATGTGATAAGACCCTG
101    AACTGCGGAAAGATTGAAGGCAGGAGGAGAAGGGATGACAGAGGATGGAA
151    GAGTTGGATGGAATCACCAACTCGATGGACATGAGTTTGAGCAAGCTTCC
201    AGGAGTTGGTAATGGGCAGGGAAGCCTGGCGTGCTGCAGTCCATGGGGTT
251    GCAAAGAGTTGGACACTACTGAGTGACTGAACTGAACTGATAGTGTAATC
301    CATGGTACAGAATATAGGATAAAAAAGAGGAAGAGTTTGCCCTGATTCTG
351    AAGAGTTGTAGGATATAAAAGTTTAGAATACCTTTAGTTTGGAAGTCTTA
401    AATTATTTACTTAGGATGGGTACCCACTGCAATATAAGAAATCAGGCTTT
451    AGAGACTGATGTAGAGAGAATGAGCCCTGGCATAACCAGAAGCTAACAGCT
501    ATTGGTTATAGCTGTTATAACCAATATATAACCAATATATTGGTTATATA
551    GCATGAAGCTTGATGCCAGCAATTTGAAGGAACCATTTAGAACTAGTATC
601    CTAAACTCTACATGTTCCAGGACACTGATCTTAAAGCTCAGGTTCAGAAT
651    CTTGTTTTATAGGCTCTAGGTGTATATTGTGGGGCTTCCCTGGTGGCTCA
701    GATGGTAAAGTGTCTGCCTGCAATGTGGGTGATCTGGGTTTCGATCCCTGG
751    CTTGGGAAGATCCCCTGGAGAAGGAAATGGCAACCCACTCTAGTACTCTT
801    ACCTGGAAAATTCCATGGACAGAGGAGCCTTGTAAGCTACAGTCCATGGG
851    ATTGCAAAGAGTTGAACACAACCTGAGCAACTAAGCACAGCACAGTACAGT
901    ATACACCTGTGAGGTGAAGTGAAGTGAAGGTTCAATGCAGGGTCTCCTGC
951    ATTGCGAGAAAGATTCTTTACCATCTGAGCCACCAGGGAAGCCCAAGAATA
1001   CTGGAGTGGGTAGCCTATTCTTCTCCAGGGGATCTTCCCATCCCAGGAA
1051   TTGAACTGGAGTCTCCTGCATTTTCAGGTGGATTCTTCACCAGCTGAACTA
1101   CCAGGTGGATACTACTCCAATATTAAAGTGCTTAAAGTCCAGTTTTCCCA
1151   CCTTTCCCAAAAAGGTTGGGTCACTCTTTTTTAACCTTCTGTGGCCTACT
1201   CTGAGGCTGTCTACAAGCTTATATATTTATGAACACATTTATTGCAAGTT
1251   GTTAGTTTTAGATTTACAATGTGGTATCTGGCTATTTAGTGGTATTGGTG
1301   GTTGGGGATGGGGAGGCTGATAGCATCTCAGAGGGCAGCTAGATACTGTC
1351   ATACACACTTTTCAAGTTCTCCATTTTTGTGAAATAGAAAGTCTCTGGAT
1401   CTAAGTTATATGTGATTCTCAGTCTCTGTGGTCATATTCTATTCTACTCC
1451   TGACCACTCAACAAGGAACCAAGATATCAAGGGACACTTGTTTTGTTCAT
1501   TGCCTGGGTTGAGTGGGCCATGACATATGTTCTGGGCCTTGTTACATGGC
1551   TGGATTGGTTGGACAAGTGCCAGCTCTGATCCTGGGACTGTGGCATGTGA
1601   TGACATACACCCCTCTCCACATTCTGCATGTCTCTAGGGGGGAAGGGGG
1651   AAGCTCGGTATAGAACCCTTTATTGTATTTTCTGATTGCCTCACTTCTTAT
1701   ATTGCCCCCATGCCCTTCTTTGTTCTCAAGTAACCAGAGACAGTGCTTC
1751   CCAGAACCAACCCTACAAGAAACAAAGGGCTAAACAAAGCCAAATGGGAA
1801   GCAGGATCATGGTTTGAACCTTTCTGGCCAGAGAACAATACCTGCTATG
1851   GACTAGATACTGGGAGAGGGAAAGGAAAAGTAGGGTGAATTATGGAAGGA
1901   AGCTGGCAGGCTCAGCGTTTCTGTCTTGGCATGACCAGTCTCTCTTCATT
1951   CTCTTCCTAGATGTAGGGCTTGGTACCAGAGCCCCTGAGGCTTTCTGCAT
2001   GAATATAAATATATGAACTGAGTGATGCTTCCATTTTCAGGTTCTTGGGG
2051   GCGCCGAATTGAGCTCGGTACCCGGGGATCTCGACGGATCCGATTACTT
2101   ACTGGCAGGTGCTGGGGGCTTCCGAGACAATCGCGAACATCTACACCACA
2151   CAACACCGCCTCGACCAGGGTGAGATATCGGCCGGGGACGCGCGGCTGGT
2201   AATTACAAGCGAGATCCGATTACTTACTGGCAGGTGCTGGGGGCTTCCGA
2251   GACAATCGCGAACATCTACACCACACAACACCGCCTCGACCAGGGTGAGA
2301   TATCGGCCGGGGACGCGCGGTGGTAATTACAAGCGAGATCTCGAGTTAA
2351   CAGATCTAGGCCTCCTAGGTGACGATCCCCGGGAATTCGGCGCCGCCA
2401   CCATGATGTCCTTTGTCTCTCTGCTCCTGGTAGGCATCCTATTCCATGCC
2451   ACCCAGGCCCAGGTCCAACCTGCAGCAGTCTGGGCCTGAGCTGGTGAAGCC
2501   TGGGACTTCAGTGAGGATATCCTGCAAGGCTTCTGGCTACACCTTCACAA
2551   GCTACTATTTACACTGGGTGAAGCAGAGGCCTGGACAGGGACTTGAGTGG
2601   ATTGCAATGGATTTATCCTGGAAATGTTATTACTACGTACAATGAGAAGTT
2651   CAAGGGCAAGGCCACACTGACTGCAGACAAATCCTCCAGCACAGCCTACA
2701   TGCACCTCAACAGCCTGACCTCTGAGGACTCTGCGGTCTATTTCTGTGCA
2751   AGGGGTGACCATGATCTTGACTACTGGGGCCAAGGCACCACTCTCACAGT
2801   CTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCCTGGAT
```

Figure 14b

2851 CTGCTGCCCCAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGC
2901 TATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCCCTGTCCAG
2951 CGGTGTGCACACCTTCCCAGCTGTCCCTGCAGTCTGACCTCTACACTCTGA
3001 GCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACC
3051 TGCAACGTTGCCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGT
3101 GCCCAGGGATTGTACTAGTGGAGGTGGAGGTAGCTAAGGGAGATCTCGAC
3151 GGATCCCCCGGAATTCGCCCCCTCTCCCTCCCCCCCCCTAACGTTACTGG
3201 CCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTT
3251 CCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCT
3301 GTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAAT
3351 GCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTT
3401 GAAGACAAACAACGTCTGTAGCGACCCCTTTCAGGCAGCGGAACCCCCCA
3451 CCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACC
3501 TGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGG
3551 AAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGAT
3601 GCCCAGAAGGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTGCAC
3651 ATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCCTAGGCCCCCGA
3701 ACCACGGGGACGTGGTTTTCTTTGAAAACACGATGATAATATGGCCTC
3751 CTTTGTCTCTCTGCTCCTGGTAGGCATCCTATTCCATGCCACCCAGGCCG
3801 ACATTGTGCTGACACAATCTCCAGCAATCATGTCTGCATCTCCAGGGGAG
3851 AAGGTCACCATGACCTGCAGTGCCACCTCAAGTGTAAGTTACATACACTG
3901 GTACCAGCAGAAGTCAGGCACCTCCCCAAAAGATGGATTTATGACACAT
3951 CCAAACCTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGG
4001 ACCTCTCACTCTCTCACACTCAGCAGCATGGAGGCTGAAGATGCTGCCAC
4051 TTATTACTGCCAGCAGTGGGGTAGTTACCTCACGTTCCGGTGCGGGGACCA
4101 AGCTGGAGCTGAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCA
4151 CCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTT
4201 GAACAACCTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCA
4251 GTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAA
4301 GACAGCACCTACAGCATGAGCAGCACCCCTCACGTTGACCAAGGACGAGTA
4351 TGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTT
4401 CACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAATAGGGGAGATCT
4451 CGACATCGATAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTG
4501 GTATTCTTAACATATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTA
4551 ATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTC
4601 CTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTG
4651 TCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACT
4701 GGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTTCGCTTT
4751 CCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCTGCTTGGCCGCT
4801 GCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCGGTGGTGTGTCG
4851 GGGAAATCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGAT
4901 TCTGCGCGGGACGTCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGG
4951 ACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTT
5001 CGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCTCCCCGCC
5051 TGATCGATAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAAT
5101 GAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTT
5151 GCAAGGCATGGAAAAATACATAACTGAGAATAGAGAAGTTCAGATCAAGG
5201 TCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGT
5251 AAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAAT
5301 ATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGG
5351 CCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGA
5401 GAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTG
5451 CCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTGCGCGCT
5501 TCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGC
5551 CAGTCCTCCGATTGACTGAGTCGCCCCGGGTACCCGTGTATCCAATAAACC
5601 CTCTTGCAAGTTGCATCCGACTTGTGGTCTCGCTGTTCTTGGGAGGGTCT

25/35

Figure 14c

5651 CCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATT

1 - 2053	Bovine/Human Alpha-lactalbumin 5' flanking region
2093 - 2336	Double mutated PPE sequence
2403 - 2459	Bovine alpha-lactalbumin signal peptide coding region
2460 - 3137	Yersenia pestis heavy chain Fab gene coding region
3167 - 3742	EMCV IRES
3743 - 3799	Bovine alpha-lactalbumin signal peptide coding region
3800 - 4441	Yersenia pestis light chain Fab gene coding region
4461 - 5052	WPRE sequence
5098 - 5691	Moloney murine leukemia virus 3' LTR

26/35

Figure 15
SEQ ID NO:12
IRES-Casein Signal Peptide Sequence

```
1   GGAATTCGCCCCTCTCCCTCCCCCCCCCTAACGTTACTGGCCGAAGCCG
51  CTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATAT
101 TGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTG
151 ACGAGCATTCCTAGGGGTCTTTCCTCTCGCCAAAGGAATGCAAGGTCT
201 GTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAA
251 CAACGTCTGTAGCGACCCTTTCAGGCAGCGGAACCCCCCACCTGGCGAC
301 AGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGC
351 GGCACAACCCCAAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCA
401 AATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAG
451 GTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTAC
501 ATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCGAACCACGGGG
551 ACGTGGTTTTCTTTGAAAAACACGATGATAATATGGCCTTGCTCATCCT
601 TACCTGTCTTGTGGCTGTTGCTCTTGCCGGCGCCATGGGATATCTAGATC
651 TCGAGCTCGCGAAAGCTT
```

1 - 583	IRES
584 - 628	Modified bovine alpha-S1 casein signal peptide coding region
629 - 668	Multiple cloning site

27/35

Figure 16a

SEQ ID NO: 13

LNBOTDC Vector

```
1   TTTGAAAGACCCACCCGTAGGTGGCAAGCTAGCTTAAGTAACGCCACTT
51  TGCAAGGCATGGAAAAATACATAACTGAGAATAGAAAAGTTCAGATCAAG
101 GTCAGGAACAAAGAAACAGCTGAATACCAAACAGGATATCTGTGGTAAGC
151 GGTTCCCTGCCCCGGCTCAGGGCCAAGAACAGATGAGACAGCTGAGTGATG
201 GGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCGGGGCCA
251 AGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGTGAA
301 TCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAAATGACCCTGTACC
351 TTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTC
401 CGCTCTCCGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGCGCGCCA
451 GTCTTCCGATAGACTGCGTCGCCCCGGGTACCCGTATTCCCAATAAAGCCT
501 CTTGCTGTTTGCATCCGAATCGTGGTCTCGCTGTTCCCTTGGGAGGGTCTC
551 CTCTGAGTGATTGACTACCCACGACGGGGGTCTTTCATTTGGGGGCTCGT
601 CCGGGATTGAGAGACCCCTGCCAGGGACCACCGACCCACCACCGGGAGG
651 TAAGCTGGCCAGCAACTTATCTGTGTCTGTCCGATTGTCTAGTGTCTATG
701 TTTGATGTTATGCGCCTGCGTCTGTACTAGTTAGCTAACTAGCTCTGTAT
751 CTGGCGGACCCGTGGTGGAAGTACGAGTTCTGAACACCCGGCCGCAACC
801 CTGGGAGACGTCCCAGGGACTTTGGGGGCCGTTTTTGTGGCCCGACCTGA
851 GGAAGGGAGTCGATGTGGAATCCGACCCCGTCAGGATATGTGGTTCTGGT
901 AGGAGACGAGAACCTAAAACAGTTCGCCCTCCGTCTGAATTTTTGCTTT
951 CGGTTTGGAACCGAAGCCGCGCGTCTTGTCTGCTGCAGCGCTGCAGCATC
1001 GTTCTGTGTTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATTA
1051 GGGCCAGACTGTTACCACTCCCTTAAGTTTGACCTTAGGTCAGTGGAAAG
1101 ATGTCGAGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAGAGACGT
1151 TGGGTTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGCC
1201 GCGAGACGGCACCTTTAACCGAGACCTCATCACCAGGTTAAGATCAAGG
1251 TCTTTTACCTGGCCCGCATGGACACCCAGACAGGTCCCCTACATCGTG
1301 ACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTTTGT
1351 ACACCCTAAGCCTCCGCCTCCTCTTCCCTCCATCCGCCCGTCTCTCCCCC
1401 TTGAACCTCCTCGTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCCTC
1451 ACTCCTTCTCTAGGCGCCGGAATTCCGATCTGATCAAGAGACAGGATGAG
1501 GATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCC
1551 GCTTGGGTGGAGAGGCTATTCCGGCTATGACTGGGCACAACAGACAATCGG
1601 CTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTT
1651 TTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAG
1701 GCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGT
1751 GCTCGACGTTGTCAGTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAG
1801 TGCCGGGGCAGGATCTCCTGTCTATCTCACCTTGCTCCTGCCGAGAAAGTA
1851 TCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTAC
1901 CTGCCCATTTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTC
1951 GGATGGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAG
2001 GGGCTCGCGCCAGCCGAAGTTCGCCAGGCTCAAGGCGCGCATGCCCGA
2051 CGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCA
2101 TGGTGGAAAATGGCCGCTTTTCTGGATTTCATCGACTGTGGCCGGCTGGGT
2151 GTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGA
2201 AGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCG
2251 CCGCTCCCGATTTCGAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTC
2301 TTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCAAC
2351 CTGCCATCACGAGATTTTCGATTCCACCGCCGCTTCTATGAAAGGTTGGG
2401 CTTCCGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGG
2451 ATCTCATGCTGGAGTTCTTCGCCCACCCCGGGCTCGATCCCCTCGCGAGT
2501 TGGTTCAGCTGCTGCCTGAGGCTGGACGACCTCGCGGAGTTCTACCGGCA
2551 GTGCAAATCCGTCGGCATCCAGGAAACCAGCAGCGGCTATCCGCGCATCC
2601 ATGCCCCCGAAGTGCAGGAGTGGGGAGGCACGATGGCCGCTTTGGTTCGAG
2651 GCGGATCCGGCCATTAGCCATATTATTCATTGGTTATATAGCATAAATCA
2701 ATATTGGCTATTGGCCATTGCATACGTTGTATCCATATCATAATATGTAC
2751 ATTTATATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATT
```

28/35

Figure 16b

2801 GACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCAT
2851 ATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGA
2901 CCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCAT
2951 AGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTAC
3001 GGTAAACTGCCCCTTGGCAGTACATCAAGTGTATCATATGCCAAGTACG
3051 CCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCA
3101 GTACATGACCTTATGGGACTTTTCTACTTGGCAGTACATCTACGTATTAG
3151 TCATCGCTATTACCATGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGT
3201 GGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGT
3251 CAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTC
3301 GTAACAACCTCCGCCCCATTGACGCAAATGGGCGGTAGGCATGTACGGTGG
3351 GAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAG
3401 ACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCA
3451 GCCTCCGCGGGCCCCAAGCTTCTCGACGGATCCCCGGGAATTCAGGCCATC
3501 GATCCCGCGCGCCACCATGGAATGGAGCTGGGTCTTTCTCTTCTTCTGTC
3551 AGTAACTACAGGTGTCCACTCCGACATCCAGATGACCCAGTCTCCAGCCT
3601 CCCTATCTGCATCTGTGGGAGAACTGTCACTATCACATGTGAGCAAGT
3651 GGGAAATATTACAATTATTTAGCATGGTATCAGCAGAAACAGGGAAAATC
3701 TCCTCAGCTCCTGGTCTATAATGCAAAAACCTTAGCAGATGGTGTGCCAT
3751 CAAGGTTCAAGTGGCAGTGGATCAGGAACACAATATTCTCTCAAGATCAAC
3801 AGCCTGCAGCCTGAAGATTTTGGGAGTTATTACTGTCAACATTTTGGAG
3851 TACTCCGTGGACGTTCCGGTGGAGGCACCAAGCTGGAAATCAAACGGGCTG
3901 ATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACA
3951 TCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACCTTCTACCCCAAAGA
4001 CATCAATGTCAAGTGAAGATTGATGGCAGTGAACGACAAAATGGCGTCC
4051 TGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGC
4101 AGCACCCCTCACATTGACCAAGGACGAGTATGAACGACATAACAGCTATAC
4151 CTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCA
4201 ACAGGAATGAGTGTTGAAAGCATCGATTTCCCCTGAATTCGCCCCCTCTCC
4251 CTCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGT
4301 GTGCGTTTGTCTATATGTTATTTCCACCATATTGCCGTCTTTTGGCAAT
4351 GTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGG
4401 TCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGG
4451 AAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACC
4501 CTTTGCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCA
4551 AAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAAGTGCC
4601 ACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGC
4651 GTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTTGTATGGG
4701 ATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGT
4751 TAAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCTTTGA
4801 AAAACACGATGATAATATGGCCTCCTTTGTCTCTCTGCTCCTGGTAGGCA
4851 TCCTATTCCATGCCACCCAGGCCGAGGTTCAAGCTTCAGCAGTCTGGGGCA
4901 GAGCTTGTGAAGCCAGGGGCCTCAGTCAAGTTGTCTGACAGCTTCTGG
4951 CTTCAACATTAAAGACACCTTTATGCACTGGGTGAAGCAGAGGCCTGAAC
5001 AGGGCCTGGAGTGGATTGGAAGGATTGATCCTGCGAATGGGAATACTGAA
5051 TATGACCCGAAGTTCCAGGGCAAGGCCACTATAACAGCAGACACATCCTC
5101 CAACACAGTCAACCTGCAGCTCAGCAGCCTGACATCTGAGGACACTGCCG
5151 TCTATTACTGTGCTAGTGGAGGGGAACTGGGGTTTCTTACTGGGGCCAA
5201 GGGACTCTGGTCACTGTCTCTGCAGCCAAAACGACACCCCCATCTGTCTA
5251 TCCACTGGCCCCTGGATCTGCTGCCCAAATAACTCCATGGTGACCCTGG
5301 GATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAAC
5351 TCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTC
5401 TGACCTCTACACTCTGAGCAGCTCAGTGAAGTGTCCCCTCCAGCACCTGGC
5451 CCAGCGAGACCGTCACCTGCAACGTTGCCCAACCGGCCAGCAGCACCAAG
5501 GTGGACAAGAAAATTGTGCCAGGGATTGTACTAGTGGAGGTGGAGGTAG
5551 CCACCATCACCATCACCATTAATCTAGAGTTAAGCGGCCGTCGAGATCTA
5601 GGCCTCCTAGGTGACATCGATAAAATAAAAGATTTTATTAGTCTCCAG
5651 AAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTA
5701 AGTAACGCCATTTTGAAGGCATGGAAAAATACATAACTGAGAATAGAGA
5751 AGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAAC
5801 AGGATATCTGTGGTAAGCAGTTCCTGCCCGGCTCAGGGCCAAGAACAGA
5851 TGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCT

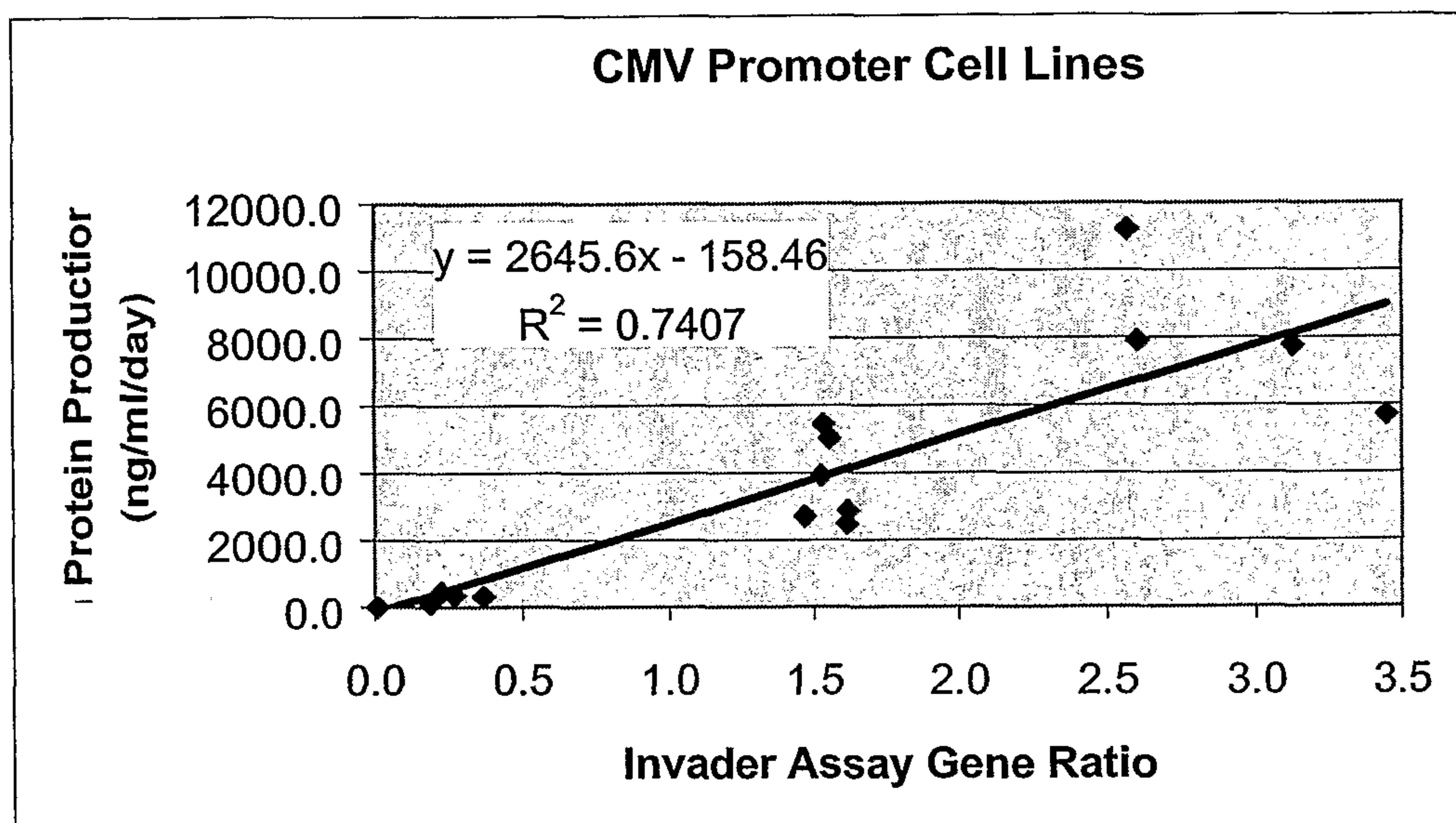
29/35

Figure 16c

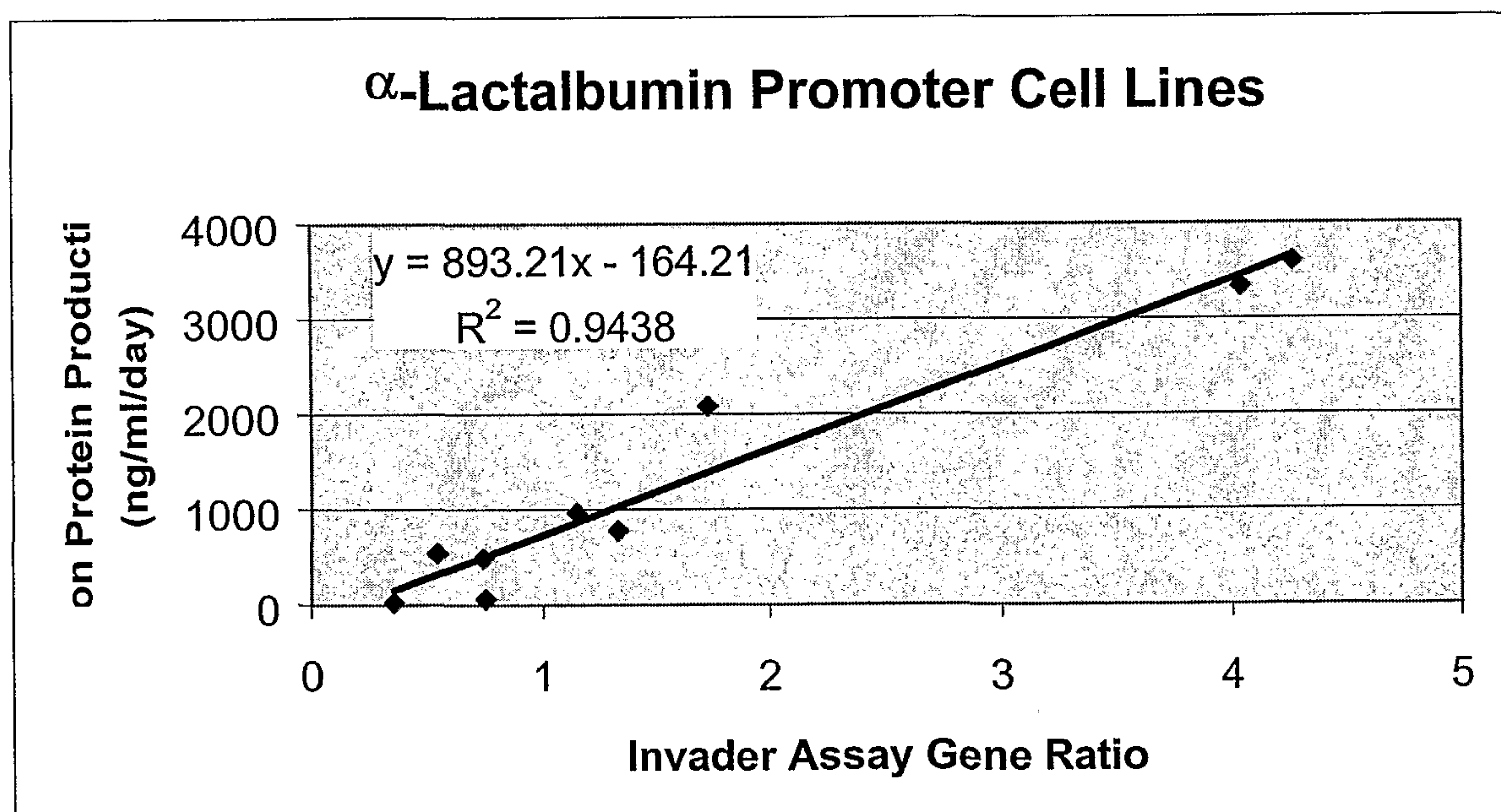
```
5901  GCGGCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCT
5951  CAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCT
6001  GAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCT
6051  TCTGTTGCGCGGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACCC
6101  CTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCCGGGTACCCGT
6151  GTATCCAATAAACCTCTTGCACTTGCATCCGACTTGTGGTCTCGCTGTT
6201  CCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTT
      TCATT
```

Moloney Murine Sarcoma Virus 5' LTR	1 - 589
Moloney Murine Leukemia Virus Extended Packaging Region	659 - 1468
Neomycin Resistance Gene	1512 - 2306
CMV Promoter	2656 - 3473
cc49 Signal Peptide Coding Region	3516 - 3572
Bot Fab 5 Light Chain	3573 - 4217
EMCV IRES (Clonetech)	4235 - 4816
Modified Bovine α -LA Signal Peptide Coding Region	4817 - 4873
Bot Fab 5 Heavy Chain	4874 - 5572
Moloney Murine Leukemia Virus 3' LTR	5662 - 6255

30/35

Figure 17. CMV construct containing cell lines.

31/35

Figure 18: α -Lactalbumin construct containing cell lines

32/35

Figure 19a
SEQ ID NO: 34
LNBOTDC Vector

```
1 GAATTAATTCATACCAGATCACCGAAAACGTGCTCCTCCAAATGTGTCCCCC
51 TCACACTCCCAAATTCGCGGGCTTCTGCCTCTTAGACCACTCTACCCTAT
101 TCCCCACACTCACCGGAGCCAAAGCCGCGGCCCTTCCGTTTCTTTGCTTT
151 TGAAAGACCCCACCCGTAGGTGGCAAGCTAGCTTAAGTAACGCCACTTTG
201 CAAGGCATGGAAAAATACATAACTGAGAATAGAAAAGTTCAGATCAAGGT
251 CAGGAACAAAGAAACAGCTGAATACCAAACAGGATATCTGTGGTAAGCGG
301 TTCCTGCCCCGGCTCAGGGCCAAGAACAGATGAGACAGCTGAGTGATGGG
351 CCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCGGGGCCAAG
401 AACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGTGAATC
451 ATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAAATGACCCTGTACCTT
501 ATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCCG
551 CTCTCCGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGCGCGCCAGT
601 CTTCCGATAGACTGCGTCGCCCCGGGTACCCGTATTCCCAATAAAGCCTCT
651 TGCTGTTTGCATCCGAATCGTGGTCTCGCTGTTTCTTGGGAGGGTCTCCT
701 CTGAGTGATTGACTACCCACGACGGGGGTCTTTCATTTGGGGGCTCGTCC
751 GGGATTTGGAGACCCCTGCCCAGGGACCACCGACCCACCACCGGGAGGTA
801 AGCTGGCCAGCAACTTATCTGTGTCTGTCCGATTGTCTAGTGTCTATGTT
851 TGATGTTATGCGCCTGCGTCTGTACTAGTTAGCTAACTAGCTCTGTATCT
901 GGCGGACCCGTGGTGGAACCTGACGAGTTCTGAACACCCGGCCGCAACCTT
951 GGGAGACGTCCCAGGGACTTTGGGGGCCGTTTTTGTGGCCCGACCTGAGG
1001 AAGGGAGTCGATGTGGAATCCGACCCCGTCAGGATATGTGGTTCTGGTAG
1051 GAGACGAGAACCTAAAACAGTTCCCGCCTCCGTCTGAATTTTGTCTTTCG
1101 GTTTGGAACCGAAGCCGCGCGTCTTGTCTGCTGCAGCGCTGCAGCATCGT
1151 TCTGTGTTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATTAGG
1201 GCCAGACTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGAT
1251 GTCGAGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAGAGACGTTG
1301 GGTTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGCCGC
1351 GAGACGGCACCTTTAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTC
1401 TTTTCACCTGGCCCGCATGGACACCCAGACAGGTCCCCTACATCGTGAC
1451 CTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTTTGTAC
1501 ACCCTAAGCCTCCGCTCCTCTTCCCTCCATCCGCCCCGTCTCTCCCCCTT
1551 GAACCTCCTCGTTGACCCCGCCTCGATCCTCCCTTTATCCAGCCCTCAC
1601 TCCTTCTCTAGGCGCCGGAATTCCGATCTGATCAAGAGACAGGATGAGGG
1651 AGCTTGATATATCCATTTTTCGGATCTGATCAGCACGTGTTGACAATTAATC
1701 ATCGGCATAGTATATCGGCATAGTATAATACGACAAGGTGAGGAACTAAA
1751 CCATGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCATTGAAAGAGCA
1801 ACGGCTACAATCAACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAG
1851 CGCAGCTCTCTCTAGCGACGGCCGCATCTTCACTGGTGTCAATGTATATC
1901 ATTTTACTGGGGGACCTTGTGCAGAACTCGTGGTGCTGGGCACTGCTGCT
1951 GCTGCGGCAGCTGGCAACCTGACTTGTATCGTCGCGATCGGAAATGAGAA
2001 CAGGGGCATCTTGAGCCCTGCGGACGGTGTGACAGGTGCTTCTCGATC
2051 TGCATCCTGGGATCAAAGCGATAGTGAAGGACAGTGATGGACAGCCGACG
2101 GCAGTTGGGATTCGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTA
2151 AGCACTTCGTGGCCGAGGAGCAGGACTGACACGTGCTACGAGATTTTCGAT
2201 TCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGA
2251 CGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCG
2301 CCCACCCCAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAAT
2351 AGCATCACAAATTTACAAATAAAGCATTTTTTTTCACTGCATTCTAGTTG
2401 TGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGTACGAGTTGGT
2451 TCAGCTGCTGCCTGAGGCTGGACGACCTCGCGGAGTTCTACCGGCAGTGC
2501 AAATCCGTCGGCATCCAGGAAACCAGCAGCGGCTATCCGCGCATCCATGC
2551 CCCCGAAGTGCAGGAGTGGGGAGGCACGATGGCCGCTTTGGTCGAGGCGG
2601 ATCCGGCCATTAGCCATATTATTCAATTGGTTATATAGCATAAATCAATAT
2651 TGGCTATTGGCCATTGCATACGTTGTATCCATATCATAATATGTACATTT
2701 ATATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATTGACT
2751 AGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATAT
2801 GGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGC
2851 CCAACGACCCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTA
```

33/35

Figure 19b

2901 ACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTA
2951 AACTGCCCACCTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC
3001 CTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTAC
3051 ATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCAT
3101 CGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGAT
3151 AGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTTGACGTCAAT
3201 GGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAATGTCTGTA
3251 CAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCATGTACGGTGGGAGG
3301 TCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGC
3351 CATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCT
3401 CCGCGGCCCCAAGCTTCTCGAGTTAACAGATCTAGGCTGGCAGCAGGT
3451 TTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAG
3501 CTCACCTATTAGGCACCCCAAGGCTTTACACTTTATGCTTCCGGCTCGTAT
3551 GTTGTGTGGAATTGTGAGCGGATAACAATTTACACACAGGAAACAGCTATG
3601 ACCATGATTACGCCAAGCTTGGCTGCAGGTGCAGGATCCACTAGTAACG
3651 GCCGCCAGTGTGCTGGAATTCACCATGGGGCAACCCGGGAACGGCAGCGC
3701 CTTCTTGCTGGCACCCAATGGAAGCCATGCGCCGGACCACGACGTCACGC
3751 AGCAAAGGGACGAGGTGTGGGTGGTGGGCATGGGCATCGTCATGTCTCTC
3801 ATCGTCCTGGCCATCGTGTTTGGCAATGTGCTGGTCATCACAGCCATTGC
3851 CAAGTTCGAGCGTCTGCAGACGGTCACCAACTACTTCATCACAAGCTTGG
3901 CCTGTGCTGATCTGGTCATGGGGCTAGCAGTGGTGCCCTTTGGGGCCGCC
3951 CATATTCTCATGAAAATGTGGACTTTTGGCAACTTCTGGTGCGAGTTCTG
4001 GACTTCCATTGATGTGCTGTGCGTCACGGCATCGATTGAGACCTGTGCG
4051 TGATCGCAGTCGACCGCTACTTTGCCATTACTAGTCCTTTCAAGTACCAG
4101 AGCCTGCTGACCAAGAATAAGGCCCGGGTGATCATTTCTGATGGTGTGGAT
4151 TGTGTCAGGCCTTACCTCCTTCTTGCCCATTCAGATGCACTGGTACAGGG
4201 CCACCCACCAGGAAGCCATCAACTGCTATGCCAATGAGACCTGCTGTGAC
4251 TTCTTCACGAACCAAGCCTATGCCATTGCCTCTTCCATCGTGTCTTCTA
4301 CGTTCCCCTGGTGATCATGGTCTTCTGCTACTCCAGGGTCTTTCAGGAGG
4351 CCAAAGGCAGCTCCAGAAGATTGACAAATCTGAGGGCCGCTTCCATGTC
4401 CAGAACCTTAGCCAGGTGGAGCAGGATGGGCGGACGGGGCATGGACTCCG
4451 CAGATCTTCCAAGTTCTGCTTGAAGGAGCACAAAGCCCTCAAGACGTTAG
4501 GCATCATCATGGGCACTTTACCCCTCTGCTGGCTGCCCTTCTTCATCGTT
4551 AACATTGTGCATGTGATCCAGGATAACCTCATCCGTAAGGAAGTTTACAT
4601 CCTCCTAAATTGGATAGGCTATGTCAATTCTGGTTTCAATCCCCTTATCT
4651 ACTGCCGGAGCCCAGATTTTCAAGGATTGCCTTCCAGGAGCTTCTGTGCCTG
4701 CGCAGGTCTTCTTTGAAGGCCTATGGCAATGGCTACTCCAGCAACGGCAA
4751 CACAGGGGAGCAGAGTGGATATCACGTGGAACAGGAGAAAGAAAATAAAC
4801 TGCTGTGTGAAGACCTCCCAGGCACGGAAGACTTTGTGGGCCATCAAGGT
4851 ACTGTGCCTAGCGATAACATTGATTACAAAGGGAGGAATTGTAGTACAAA
4901 TGACTCACTGCTCTCGAGAATCGAGGGGCGGCACCACCATCATCACCACG
4951 TCGACCCCGGGGACTACAAGGATGACGATGACAAGTAAGCTTTATCCATC
5001 AACTGGCGGCGCTCGAGCATGCATCTAGCGGCCGCTCGAGGCCGGCAA
5051 GGCCGGATCCCCGGGAATTCGCCCTCTCCCTCCCCCCCCCTAACGTTA
5101 CTGGCCGAAGCCGCTTGGAAATAAGGCCGGTGTGCGTTTGTCTATATGTTA
5151 TTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGG
5201 CCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCTCTCGCCAAAG
5251 GAATGCAAGGTCTGTTGAATGTGCTGAAGGAAGCAGTTCCTCTGGAAGCT
5301 TCTTGAAGACAAACAACGTCTGTAGCGACCTTTGCAAGGAGCGGAACCC
5351 CCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATA
5401 CACCTGCAAAGGCGGCACAACCCAGTGCCACGTTGTGAGTTGGATAGTT
5451 GTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAA
5501 GGATGCCCAGAAGGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGT
5551 GCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCC
5601 CCGAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATGATAATATGG
5651 CCTCCTTTGTCTCTCTGCTCCTGGTAGGCATCCTATTCCATGCCACCCAG
5701 GCCGAGCTCACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAG
5751 GGCCACCATCAACTGCAAGTCCAGCCAGAGTGTTTTGTACAGCTCCAACA
5801 ATAAGAACTATTTAGCTTGGTATCAGCAGAAACCAGGACAGCCTCCTAAG
5851 CTGCTCATTTACTGGGCATCTACCCGGGAATCCGGGGTCCCTGACCGATT
5901 CAGTGGCAGCGGGTCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGC
5951 AGGCTGAAGATGTGGCAGTTTATTACTGTCAGCAATATTATAGTACTCAG

34/35

Figure 19c

6001 ACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGAACTGTGGCTGCACC
6051 ATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTG
6101 CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTA
6151 CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGT
6201 CACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGA
6251 CGCTGAGCAAAGCAGACTACGAGAAACACAAACTCTACGCCTGCGAAGTC
6301 ACCCATCAGGGCCTGAGATCGCCCGTCACAAAGAGCTTCAACAAGGGGAG
6351 AGTGTTAGTTCTAGATAATTAATTAGGAGGAGATCTCGAGCTCGCGAAAG
6401 CTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCCTGGCG
6451 TTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCCTCCTA
6501 GGTCGACATCGATAAAATAAAAGATTTTATTAGTCTCCAGAAAAGGGG
6551 GGAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCC
6601 ATTTTGCAAGGCATGGAAAAATACATAACTGAGAATAGAGAAGTTCAGAT
6651 CAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCT
6701 GTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGC
6751 TGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCT
6801 CAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTT
6851 CTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACC
6901 CTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGC
6951 GCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCAACCCCTCACTCGG
7001 GGCGCCAGTCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAAT
7051 AAACCCCTCTTGCAAGTTCATCCGACTTGTGGTCTCGCTGTTCCCTGGGAG
7101 GGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATTTGGG
7151 GGCTCGTCCGGGATCGGGAGACCCCTGCCAGGGACCACCGACCCACCAC
7201 CGGGAGGTAAGCTGGCTGCCTCGCGCGTTTCGGTGATGACGGTGAAAACC
7251 TCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGAT
7301 GCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTG
7351 TCGGGGCGCAGCCATGACCCAGTCACGTAGCGATAGCGGAGTGTATACTG
7401 GCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGC
7451 GGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGC
7501 TCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTGTTCCGGCTGCG
7551 GCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAAT
7601 CAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCC
7651 AGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCC
7701 CCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACC
7751 CGACAGGACTATAAAGATACCAGGCGTTTTCCCCCTGGAAGCTCCCTCGTG
7801 CGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCT
7851 CCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCA
7901 GTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCC
7951 GTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAA
8001 CCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGA
8051 TTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGG
8101 CCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCT
8151 GAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAC
8201 AAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACG
8251 CGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTC
8301 TGACGCTCAGTGGAAACGAAACTCACGTTAAGGGATTTTGGTCATGAGAT
8351 TATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTT
8401 AAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATG
8451 CTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCA
8501 TAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTA
8551 CCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGC
8601 TCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAA
8651 GTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGG
8701 GAAGCTAGAGTAAGTAGTTCCGCCAGTTAATAGTTTTCGCAACGTTGTTGC
8751 CATTGCTGCAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCAT
8801 TCAGCTCCGGTTCCTAACGATCAAGGCGAGTTACATGATCCCCCATGTTG
8851 TGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAA
8901 GTTGGCCGCAAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTC
8951 TTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCA
9001 ACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCC
9051 GGCGTCAACACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGC

35/35

Figure 19d

```
9101 TCATCATTTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCG
9151 CTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCAACTGATCTTC
9201 AGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGC
9251 AAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTC
9301 ATACTCTTCCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCT
9351 CATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGG
9401 TTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATT
9451 ATTATCATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTCG
1. TCTTCAAGAAT
```

Features:

```
149-737 Moloney murine sarcoma virus 5' LTR
807-1616 Extended Packaging Region
1680-1735 EM7 promoter (bacteriophage T7 promoter)
1754-2151 Blasticidin resistance gene coding sequence
2310-2440 SV40 poly A signal and site
2603-3420 CMV IE promoter
3675-4988 G-protein-coupled receptor (GPCR)
5071-5646 IRES
5647-5703 Bovine a-lactalbumin signal peptide
5704-6372 'humanized' antibody light chain
6553-7146 MoMuLV 3' LTR
7683Origin of replication
9302-8442 b-Lactmase coding sequence
```