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(56) Fremdragne publikationer:
US-A1- 2005 186 180
US-A1- 2005 208 066
US-A1- 2011 201 086
US-B2- 8 703 469
ALEXANDRA SPENGER ET AL: "Altering the surface properties of baculovirus Autographa californica NPV by insertional mutagenesis of the envelope protein gp64", EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 269, no. 18, 1 September 2002 (2002-09-01), pages 4458-4467, XP055331498, GB ISSN: 0014-2956, DOI: 10.1046/j.1432-1033.2002.03135.x
ZHOU J ET AL: "Mapping the conformational epitope of a neutralizing antibody (AcV1) directed against the AcMNPV GP64 protein", VIROLOGY, ELSEVIER, AMSTERDAM, NL, vol. 352, no. 2, 1 September 2006 (2006-09-01), pages 427-437, XP024896411, ISSN: 0042-6822, DOI: 10.1016/J.VIROL.2006.04.041 [retrieved on 2006-09-01]
SPENGER ET AL.: 'Altering the surface properties of baculovirus Autographa californica NPV by insertional mutagenesis of the envelope protein gp64' EUR. J. BIOCHEM vol. 269, no. 18, 28 August 2002, pages 4458 - 4467, XP055331498

Fortsættes ...

DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention relates generally to recombinant baculoviruses.

BACKGROUND OF THE INVENTION

[0002] Baculovirus infects insects and is non-pathogenic to humans, but can transduce a broad range of mammalian and avian cells. Thanks to the biosafety, large cloning capacity, low cytotoxicity and non-replication nature in the transduced cells as well as the ease of manipulation and production, baculovirus has gained explosive popularity as a gene delivery vector for a wide variety of applications such as antiviral therapy, cancer therapy, regenerative medicine and vaccine.

[0003] U.S. Patent No. 7,527,967 (or US 2005/0208066) discloses a recombinant baculovirus that displays a fusion heterologous polypeptide on the surface of the baculovirus for use in generating an antibody or an immune response against a heterologus protein or virus in a subject in need thereof. The fusion heterologous polypeptides therein is made by fusing a heterologus antigen with the carboxyl terminal amino acids from 227 to 529 of baculovirus GP64 protein (FIG. 2C). The construct therein contains a substantial portion of the extracellular domain of GP64 including B12D5 binding site. When it is used in immunization, the extracellular domain of GP64 may elicit an immune resposne and produce unintended antibodies such as useless anti-GP64 B12D5 antibody (Zhou et al. Virology 2006; 352(2): 427-437). GP64 B12D5 antibody is a neutralization antibody against baculovirus itself instead of a foreign antigen of interest. In addition, baculovirus is slightly immunogenic to porcine.

[0004] Taiwanese Patent No. I368656 discloses a method of using the signal peptide, transmembrane domain and the cytoplasmic transduction domain from GP64 to present an antigen. The construct therein contains only the transmembrane domain and cytoplasmic trunsduction domain without the extracellular domain of GP64 (FIG. 2D). It has less expression of foreign antigen and is sensitive to inactivation reagents (Premanand et al. PLoS ONE 2013; 8(2): e55536).

[0005] Therefore, a heretofore unaddressed need exists in the art to address the aforementioned deficiencies and inadequacies, especially in connection with a baculovirus vector that is insensitive to inactivation reagent and has improved immunogenicity.

[0006] Spenger et al. (Eur. J. Biochem. 269, 4458-4467, 2002) disclose altering the surface properties of baculovirus Autographa californica NPV by insertional mutagenesis of the envelope protein gp64.

SUMMARY OF THE INVENTION

[0007] In one aspect, the invention relates to a vector comprising a transgene encoding a fusion protein, the fusion protein comprising: (a) a signal peptide located at the N-terminus of the fusion protein; (b) an antigen; and (c) a C-terminal region of baculovirus envelope GP64 protein, comprising at least 100 amino acid residues in length and lacking a B12D5 binding epitope consisting of the amino acid sequence of SEQ ID NO: 2 or 3 located within the central basic region of the GP64 protein; wherein the antigen is located at the C-terminus of the signal peptide.

[0008] In another aspect, the invention relates to a vector comprising a transgene encoding a fusion protein, the fusion protein comprising:

1. (a) a signal peptide located at the N-terminus of the fusion protein;
2. (b) an antigen; and
3. (c) a C-terminal region of baculovirus envelope GP64 protein, having at least 100 amino acid residues in length and lacking a B12D5 binding epitope located within the GP64 protein's central basic region consisting of the amino acid sequence of SEQ ID NO: 4;

wherein the antigen is located at the C-terminus of the signal peptide.

[0009] In one embodiment of the invention, the vector of the invention is a recombinant baculovirus.

[0010] In another aspect, the invention relates to a recombinant baculovirus displaying on its envelope a fusion protein, the fusion protein comprising: (i) a heterologous antigen; and (ii) a C-terminal region of baculovirus envelope GP64 protein, comprising at least 100 amino acid residues in length and lacking a B12D5 binding epitope consisting of the amino acid sequence of SEQ ID NO: 2 or 3 located within the central basic region of the GP64 protein; wherein the heterologous antigen is located at the N-terminus of the fusion protein displaying on the envelope of the recombinant baculovirus.

[0011] Further in another aspect, the invention relates to a recombinant baculovirus displaying on its envelope a fusion protein, the fusion protein comprising:

1. (i) a heterologous antigen; and
2. (ii) a C-terminal region of baculovirus envelope GP64 protein, comprising at least 100 amino acid residues in length and lacking a B12D5 binding epitope located within the GP64 protein's central basic region consisting of the amino acid sequence of SEQ ID NO: 4;

wherein the heterologous antigen is located at the N-terminus of the fusion protein displaying on the envelope of the recombinant baculovirus.

[0012] In one embodiment of the invention, the genome of the recombinant baculovirus comprises a transgene encoding a fusion protein comprising: (a) a signal peptide; (b) the heterologous antigen; and (c) the C-terminal region of the baculovirus envelope GP64 protein; wherein the antigen is located at the C-terminus of the signal peptide.

[0013] In another embodiment of the invention, the transgene is operably linked to a promoter. In another embodiment of the invention, the promoter is polyhedrin.

[0014] In another embodiment of the invention, the C-terminal region of the GP64 protein has from 186 to 220 amino acids in length.

[0015] In another embodiment of the invention, the C-terminal region of the GP64 protein lacks the amino acid sequence of SEQ ID NO: 2, 3, or 4.

[0016] In another embodiment of the invention, the C-terminal region of the GP64 protein comprises the amino acids from 293 to 512 of SEQ ID NO: 1.

[0017] In another embodiment of the invention, the C-terminal region of the GP64 protein comprises amino acids from 327 to 512 of SEQ ID NO: 1.

[0018] In another embodiment of the invention, the C-terminal region of the GP64 protein has an N-terminus starting from between amino acid residues 292 and 328 of SEQ ID NO: 1.

[0019] In another embodiment of the invention, the signal peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, and 12.

[0020] Further in another aspect, the invention relates to an insect cell or a cell comprising the vector or the recombinant baculovirus of the invention.

[0021] In another embodiment of the invention, the antigen is at least one selected from the group consisting of a pathogen protein, a cancer cell protein, and an immune checkpoint protein.

[0022] The pathogen may be at least one selected from the group consisting of human papillomavirus, porcine reproductive and respiratory syndrome virus, human immunodeficiency virus-1, Dengue virus, hepatitis C virus, hepatitis B virus, porcine circovirus 2, classical swine fever virus, foot-and-mouth disease virus, Newcastle disease virus, transmissible gastroenteritis virus, porcine epidemic diarrhea virus, influenza virus, pseudorabies virus, parvovirus, swine vesicular disease virus, poxvirus, rotavirus, Mycoplasma pneumonia, herpes virus, infectious bronchitis, and infectious bursal disease virus. The cancer may be at least one selected from the group consisting of non-small cell lung cancer, breast carcinoma, melanoma, lymphomas, colon carcinoma, hepatocellular carcinoma, and any combination thereof. The immune check point may be at least one selected from the group consisting of PD-1, PD-L1, PD-L2, and CTLA-4.

[0023] In another embodiment of the invention, the antigen is at least one selected from the group consisting of classical swine fever virus envelope glycoprotein E2, porcine epidemic diarrhea virus S1 protein, programmed cell death protein 1, and a tumor-associated antigen.

[0024] Yet in another aspect, the invention relates to a vector or a recombinant baculovirus according to the invention for use in treatment in eliciting an antigen-specific immunogenic response in a subject in need thereof.

[0025] These and other aspects will become apparent from the following description of the preferred embodiment taken in conjunction with the following drawings.The accompanying drawings illustrate one or more embodiments of the invention and, together with the written description, serve to explain the principles of the invention. Wherever possible, the same reference numbers are used throughout the drawings to refer to the same or like elements of an embodiment.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026]

FIG. 1A is a schematic drawing illustrating a baculovirus vector platform design according to one embodiment of the invention.

FIG. 1B is a schematic drawing illustrating a foreign antigen or foreign genes not only can be anchored onto the virus envelope but also expressed in the membrane fraction of insect cells by a gBac surface display platform. The rectangle represents a virus.

FIG. 2A is a schematic drawing showing a full-length GP64 protein. GP64 minimum ($GP64_{327-482}$), GP64 transmembrane domain (TM) ($GP64_{483-505}$); cytoplasmic transduction domain (CTD) ($GP64_{506-512}$).

FIG. 2B is a schematic drawing showing a baculovirus vector according to one embodiment of the invention. SP: signal peptide; TM: transmembrane domain, CTD: cytoplasmic transduction domain.

FIG. 2C is a schematic drawing showing a baculovirus vector design disclosed in United States Patent No. 7527967.

FIG. 2D is a schematic drawing showing a baculovirus vector design disclosed in Taiwan Patent No. 1368656.

FIG. 3 is a graph showing an immune response induced by baculovirus vectors in mice post vaccination (left panel) and neutralization serum (SN) titer measurement of E2-gBac subunit vaccine (right panel) in mice. Each ELISA value shown is an average value from 5 mice. The

difference between the gBac and Bac groups is statistically significant, P≦0.05. The strain of baculovirus used was AcNPV. The term "SN" stands for neutralization serum. The term "E2-gBac" stands for "baculovirus vector CSFV E2-gBac" according to the vector design of FIG. 2B. The term "E2-gBac (inactivated)" means the baculovirus was inactivated before it was used for immunization. The term "E2-Bac" stands for "baculovirus vector CSFV E2-Bac" according to the vector design of FIG. 2D.

FIG. 4 is a graph showing an immune response induced by baculovirus vectors in pigs post vaccination. Each ELISA value shown is an average value from 3 pigs. The difference between the gBac and gp64 groups is statistically significant, P≦0.05. The term "E2-gp64" stands for "baculovirus vector CSFV E2-gp64" according to the vector design of FIG. 2C.

FIG. 5 is a graph showing antibody titers against porcine epidemic diarrhea virus (PEDV) in 3 specific pathogen-free (SPF) pigs post vaccination with the baculovirus vector PEDV S1-gBac. The 3 SPF pigs were labeled as number 73, 74 and 75, respectively. The tem "IFA" stands for immunofluorescent antibody. The results indicated that the serum from the vaccinated pigs could be recognized by PED virus.

FIGs. 6A-B are photographs of western blot showing that the antigens E2 of CSFV E2-gBac (FIG. 6A) and S1 of PEDV S1-gBac (FIG. 6B) from the Sf9 cell samples were detected.

FIGs. 6C-D are photographs of western blot showing that the antigens of hPD-1-gBac in the samples were recognized and detected by anti-gp64 mAb (left panel) and human PD-1 antibody (right panel), respectively.

DETAILED DESCRIPTION OF THE INVENTION

[0027] The present invention is more particularly described in the following examples that are intended as illustrative only. Various embodiments of the invention are now described in detail.

DEFINITIONS

[0028] A vector is a vehicle used to transfer genetic material to a target cell. A viral vector is a virus modified to deliver foreign genetic material into a cell.

[0029] The term "gene transduction", " transduce", or "transduction" is a process by which a foreign DNA is introduced into another cell via a viral vector.

[0030] A signal sequence or signal peptide (sometimes referred to as signal sequence, targeting signal, localization signal, localization sequence, transit peptide, leader sequence or leader peptide) is a short (5-30 amino acids long) peptide present at the N-terminus of the

majority of newly synthesized proteins that are destined towards the secretory pathway. These proteins include those that reside either inside certain organelles (the endoplasmic reticulum, golgi or endosomes), secreted from the cell, or inserted into most cellular membranes.

[0031] The term "B12D5" refers to a monoclonal antibody against gp64. B12D5 has a binding epitope of KKRPPTWRHNV (SEQ ID NO: 3) at 277-287 of gp64 (SEQ ID NO: 1), or HRVKKRPPTW (SEQ ID NO: 2) located within the central region from residues 271 to 292 (SEQ ID NO: 4) of gp64 (SEQ ID NO: 1). *See* Zhou et al. (2006) *Supra*; Wu et al (2012) "A pH-Sensitive Heparin-Binding Sequence from Baculovirus gp64 Protein Is Important for Binding to Mammalian Cells but Not to Sf9 Insect Cells" Journal of Virology, Vol. 86 (1) 484-491.

[0032] Programmed cell death protein 1, also known as PD-1 and CD279 (cluster of differentiation 279), is a protein that in humans is encoded by the *PDCD1* gene. PD-1 is a cell surface receptor that belongs to the immunoglobulin superfamily and is expressed on T cells and pro-B cells. PD-1 binds two ligands, PD-L1 and PD-L2. PD-1, functioning as an immune checkpoint, plays an important role in down regulating the immune system by preventing the activation of T-cells, which in turn reduces autoimmunity and promotes self-tolerance. The inhibitory effect of PD-1 is accomplished through a dual mechanism of promoting apoptosis (programmed cell death) in antigen specific T-cells in lymph nodes while simultaneously reducing apoptosis in regulatory T cells (suppressor T cells). A new class of drugs that block PD-1, the PD-1 inhibitors, activate the immune system to attack tumors and are therefore used with varying success to treat some types of cancer.

[0033] An antigen may be a pathogenic protein, polypeptide or peptide that is responsible for a disease caused by the pathogen or is capable of inducing an immunological response in a host infected by the pathogen, or tumor-associated antigen (TAA) which is a polypeptide specifically expressed in tumor cells. The antigen may be selected from a pathogen or cancer cells including, but not limited to, Human Papillomavirus (HPV), Porcine reproductive and respiratory syndrome virus (PRRSV), Human immunodeficiency virus-1 (HIV-1), Dengue virus, Hepatitis C virus (HCV), Hepatitis B virus (HBV), Porcine Circovirus 2 (PCV2), Classical Swine Fever Virus (CSFV), Foot-and-mouth disease virus (FMDV), Newcastle disease virus (NDV), Transmissible gastroenteritis virus (TGEV), Porcine epidemic diarrhea virus (PEDV), Influenza virus, Pseudorabies virus, Parvovirus, Pseudorabies virus, Swine vesicular disease virus (SVDV), Poxvirus, Rotavirus, Mycoplasma pneumonia, Herpes virus, infectious bronchitis, or infectious bursal disease virus, non-small cell lung cancer, breast carcinoma, melanoma, lymphomas, colon carcinoma, hepatocellular carcinoma and any combination thereof. For example, HPV E7 protein (E7), HCV core protein (HCV core), HBV X protein (HBx) were selected as antigens for vaccine development. The antigen may be a fusion antigen from a fusion of two or more antigens selected from one or more pathogenic proteins. For example, a fusion antigen of PRRSV ORF6 and ORF5 fragments, or a fusion of antigens from PRRSV and PCV2 pathogens.

[0034] Alternatively, an antigen may be an inhibitory immune checkpoint protein such as PD-1, PD-L1, PD-L2, and CTLA-4, etc.

[0035] The terms "immune checkpoint protein" and "Immune checkpoint" are interchangeable. Immune checkpoints affect immune system functioning. Immune checkpoints can be stimulatory or inhibitory. Tumors can use these checkpoints to protect themselves from immune system attacks. Checkpoint therapy can block inhibitory checkpoints, restoring immune system function. One ligand-receptor interaction under investigation is the interaction between the transmembrane programmed cell death 1 protein (PDCD1, PD-1; also known as CD279) and its ligand, PD-1 ligand 1 (PD-L1, CD274). PD-L1 on the cell surface binds to PD1 on an immune cell surface, which inhibits immune cell activity. Among PD-L1 functions is a key regulatory role on T cell activities. It appears that (cancer-mediated) upregulation of PD-L1 on the cell surface may inhibit T cells that might otherwise attack. Antibodies that bind to either PD-1 or PD-L1 and therefore block the interaction may allow the T-cells to attack the tumor. Ipilimumab is the first checkpoint antibody approved by the FDA. It blocks inhibitory immune checkpoint CTLA-4.

[0036] The term "treating" or "treatment" refers to administration of an effective amount of the fusion protein to a subject in need thereof, who has cancer or infection, or a symptom or predisposition toward such a disease, with the purpose of cure, alleviate, relieve, remedy, ameliorate, or prevent the disease, the symptoms of it, or the predisposition towards it. Such a subject can be identified by a health care professional based on results from any suitable diagnostic method.

[0037] The term "an effective amount" refers to the amount of an active compound that is required to confer a therapeutic effect on the treated subject. Effective doses will vary, as recognized by those skilled in the art, depending on rout of administration, excipient usage, and the possibility of co-usage with other therapeutic treatment.

EXAMPLES

EXAMPLE 1

Construction of Vectors and Generation of Recombinant Baculoviruses, Virus-like Particles, and Proteins

[0038] FIG. 1A shows a gBac platform. A foreign gene (e.g., optimized classical swine fever virus (CSFV) E2 or porcine epidemic diarrhea virus (PEDV) spike genes) may be obtained by PCR synthesis and then cloned into a gBac vector through restriction enzyme sites (e.g., SacI/ NotI) using IN-FUSION® cloning kit (Clontech). The gBac vector is derived from baculovirus transfer vector pBACPAK8™ (GENBANK™ accession No. U02446). Using the gBac surface display platform of FIG. 1A, a foreign antigen or foreign genes can be anchored onto the virus

envelope and also expressed in the membrane fraction of insect cells (FIG. 1B). FIG. 2A shows a full-length GP64. The baculovirus GP64 envelope fusion protein (GP64 EFP) is the major envelope fusion glycoprotein in some, though not all, baculoviruses. It is found on the surface of both infected cells and budded virions as a homotrimer. Baculovirus enters its host cells by endocytosis followed by a low-pH-induced fusion of the viral envelope with the endosomal membrane, allowing viral entrance into the cell cytoplasm. This membrane fusion, and also the efficient budding of virions from the infected cell, is dependent on GP64.

[0039] FIGs. 2B-D show comparisons of three vectors, gBac (or Reber-gBac), antigen-gp64 (or abbreviated as "gp64" disclosed in US 7527967), and Antigen-Bac (or abbreviated as "Bac" disclosed in TW 1368656). The gBac vector was constructed using the signal peptide (SP) and a C-terminal region of GP64, which is from amino acids 327 to 512 of SEQ ID NO: 1. The gp64 vector was constructed using the SP and a C-terminal region of GP64, which is from amino acids 227 to 512 of SEQ ID NO: 1. The Bac vector comprises the insect signal peptide (SP), the TM (transmembrane domain) and CTD (cytoplasmic transduction domain) of GP64 and does not comprise the GP64 extracellular domain amino acids. The symbol triangle before the "SP" represents the polyhedrin promoter, which is located -1 site of the insert gene's start codon.

[0040] A DNA fragment encoding the SP was generated and amplified by PCR with forward and reverse pirmers 5'-GAGCTCATGGTAAGC-3' (SEQE ID NO: 19) and 5'-AGGCAGAATGCG-3' (SEQ ID NO: 20), respectively. The C-terminal portion of the gp64 gene (encoding GP64 from aa 327 to aa 512 of SEQ ID NO: 1) was generated and amplified by PCR using the forward and reverse pirmers 5'-GCGTGTCTGCTCA-3' (SEQ ID NO: 21) and 5'-TTAATATTGTCTA-3' (SEQ ID NO: 22), respectively. The C-terminal portion of the gp64 gene (encoding GP64 from aa 227 to aa 512 of SEQ ID NO: 1) was generated and amplified by PCR using the forward and reverse pirmers 5'-ATCAACAAGCTAA-3' (SEQ ID NO: 23) and 5'-TTAATATTGTCTA-3' (SEQ ID NO: 24), respectively. The above foreign genes were resepctively cloned into the pBACPAK8™ transfer vector.

[0041] Using the gBac platform of 2B (or FIG. 1A), we generated two baculovirus vectors: the baculovirus vector CSFV E2-gBac and the baculovirus vector PEDV S1-gBac (FIG. 2B design). The former transduces a gene encoding classical swine fever virus (CSFV) envelope glycoprotein E2, and the latter transduces a gene encoding porcine epidemic diarrhea virus S1 Protein.

[0042] For a parallel comparison, we have also used the vectors of FIGs. 2C and 2D and generated two separate baculovirus vectors: the baculovirus vector CSFV E2-gp64 (FIG. 2C design), and the baculovirus vector CSFV E2-Bac (FIG. 2D design) for transducing a foreign gene encoding the antigen CSFV E2.

[0043] The fusion genes in the gBac, gp64, and Bac vectors (FIGs. 2B-D designs, respectively) were sequenced to confirm their identities. Recombinant baculovirus viruses containing the recombinant GP64 gene without the transfer vector backbone were generated

by homologous recombination. Methods for this construction and recombination are well known in the art. These recombinant viruses were used to prepare antigens. The modified GP64 gene was inserted into a baculovirus transfer vector under the polyhedron promoter by PCR to form the gBac vector. Because the gBac vector has two homologous recombination sites, the original polyhedron locus of wild-type baculovirus is replaced by gBac sequence when co-transfection with gBac plasmid and wild-type baculovirus.

EXAMPLE 2

Protein expression and purification of antigens

[0044] Sf9 cells were used for propagaton and infection of recombiant baculovirus. Baculovirus was propagated in Spodoptera frugiperda Sf-9 cell lines and grown at 26°C in a serum-free medium. The recombinant baculoviruses were produced by infecting Sf-9 cells at an MOI of 5-10 and harvested 3 days after infection. To produce baculovirus on a large scale, the cells may be cultured and infected in bioreactors. The baculovirus fraction were isolated from the culture supernatant of the infected cell. The baculovirus particles were collected by Tangential Flow Filtration (TFF) using a suitable protein cut-off membrane and 0.45 μm sterile membrane.

[0045] To test the production of the antigens, the partially purified baculoviruses or infected cell lysates were subjected to 8-10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. The foreign antigens were detected by mouse anti-gp64 mAb (available from commercial sources and used at 1:5,000 dilution) as the primary antibody. The secondary antibodies were goat anti-rabbit or goat anti-mouse mAb conjugated with alkaline phosphatase (1:5,000 dilution). The protein bands were visualized by ECL PLUS™ Western Blotting Detection Reagents (GE Healthcare). In the baculovirus surface display system, the forein antigens can be firstly expressed on insect cell membrane, then the budding baculovirus take some of cell membrane to form its envelope. As the result, baculovirus display antigens can be harvested from infected cell lysate (mambrane fraction) and virus surface.

EXAMPLE 3

Expression of the antigens by recombinant viruses

[0046] Before immunization with gBac vaccines, to confirm whether the fusion protein CSFV E2-gBac or PEDV S1-gBac were successfully displayed on the baculovirus envelope, recombinant baculoviruses were collected by centrifugation and TFF. The recombinant baculoviruses were resuspended in PBS at different concentrations of p.f.u/μl. Incorporation of

recombinant proteins into the baculovirus particles were analyzed by Western blotting using anti-gp64 mAb (eBioscience). The fusion protein CSFV E2-gBae was detected at a molecular weight of about 80 kDa and the fusion protein PEDV S1-gBac was detected at a molecular weight of 130 kDa.

EXAMPLE 4

Vaccine preparation and immunization

[0047] The recombinant baculoviruses displaying antigens were mixed with water-in-oil-in-water (w/o/w) adjuvant (MONTANIDE ISA 206 VG. SEPPIC.) for vaccine preparation. Female BALB/c mice of 4-week old, purchased from National Experimental Animal Center, Taiwan, R.O.C., were divided into four groups with 5 mice per group. Mice were immunized subcutaneously twice on day 0 and day-14 (FIG. 3) with 200 µl of a solution containing with 10^7 p.f.u. recombinant bactilovirus. The virus of E2-gBac, E2-gp64, and E2-Bac. (FIGs. 2B-D), containing CSFV envelope glycoprotein E2, were inactivated and formulated as vaccines. As negative controls, mice were injected with wild type baculovirus (10^7 p.f.u.) or PBS. The blood samples were collected from the caudal vein at week 6. Binary ethylenimine (BEI) was used to inactivate baculoviruses. It was prepared by dissolving the 0.1 M 2-bromoethylamine hydrobromide (BEA) in 0.2 N NaOH at 37°C for 1 h. To inactivate the baculovirus, the viral medium was added 4 mM BEI and virus incubated for 16 h at 37°C. After inactivation, sodium thiosulfate ($Na_2S_2O_3$) was added to the medium at a final concentration of 10 times the final BEI concentration to stop the inactivation.

EXAMPLE 5

Immunogenicity of antigens produced by recombinant viruses

[0048] After immunization, the sera of mice were analyzed for the presence of anti-CSFV E2 antibody using the IDEXX CSFV Ab Test Kit (IDEXX) to detect classical swine fever virus (CSFV) antibodies. The degree of CSFV E2-specific antibodies in the serum was calculated as positive when the blocking percentage was above 40% (FIG. 3). The results indicate that the baculovirus vector displayed the fusion protein CSFV E2-Bac. FIG. 3 show that the baculovirus vector CSFV E2-gBac, whether the baculovirus was inactivated or not, induced a much higher anti-CSFV E2 antibody titer than the baculovirus vector CSFV E2-Bac in mice.

[0049] We have also compared the effects of the three baculovirus vectors in inducing immunogenic responses in pigs. Three SPF (specific pathogen free) pigs were immunized

twice (6-week-old and 9-week-old) via intramuscular route with 10^8 pfu of recombinant baculovirus vectors E2-gBac, E2-gp64, E2-Bac, and PBS, respectively. After immunization, the sera of piglets were analyzed for the presence of CSFV E2 antibody using IDEXX CSFV Ab Test Kit (IDEXX). The degree of CSFV E2 specific neutralization antibody in the serum was calculated as positive and efficient when the blocking percentage was above 43%.

[0050] As shown in FIG. 4, pigs immunized with E2-gBac were able to survive the CSFV challenge. It indicates that competent neutralization antibody had been induced in the pigs (SN titer ≥16, IDEXX blocking ratio ≥ 43%). This proves that the gBac platform is a vaccine platform. FIG. 4 shows that the baculovirus vector CSFV E2-gBac induced a much higher anti-CSFV E2 antibody titer than the baculovirus vectors CSFV E2-Bac and CSFV E2-gp64 in pigs. The results indicate that the gBac surface display platform of the invention can induce a stronger immunity than the prior art baculovirus vectors.

[0051] We have also gerenated baculovirus vector for transducing porcine epidemic diarrhea virus S1 protein (PEDV S1) into cells for vaccination applications. We tested its effects in inducing an immunogenic response. Briefly, three 9-week-old SPF pigs (labeled as number 73, 74 and 75, respectively) were each immunized twice via an intramuscular route with 10^8 pfu of recombinant baculovirus vector PEDV S1-gBac or PBS. After immunization, the sera of pigs (from 1 to 4 weeks post vaccination) were analyzed for the presence of anti-PEDV antibodies using an ELISA assay of PED virus. FIG. 5 shows that the baculovirus vector PEDV S1-gBac induced antibody titers against porcine epidemic diarrhea virus in all 3 pigs.

[0052] FIGs. 6A-B are photographs of Western blots showing that the antigens E2 of CSFV E2-gBac (FIG. 6A) and S1 of PEDV S1-gBac (FIG. 6B) from the Sf9 cell samples were detected. The cell lysates and collected virus were loaded by different cell number and virus titer. We have also constructed the baculovirus vector human programmed cell death protein 1 (hPD-1gBac) -gBac (hPD-1gBac). To test the production of the antigen, the recombinant baculoviruses or infected cell lysates were subjected to 8-10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. The foreign antigens were detected by a mouse anti-gp64 mAb (FIG. 6C) and anti-PDCD-1 antibody (FIG. 6D) (Santa Cruz Biotechnology, Santa Cruz, CA) as the primary antibodies. The protein bands were visualized by ECL PLUS™ Western Blotting Detection Reagents (GE Healthcare). FIG. 6D shows that the baculovirus displayed human PD-1 antigen on the envelope, and the displayed PD-1 antigen could be recognized by commercial anti-PDCD-1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

[0053] In summary, the vector of the invention is insensitive to inactivation reagents (FIG. 3) and exhibits higher immunogenicity (FIG. 4). Table 1 shows peptide sequences and SEQ ID NOs.

Table 1

Protein or peptide	Amino acid sequence* (SEQ ID NO:)	a.a. length
Full length GP64	*MVSAIVLYVLLAAAAHSAFA*AEHCNAQMKTGPYKIKNLDITPPKET LQKDVEITIVETDYNENVIIGYKGYYQAYAYNGGSLDPNTRVEE TMKTLNVGKEDLLMWSIRQQCEVGEELIDRWGSDSDDCFRDNE GRGQWVKGKELVKRQNNNHFAHHTCNKSWRCGISTSKMYSRL ECQDDTDECQVYILDAEGNPINVTVDTVLHRDGVSMILKQKSTF TTRQIKAACLLIKDDKNNPESVTREHCLIDNDIYDLSKNTWNCK FNRCIKRKVEHRVKKRPPTWRHNVRAKYTEGDTATKGDLMHIQ EELMYENDLLKMNIELMHAHINKLNNMLHDLIVSVAKVDERLI GNLMNNSVSSTFLSDDTFLLMPCTNPPAHTSNCYNNSIYKEGRW VANTDSSQCIDFSNYKELAIDDDVEFWIPTIGNTTYHDSWKDAS GWSFIAQQKSNLITTMENTKFGGVGTSLSDITSMAEGELAAKLT SFMFGHVVNFVIILIVILFLYCMI*RNRNRQY* (SEQ ID NO: 1)	512
B12D5 binding epitope	HRVKKRPPTW (SEQ ID NO: **2**; 274-283 of GP64).	
B12D5 binding epitope	KKRPPTWRHNV (277-287 of gp64; SEQ ID NO: **3**)	
Gp64 central basic region	KVEHRVKKRPPTWRHNVRAKYT (271-292 of gp64; SEQ ID NO: **4**)	
GP64 signal peptide (SP)	MVSAIVLYVLLAAAAHSAFA ($GP64_{1-20}$; SEQ ID NO: **5**)	20
30K protein *Bombyx mori*	MRLTLFAFVLAVCALASNA (SEQ ID NO: **6**)	
SP1 *Bombyx mori*	MRVLVLLACLAAASA (SEQ ID NO: **7**)	
SP2 *Bombyx mori*	MKSVLILAGLVAVALSSAVPKP (SEQ ID NO: **8**)	
Bombyxin A-4	MKILLAIALMLSTVMWVST (SEQ ID NO: **9**)	
Vitellogenin	MKLFVLAAIIAAVSS (SEQ ID NO: **10**)	
Chitinase precursor	MRAIFATLAVLASCAALVQS (SEQ ID NO: **11**)	
Adipokinetic hormone	MYKLTVFLMFIAF VIIAGAQSMASLTRQDLA (SEQ ID NO: **12**)	
CSFV E2 (Classical swine fever virus (CSFV) envelope	MLRGQVVQGIIWLLLVTGAQGRLSCKEDHRYAISSTNEIGPLGA EGLTTTWKEYNHGLQLDDGTVRAICIAGSFKVTALNVVSRRYL ASLHKRALPTSVTFELLFDGTSPAIEEMGDDFGFGLCPFDTTPVV	363

Protein or peptide	Amino acid sequence* (SEQ ID NO:)	a.a. length
glycoprotein E2) *CSFV 96TD*	KGKYNTTLLNGSAFYLVCPIGWTGVIECTAVSPTTLRTEVVKTF KREKPFPHRVDCVTTIVEKEDLFYCKLGGNWTCVKGNPVTYTG GQVRQCRWCGFDFKEPDGLPHYPIGKCILTNETGYRVVDSPDCN RDGVVISTEGEHECLIGNTTVKVHALDGRLAPMPCRPKEIVSSA GPVRKTSCTFNYTKTLRNKYYEPRDSYFQQYMLKGEYQYWFDL DVTDHHTDYFAEF (SEQ ID NO: **13**)	
PEDV S 1	CSANTNFRRFFSKFNVQAPAVVVLGGYLPIGENQGVNSTWYCA	708
(Porcine Epidemic Diarrhea Virus S1 Protein) *PEDV USAIowa11898 4l2013*	GQHPTASGVHGIFVSHIRGGHGFEIGISQEPFDPSGYQLYLHKAT NGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAH MSEHSVVGITWDNDRVTVFSDKIYYFYFKNDWSRVATKCYNSG GCAMQYVYEPTYYMLNVTSAGEDGISYQPCTANCIGYAANVFA TEPNGHIPEGFSFNNWFLLSNDSTLVHGKVVSNQPLLVNCLLAIP KIYGLGQFFSFNQTIDGVCNGAAVQRAPEALRFNINDTSVILAEG SIVLHTALGTNFSFVCSNSSNPHLATFAIPLGATQVPYYCFFKVD TYNSTVYKFLAVLPPTVREIVITKYGDVYVNGFGYLHLGLLDAV TINFTGHGTDDDVSGFWTIASTNFVDALIEVQGTAIQRILYCDDP VSQLKCSQVAFDLDDGFYPISSRNLLSHEQPISFVTLPSFNDHSFV NITVSASFGGHSGANLIASDTTINGFSSFCVDTRQFTISLFYNVTN SYGYVSKSQDSNCPFTLQSVNDYLSFSKFCVSTSLLASACTIDLF GYPEFGSGVKFTSLYFQFTKGELITGTPKPLEGVTDVSFMTLDVC TKYTIYGFKGEGIITLTNSSFLAGVYYTSDSGQLLAFKNVTSGAV YSVTPCSFSEQAAYVDDDIVGVISSLSSSTFNSTREIPG (SEQ ID NO: **14**)	
Human PD-1 (Programmed cell death protein 1)	QIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFFPALLVV TEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQ PGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPK AQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTDIY (SEQ ID NO: **15**)	170
B2-gBac	*MVSAIVLYVLLAAAAHSAFA*MLRGQVVQGIWLLLVTGAQGRLSC KEDHRYAISSTNEIGPLGAEGLTTTWKEYNHGLQLDDGTVRAICI AGSFKVTALNVVSRRYLASLHKRALPTSVTFELLFDGTSPAIEEM GDDFGFGLCPFDTTPVVKGKYNTTLLNGSAFYLVCPIGWTGVIE CTAVSPTTLRTEVVKTFKREKPFPHRVDCVTTIVEKEDLFYCKLG	

Protein or peptide	Amino acid sequence* (SEQ ID NO:)	a.a. length
protein (SP-E2-GP64 mini-TM/CTD)	GNWTCVKGNPVTYTGGQVRQCRWCGFDFKEPDGLPHYPIGKCI LTNETGYRVVDSPDCNRDGVVISTEGEHECLIGNTTVKVHALDG RLAPMPCRPKEIVSSAGPVRKTSCTFNYTKTLRNKYYEPRDSYF QQYMLKGEYQYWFDLDVTDHHTDYFAEFINKLNNMLHDLIVS VAKVDERLIGNLMNNSVSSTFLSDDTFLLMPCTNPPAHTSNCYN NSIYKEGRWVANTDSSQCIDFSNYKELAIDDDVEFWIPTIGNTTY HDSWKDASGWSFIAQQKSNLITTMENTKFGGVGTSLSDITSMAE	569
	GELAAKLTS**FMFGHVVNFVIILIVILFLYCMI***RNRNRQY* (SEQ ID NO: **16**)	
S 1-gBac protein (SP-S1-GP64 mini-TM/CTD)	*MVSAIVLYVLLAAAAIHSAFA*CSANTNFRRFFSKFNVQAPAVVVLGG YLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGIS QEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANN DVTTGRNCLFNKAIPAHMSEHSVVGITWDNDRVTVFSDKIYYFY FKNDWSRVATKCYNSGGCAMQYVYEPTYYMLNVTSAGEDGIS YQPCTANCIGYAANVFATEPNGHIPEGFSFNNWFLLSNDSTLVH GKVVSNQPLLVNCLLAIPKIYGLGQFFSFNQTIDGVCNGAAVQR APEALRFNINDTSVILAEGSIVLHTALGTNFSFVCSNSSNPHLATF AIPLGATQVPYYCFFKVDTYNSTVYKFLAVLPPTVREIVITKYGD VYVNGFGYLHLGLLDAVTINFTGHGTDDDVSGFWTIASTNFVD ALIEVQGTAIQRILYCDDPVSQLKCSQVAFDLDDGFYPISSRNLLS HEQPISFVTLPSFNDHSFVNITVSASFGGHSGANLIASDTTINGFSS FCVDTRQFTISLFYNVTNSYGYVSKSQDSNCPFTLQSVNDYLSFS KFCVSTSLLASACTIDLFGYPEFGSGVKFTSLYFQFTKGELITGTP KPLEGVTDVSFMTLDVCTKYTIYGFKGEGIITLTNSSFLAGVYYT SDSGQLLAFKNVTSGAVYSVTPCSFSEQAAYVDDDIVGVISSLSS STFNSTRELPGINKLNNMLHDLIVSVAKVDERLIGNLMNNSVSST FLSDDTFLLMPCTNPPAHTSNCYNNSIYKEGRWVANTDSSQCID FSNYKELAIDDDVEFWIPTIGNTTYHDSWKDASGWSFIAQQKSN LITTMENTKFGGVGTSLSDITSMAEGELAAKLTS**FMFGHVVNFV IILIVILFLYCMI***RNRNRQY* (SEQ ID NO: **17**)	914
hPD 1-gBac	*MFSAIVLYVLLAAAAIHSAFA*QIPQAPWPVVWAVLQLGWRPGWFL DSPDRPWNPPTFFPALLVVTEGDNATFTCSFSNTSESFVLNWYR MSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVR ARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPS	

Protein or peptide	Amino acid sequence* (SEQ ID NO:)	a.a. length
protein (SP-hPD1-GP64 mini-TM/CTD)	PSPRPAGQFQTDIYINKLNNMLHDLIVSVAKVDERLIGNLMNNS VSSTFLSDDTFLLMPCTNPPAHTSNCYNNSIYKEGRWVANTDSS QCIDFSNYKELAIDDDVEFWIPTIGNTTYHDSWKDASGWSFIAQ QKSNLITTMENTKFGGVGTSLSDITSMAEGELAAKLTSFMFGHV VNFVIILIVILFLYCMI*RNRNRQY* (SEQ ID NO: 18)	376

*Full length GP64: The GP64 Signal peptide (SP) ($GP64_{1-20}$) is underlined and in italics. The GP64 minimum ($GP64_{327-482}$) is underlined without italics. The GP64 transmembrane domain (TM) ($GP64_{483-505}$) is in bold only. The GP64 cytoplasmic transduction domain (CTD) ($GP64_{506-512}$) is in italics only.
E2-gBac protein (SP-E2-GP64 mini-TM/CTD): The GP64 Signal peptide (SP) ($GP64_{1-20}$) is underlined and in italics. The GP64 minimum ($GP64_{327-482}$) is underlined without italics. The GP64 transmembrane domain (TM) ($GP64_{483-505}$) is in bold only. The GP64 cytoplasmic transduction domain (CTD) ($GP64_{506-512}$) is in italics only.
S1-gBac protein (SP-S1-GP64 mini-TM/CTD): The GP64 Signal peptide (SP) ($GP64_{1-20}$) is underlined and in italics. The GP64 minimum ($GP64_{327-482}$) is underlined without italics. The GP64 transmembrane domain (TM) ($GP64_{483-505}$) is in bold only. The GP64 cytoplasmic transduction domain (CTD) ($GP64_{506-512}$) is in italics only.
hPD1-gBac protein (SP-hPD1-GP64 mini-TM/CTD): The GP64 Signal peptide (SP) ($GP64_{1-20}$) is underlined and in italics. The GP64 minimum ($GP64_{327-482}$) is underlined without italics. The GP64 transmembrane domain (TM) ($GP64_{483-505}$) is in bold only. The GP64 cytoplasmic transduction domain (CTD) ($GP64_{506-512}$) is in italics only.

SEQUENCE LISTING

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<130> 10025-00001

<150> 62/162,139
<151> 2015-05-15

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Tyr Lys Ile Lys Asn Leu Asp Ile Thr Pro Pro Lys Glu Thr Leu Gln
        35                  40                  45

Lys Asp Val Glu Ile Thr Ile Val Glu Thr Asp Tyr Asn Glu Asn Val
    50                  55                  60

Ile Ile Gly Tyr Lys Gly Tyr Tyr Gln Ala Tyr Ala Tyr Asn Gly Gly
65                  70                  75                  80

Ser Leu Asp Pro Asn Thr Arg Val Glu Glu Thr Met Lys Thr Leu Asn
                85                  90                  95

Val Gly Lys Glu Asp Leu Leu Met Trp Ser Ile Arg Gln Gln Cys Glu
            100                 105                 110

Val Gly Glu Glu Leu Ile Asp Arg Trp Gly Ser Asp Ser Asp Asp Cys
        115                 120                 125

Phe Arg Asp Asn Glu Gly Arg Gly Gln Trp Val Lys Gly Lys Glu Leu
    130                 135                 140

Val Lys Arg Gln Asn Asn Asn His Phe Ala His His Thr Cys Asn Lys
145                 150                 155                 160

Ser Trp Arg Cys Gly Ile Ser Thr Ser Lys Met Tyr Ser Arg Leu Glu

                165                 170                 175

Cys Gln Asp Asp Thr Asp Glu Cys Gln Val Tyr Ile Leu Asp Ala Glu
            180                 185                 190

Gly Asn Pro Ile Asn Val Thr Val Asp Thr Val Leu His Arg Asp Gly
        195                 200                 205

Val Ser Met Ile Leu Lys Gln Lys Ser Thr Phe Thr Thr Arg Gln Ile
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Lys Asn Thr Trp Asn Cys Lys Phe Asn Arg Cys Ile Lys Arg Lys Val
            260                 265                 270

Glu His Arg Val Lys Lys Arg Pro Pro Thr Trp Arg His Asn Val Arg
        275                 280                 285

Ala Lys Tyr Thr Glu Gly Asp Thr Ala Thr Lys Gly Asp Leu Met His
    290                 295                 300

Ile Gln Glu Glu Leu Met Tyr Glu Asn Asp Leu Leu Lys Met Asn Ile
305                 310                 315                 320

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    370                 375                 380

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385                 390                 395                 400

Gln Cys Ile Asp Phe Ser Asn Tyr Lys Glu Leu Ala Ile Asp Asp Asp
                405                 410                 415

Val Glu Phe Trp Ile Pro Thr Ile Gly Asn Thr Thr Tyr His Asp Ser
            420                 425                 430

Trp Lys Asp Ala Ser Gly Trp Ser Phe Ile Ala Gln Gln Lys Ser Asn
        435                 440                 445

Leu Ile Thr Thr Met Glu Asn Thr Lys Phe Gly Gly Val Gly Thr Ser
    450                 455                 460

Leu Ser Asp Ile Thr Ser Met Ala Glu Gly Glu Leu Ala Ala Lys Leu
465                 470                 475                 480

Thr Ser Phe Met Phe Gly His Val Val Asn Phe Val Ile Ile Leu Ile
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            20                  25                  30
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<210> 13

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<213> Classical swine fever virus

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            20                  25                  30

Ile Ser Ser Thr Asn Glu Ile Gly Pro Leu Gly Ala Glu Gly Leu Thr

        35                  40                  45

Thr Thr Trp Lys Glu Tyr Asn His Gly Leu Gln Leu Asp Asp Gly Thr
    50                  55                  60

Val Arg Ala Ile Cys Ile Ala Gly Ser Phe Lys Val Thr Ala Leu Asn
65                  70                  75                  80

Val Val Ser Arg Arg Tyr Leu Ala Ser Leu His Lys Arg Ala Leu Pro
                85                  90                  95

Thr Ser Val Thr Phe Glu Leu Leu Phe Asp Gly Thr Ser Pro Ala Ile
            100                 105                 110

Glu Glu Met Gly Asp Asp Phe Gly Phe Gly Leu Cys Pro Phe Asp Thr
        115                 120                 125

Thr Pro Val Val Lys Gly Lys Tyr Asn Thr Thr Leu Leu Asn Gly Ser
    130                 135                 140

Ala Phe Tyr Leu Val Cys Pro Ile Gly Trp Thr Gly Val Ile Glu Cys
145                 150                 155                 160

Thr Ala Val Ser Pro Thr Thr Leu Arg Thr Glu Val Val Lys Thr Phe
                165                 170                 175

Lys Arg Glu Lys Pro Phe Pro His Arg Val Asp Cys Val Thr Thr Ile
            180                 185                 190

Val Glu Lys Glu Asp Leu Phe Tyr Cys Lys Leu Gly Gly Asn Trp Thr
        195                 200                 205

Cys Val Lys Gly Asn Pro Val Thr Tyr Thr Gly Gly Gln Val Arg Gln
    210                 215                 220
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Cys Arg Trp Cys Gly Phe Asp Phe Lys Glu Pro Asp Gly Leu Pro His
225                 230                 235                 240

Tyr Pro Ile Gly Lys Cys Ile Leu Thr Asn Glu Thr Gly Tyr Arg Val
                245                 250                 255

Val Asp Ser Pro Asp Cys Asn Arg Asp Gly Val Val Ile Ser Thr Glu
            260                 265                 270

Gly Glu His Glu Cys Leu Ile Gly Asn Thr Thr Val Lys Val His Ala
        275                 280                 285

Leu Asp Gly Arg Leu Ala Pro Met Pro Cys Arg Pro Lys Glu Ile Val
    290                 295                 300

Ser Ser Ala Gly Pro Val Arg Lys Thr Ser Cys Thr Phe Asn Tyr Thr
305                 310                 315                 320

Lys Thr Leu Arg Asn Lys Tyr Tyr Glu Pro Arg Asp Ser Tyr Phe Gln
                325                 330                 335

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            20                  25                  30

Asn Gln Gly Val Asn Ser Thr Trp Tyr Cys Ala Gly Gln His Pro Thr
        35                  40                  45

Ala Ser Gly Val His Gly Ile Phe Val Ser His Ile Arg Gly Gly His
    50                  55                  60

Gly Phe Glu Ile Gly Ile Ser Gln Glu Pro Phe Asp Pro Ser Gly Tyr
65                  70                  75                  80

Gln Leu Tyr Leu His Lys Ala Thr Asn Gly Asn Thr Asn Ala Thr Ala
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Arg Leu Arg Ile Cys Gln Phe Pro Ser Ile Lys Thr Leu Gly Pro Thr
            100                 105                 110
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Ala Asn Asn Asp Val Thr Thr Gly Arg Asn Cys Leu Phe Asn Lys Ala
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Ile Pro Ala His Met Ser Glu His Ser Val Val Gly Ile Thr Trp Asp
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Asn Asp Arg Val Thr Val Phe Ser Asp Lys Ile Tyr Tyr Phe Tyr Phe
145                 150                 155                 160

Lys Asn Asp Trp Ser Arg Val Ala Thr Lys Cys Tyr Asn Ser Gly Gly
                165                 170                 175

Cys Ala Met Gln Tyr Val Tyr Glu Pro Thr Tyr Tyr Met Leu Asn Val
            180                 185                 190

Thr Ser Ala Gly Glu Asp Gly Ile Ser Tyr Gln Pro Cys Thr Ala Asn
        195                 200                 205

Cys Ile Gly Tyr Ala Ala Asn Val Phe Ala Thr Glu Pro Asn Gly His
    210                 215                 220

Ile Pro Glu Gly Phe Ser Phe Asn Asn Trp Phe Leu Leu Ser Asn Asp
225                 230                 235                 240

Ser Thr Leu Val His Gly Lys Val Val Ser Asn Gln Pro Leu Leu Val
                245                 250                 255

Asn Cys Leu Leu Ala Ile Pro Lys Ile Tyr Gly Leu Gly Gln Phe Phe
            260                 265                 270

Ser Phe Asn Gln Thr Ile Asp Gly Val Cys Asn Gly Ala Ala Val Gln
        275                 280                 285

Arg Ala Pro Glu Ala Leu Arg Phe Asn Ile Asn Asp Thr Ser Val Ile
    290                 295                 300

Leu Ala Glu Gly Ser Ile Val Leu His Thr Ala Leu Gly Thr Asn Phe
305                 310                 315                 320

Ser Phe Val Cys Ser Asn Ser Ser Asn Pro His Leu Ala Thr Phe Ala
                325                 330                 335

Ile Pro Leu Gly Ala Thr Gln Val Pro Tyr Tyr Cys Phe Phe Lys Val
            340                 345                 350

Asp Thr Tyr Asn Ser Thr Val Tyr Lys Phe Leu Ala Val Leu Pro Pro
        355                 360                 365

Thr Val Arg Glu Ile Val Ile Thr Lys Tyr Gly Asp Val Tyr Val Asn
    370                 375                 380

Gly Phe Gly Tyr Leu His Leu Gly Leu Leu Asp Ala Val Thr Ile Asn
385                 390                 395                 400

Phe Thr Gly His Gly Thr Asp Asp Asp Val Ser Gly Phe Trp Thr Ile
                405                 410                 415

Ala Ser Thr Asn Phe Val Asp Ala Leu Ile Glu Val Gln Gly Thr Ala
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            420                 425                 430

Ile Gln Arg Ile Leu Tyr Cys Asp Asp Pro Val Ser Gln Leu Lys Cys
        435                 440                 445

Ser Gln Val Ala Phe Asp Leu Asp Asp Gly Phe Tyr Pro Ile Ser Ser
    450                 455                 460

Arg Asn Leu Leu Ser His Glu Gln Pro Ile Ser Phe Val Thr Leu Pro
465                 470                 475                 480

Ser Phe Asn Asp His Ser Phe Val Asn Ile Thr Val Ser Ala Ser Phe
                485                 490                 495

Gly Gly His Ser Gly Ala Asn Leu Ile Ala Ser Asp Thr Thr Ile Asn
            500                 505                 510

Gly Phe Ser Ser Phe Cys Val Asp Thr Arg Gln Phe Thr Ile Ser Leu
        515                 520                 525

Phe Tyr Asn Val Thr Asn Ser Tyr Gly Tyr Val Ser Lys Ser Gln Asp
    530                 535                 540

Ser Asn Cys Pro Phe Thr Leu Gln Ser Val Asn Asp Tyr Leu Ser Phe
545                 550                 555                 560

Ser Lys Phe Cys Val Ser Thr Ser Leu Leu Ala Ser Ala Cys Thr Ile
                565                 570                 575

Asp Leu Phe Gly Tyr Pro Glu Phe Gly Ser Gly Val Lys Phe Thr Ser
            580                 585                 590

Leu Tyr Phe Gln Phe Thr Lys Gly Glu Leu Ile Thr Gly Thr Pro Lys
        595                 600                 605

Pro Leu Glu Gly Val Thr Asp Val Ser Phe Met Thr Leu Asp Val Cys
    610                 615                 620

Thr Lys Tyr Thr Ile Tyr Gly Phe Lys Gly Glu Gly Ile Ile Thr Leu
625                 630                 635                 640

Thr Asn Ser Ser Phe Leu Ala Gly Val Tyr Tyr Thr Ser Asp Ser Gly
                645                 650                 655

Gln Leu Leu Ala Phe Lys Asn Val Thr Ser Gly Ala Val Tyr Ser Val
            660                 665                 670

Thr Pro Cys Ser Phe Ser Glu Gln Ala Ala Tyr Val Asp Asp Asp Ile
        675                 680                 685

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    690                 695                 700

Glu Leu Pro Gly
705
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            20                  25                  30

Pro Pro Thr Phe Phe Pro Ala Leu Leu Val Val Thr Glu Gly Asp Asn
        35                  40                  45

Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val Leu
    50                  55                  60

Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala Ala
65                  70                  75                  80

Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg Val
                85                  90                  95

Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg Ala
            100                 105                 110

Arg Arg Asn Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu Ala
        115                 120                 125

Pro Lys Ala Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val Thr
    130                 135                 140

Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro Arg
145                 150                 155                 160

Pro Ala Gly Gln Phe Gln Thr Asp Ile Tyr
                165                 170
```

<210> 16

<211> 569

<212> PRT

<213> Artificial Sequence

<220>

<223> CSFV E2-gBac protein

<400> 16

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Met Val Ser Ala Ile Val Leu Tyr Val Leu Leu Ala Ala Ala Ala His
1               5                  10                  15

Ser Ala Phe Ala Met Leu Arg Gly Gln Val Val Gln Gly Ile Ile Trp
            20                  25                  30

Leu Leu Leu Val Thr Gly Ala Gln Gly Arg Leu Ser Cys Lys Glu Asp
        35                  40                  45
```

```
His Arg Tyr Ala Ile Ser Ser Thr Asn Glu Ile Gly Pro Leu Gly Ala
    50                  55                  60

Glu Gly Leu Thr Thr Thr Trp Lys Glu Tyr Asn His Gly Leu Gln Leu
65                  70                  75                  80

Asp Asp Gly Thr Val Arg Ala Ile Cys Ile Ala Gly Ser Phe Lys Val
                85                  90                  95

Thr Ala Leu Asn Val Val Ser Arg Arg Tyr Leu Ala Ser Leu His Lys
            100                 105                 110

Arg Ala Leu Pro Thr Ser Val Thr Phe Glu Leu Leu Phe Asp Gly Thr
        115                 120                 125

Ser Pro Ala Ile Glu Glu Met Gly Asp Asp Phe Gly Phe Gly Leu Cys
    130                 135                 140

Pro Phe Asp Thr Thr Pro Val Val Lys Gly Lys Tyr Asn Thr Thr Leu
145                 150                 155                 160

Leu Asn Gly Ser Ala Phe Tyr Leu Val Cys Pro Ile Gly Trp Thr Gly
                165                 170                 175

Val Ile Glu Cys Thr Ala Val Ser Pro Thr Thr Leu Arg Thr Glu Val
            180                 185                 190

Val Lys Thr Phe Lys Arg Glu Lys Pro Phe Pro His Arg Val Asp Cys
        195                 200                 205

Val Thr Thr Ile Val Glu Lys Glu Asp Leu Phe Tyr Cys Lys Leu Gly
    210                 215                 220

Gly Asn Trp Thr Cys Val Lys Gly Asn Pro Val Thr Tyr Thr Gly Gly
225                 230                 235                 240

Gln Val Arg Gln Cys Arg Trp Cys Gly Phe Asp Phe Lys Glu Pro Asp
                245                 250                 255

Gly Leu Pro His Tyr Pro Ile Gly Lys Cys Ile Leu Thr Asn Glu Thr
            260                 265                 270

Gly Tyr Arg Val Val Asp Ser Pro Asp Cys Asn Arg Asp Gly Val Val
        275                 280                 285

Ile Ser Thr Glu Gly Glu His Glu Cys Leu Ile Gly Asn Thr Thr Val
    290                 295                 300

Lys Val His Ala Leu Asp Gly Arg Leu Ala Pro Met Pro Cys Arg Pro
305                 310                 315                 320

Lys Glu Ile Val Ser Ser Ala Gly Pro Val Arg Lys Thr Ser Cys Thr
                325                 330                 335

Phe Asn Tyr Thr Lys Thr Leu Arg Asn Lys Tyr Tyr Glu Pro Arg Asp
```

```
          340                 345                 350

Ser Tyr Phe Gln Gln Tyr Met Leu Lys Gly Glu Tyr Gln Tyr Trp Phe
        355                 360                 365

Asp Leu Asp Val Thr Asp His His Thr Asp Tyr Phe Ala Glu Phe Ile
    370                 375                 380

Asn Lys Leu Asn Asn Met Leu His Asp Leu Ile Val Ser Val Ala Lys
385                 390                 395                 400

Val Asp Glu Arg Leu Ile Gly Asn Leu Met Asn Asn Ser Val Ser Ser
                405                 410                 415

Thr Phe Leu Ser Asp Asp Thr Phe Leu Leu Met Pro Cys Thr Asn Pro
            420                 425                 430

Pro Ala His Thr Ser Asn Cys Tyr Asn Asn Ser Ile Tyr Lys Glu Gly
        435                 440                 445

Arg Trp Val Ala Asn Thr Asp Ser Ser Gln Cys Ile Asp Phe Ser Asn
    450                 455                 460

Tyr Lys Glu Leu Ala Ile Asp Asp Asp Val Glu Phe Trp Ile Pro Thr
465                 470                 475                 480

Ile Gly Asn Thr Thr Tyr His Asp Ser Trp Lys Asp Ala Ser Gly Trp
                485                 490                 495

Ser Phe Ile Ala Gln Gln Lys Ser Asn Leu Ile Thr Thr Met Glu Asn
            500                 505                 510

Thr Lys Phe Gly Gly Val Gly Thr Ser Leu Ser Asp Ile Thr Ser Met
        515                 520                 525

Ala Glu Gly Glu Leu Ala Ala Lys Leu Thr Ser Phe Met Phe Gly His
    530                 535                 540

Val Val Asn Phe Val Ile Ile Leu Ile Val Ile Leu Phe Leu Tyr Cys
545                 550                 555                 560

Met Ile Arg Asn Arg Asn Arg Gln Tyr
                565
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<210> 17

<211> 914

<212> PRT

<213> Artificial Sequence

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<223> S1-gBac protein

<400> 17

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1               5                  10                  15
```

```
Ser Ala Phe Ala Cys Ser Ala Asn Thr Asn Phe Arg Arg Phe Phe Ser
            20                  25                  30

Lys Phe Asn Val Gln Ala Pro Ala Val Val Val Leu Gly Gly Tyr Leu
        35                  40                  45

Pro Ile Gly Glu Asn Gln Gly Val Asn Ser Thr Trp Tyr Cys Ala Gly
    50                  55                  60

Gln His Pro Thr Ala Ser Gly Val His Gly Ile Phe Val Ser His Ile
65                  70                  75                  80

Arg Gly Gly His Gly Phe Glu Ile Gly Ile Ser Gln Glu Pro Phe Asp
                85                  90                  95

Pro Ser Gly Tyr Gln Leu Tyr Leu His Lys Ala Thr Asn Gly Asn Thr
            100                 105                 110

Asn Ala Thr Ala Arg Leu Arg Ile Cys Gln Phe Pro Ser Ile Lys Thr
        115                 120                 125

Leu Gly Pro Thr Ala Asn Asn Asp Val Thr Thr Gly Arg Asn Cys Leu
    130                 135                 140

Phe Asn Lys Ala Ile Pro Ala His Met Ser Glu His Ser Val Val Gly
145                 150                 155                 160

Ile Thr Trp Asp Asn Asp Arg Val Thr Val Phe Ser Asp Lys Ile Tyr
                165                 170                 175

Tyr Phe Tyr Phe Lys Asn Asp Trp Ser Arg Val Ala Thr Lys Cys Tyr
            180                 185                 190

Asn Ser Gly Gly Cys Ala Met Gln Tyr Val Tyr Glu Pro Thr Tyr Tyr
        195                 200                 205

Met Leu Asn Val Thr Ser Ala Gly Glu Asp Gly Ile Ser Tyr Gln Pro
    210                 215                 220

Cys Thr Ala Asn Cys Ile Gly Tyr Ala Ala Asn Val Phe Ala Thr Glu
225                 230                 235                 240

Pro Asn Gly His Ile Pro Glu Gly Phe Ser Phe Asn Asn Trp Phe Leu
                245                 250                 255

Leu Ser Asn Asp Ser Thr Leu Val His Gly Lys Val Val Ser Asn Gln
            260                 265                 270

Pro Leu Leu Val Asn Cys Leu Leu Ala Ile Pro Lys Ile Tyr Gly Leu
        275                 280                 285

Gly Gln Phe Phe Ser Phe Asn Gln Thr Ile Asp Gly Val Cys Asn Gly
    290                 295                 300

Ala Ala Val Gln Arg Ala Pro Glu Ala Leu Arg Phe Asn Ile Asn Asp
305                 310                 315                 320
```

```
Thr Ser Val Ile Leu Ala Glu Gly Ser Ile Val Leu His Thr Ala Leu
                325                 330                 335

Gly Thr Asn Phe Ser Phe Val Cys Ser Asn Ser Ser Asn Pro His Leu
            340                 345                 350

Ala Thr Phe Ala Ile Pro Leu Gly Ala Thr Gln Val Pro Tyr Tyr Cys
        355                 360                 365

Phe Phe Lys Val Asp Thr Tyr Asn Ser Thr Val Tyr Lys Phe Leu Ala
    370                 375                 380

Val Leu Pro Pro Thr Val Arg Glu Ile Val Ile Thr Lys Tyr Gly Asp
385                 390                 395                 400

Val Tyr Val Asn Gly Phe Gly Tyr Leu His Leu Gly Leu Leu Asp Ala
                405                 410                 415

Val Thr Ile Asn Phe Thr Gly His Gly Thr Asp Asp Asp Val Ser Gly
            420                 425                 430

Phe Trp Thr Ile Ala Ser Thr Asn Phe Val Asp Ala Leu Ile Glu Val
        435                 440                 445

Gln Gly Thr Ala Ile Gln Arg Ile Leu Tyr Cys Asp Asp Pro Val Ser
    450                 455                 460

Gln Leu Lys Cys Ser Gln Val Ala Phe Asp Leu Asp Asp Gly Phe Tyr
465                 470                 475                 480

Pro Ile Ser Ser Arg Asn Leu Leu Ser His Glu Gln Pro Ile Ser Phe
                485                 490                 495

Val Thr Leu Pro Ser Phe Asn Asp His Ser Phe Val Asn Ile Thr Val
            500                 505                 510

Ser Ala Ser Phe Gly Gly His Ser Gly Ala Asn Leu Ile Ala Ser Asp
        515                 520                 525

Thr Thr Ile Asn Gly Phe Ser Ser Phe Cys Val Asp Thr Arg Gln Phe
    530                 535                 540

Thr Ile Ser Leu Phe Tyr Asn Val Thr Asn Ser Tyr Gly Tyr Val Ser
545                 550                 555                 560

Lys Ser Gln Asp Ser Asn Cys Pro Phe Thr Leu Gln Ser Val Asn Asp
                565                 570                 575

Tyr Leu Ser Phe Ser Lys Phe Cys Val Ser Thr Ser Leu Leu Ala Ser

            580                 585                 590

Ala Cys Thr Ile Asp Leu Phe Gly Tyr Pro Glu Phe Gly Ser Gly Val
        595                 600                 605
```

```
Lys Phe Thr Ser Leu Tyr Phe Gln Phe Thr Lys Gly Glu Leu Ile Thr
    610                 615                 620

Gly Thr Pro Lys Pro Leu Glu Gly Val Thr Asp Val Ser Phe Met Thr
625                 630                 635                 640

Leu Asp Val Cys Thr Lys Tyr Thr Ile Tyr Gly Phe Lys Gly Glu Gly
                645                 650                 655

Ile Ile Thr Leu Thr Asn Ser Ser Phe Leu Ala Gly Val Tyr Tyr Thr
            660                 665                 670

Ser Asp Ser Gly Gln Leu Leu Ala Phe Lys Asn Val Thr Ser Gly Ala
        675                 680                 685

Val Tyr Ser Val Thr Pro Cys Ser Phe Ser Glu Gln Ala Ala Tyr Val
    690                 695                 700

Asp Asp Asp Ile Val Gly Val Ile Ser Ser Leu Ser Ser Ser Thr Phe
705                 710                 715                 720

Asn Ser Thr Arg Glu Leu Pro Gly Ile Asn Lys Leu Asn Asn Met Leu
                725                 730                 735

His Asp Leu Ile Val Ser Val Ala Lys Val Asp Glu Arg Leu Ile Gly
            740                 745                 750

Asn Leu Met Asn Asn Ser Val Ser Ser Thr Phe Leu Ser Asp Asp Thr
        755                 760                 765

Phe Leu Leu Met Pro Cys Thr Asn Pro Pro Ala His Thr Ser Asn Cys
    770                 775                 780

Tyr Asn Asn Ser Ile Tyr Lys Glu Gly Arg Trp Val Ala Asn Thr Asp
785                 790                 795                 800

Ser Ser Gln Cys Ile Asp Phe Ser Asn Tyr Lys Glu Leu Ala Ile Asp
                805                 810                 815

Asp Asp Val Glu Phe Trp Ile Pro Thr Ile Gly Asn Thr Thr Tyr His
            820                 825                 830

Asp Ser Trp Lys Asp Ala Ser Gly Trp Ser Phe Ile Ala Gln Gln Lys
        835                 840                 845

Ser Asn Leu Ile Thr Thr Met Glu Asn Thr Lys Phe Gly Gly Val Gly
    850                 855                 860

Thr Ser Leu Ser Asp Ile Thr Ser Met Ala Glu Gly Glu Leu Ala Ala
865                 870                 875                 880

Lys Leu Thr Ser Phe Met Phe Gly His Val Val Asn Phe Val Ile Ile
                885                 890                 895

Leu Ile Val Ile Leu Phe Leu Tyr Cys Met Ile Arg Asn Arg Asn Arg
            900                 905                 910
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Gln Tyr

<210> 18

<211> 376

<212> PRT

<213> Artificial Sequence

<220>

<223> hPD1-gBac protein

<400> 18

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Ser Ala Phe Ala Gln Ile Pro Gln Ala Pro Trp Pro Val Val Trp Ala
            20                  25                  30

Val Leu Gln Leu Gly Trp Arg Pro Gly Trp Phe Leu Asp Ser Pro Asp
        35                  40                  45

Arg Pro Trp Asn Pro Pro Thr Phe Phe Pro Ala Leu Leu Val Val Thr
    50                  55                  60

Glu Gly Asp Asn Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu
65                  70                  75                  80

Ser Phe Val Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp
                85                  90                  95

Lys Leu Ala Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys
            100                 105                 110

Arg Phe Arg Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser

        115                 120                 125

Val Val Arg Ala Arg Arg Asn Asp Ser Gly Thr Tyr Leu Cys Gly Ala
    130                 135                 140

Ile Ser Leu Ala Pro Lys Ala Gln Ile Lys Glu Ser Leu Arg Ala Glu
145                 150                 155                 160

Leu Arg Val Thr Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro Ser
                165                 170                 175

Pro Ser Pro Arg Pro Ala Gly Gln Phe Gln Thr Asp Ile Tyr Ile Asn
            180                 185                 190

Lys Leu Asn Asn Met Leu His Asp Leu Ile Val Ser Val Ala Lys Val
        195                 200                 205

Asp Glu Arg Leu Ile Gly Asn Leu Met Asn Asn Ser Val Ser Ser Thr
    210                 215                 220
```

```
Phe Leu Ser Asp Asp Thr Phe Leu Leu Met Pro Cys Thr Asn Pro Pro
225                 230                 235                 240

Ala His Thr Ser Asn Cys Tyr Asn Asn Ser Ile Tyr Lys Glu Gly Arg
                245                 250                 255

Trp Val Ala Asn Thr Asp Ser Ser Gln Cys Ile Asp Phe Ser Asn Tyr
            260                 265                 270

Lys Glu Leu Ala Ile Asp Asp Asp Val Glu Phe Trp Ile Pro Thr Ile
        275                 280                 285

Gly Asn Thr Thr Tyr His Asp Ser Trp Lys Asp Ala Ser Gly Trp Ser
    290                 295                 300

Phe Ile Ala Gln Gln Lys Ser Asn Leu Ile Thr Thr Met Glu Asn Thr
305                 310                 315                 320

Lys Phe Gly Gly Val Gly Thr Ser Leu Ser Asp Ile Thr Ser Met Ala
                325                 330                 335

Glu Gly Glu Leu Ala Ala Lys Leu Thr Ser Phe Met Phe Gly His Val
            340                 345                 350

Val Asn Phe Val Ile Ile Leu Ile Val Ile Leu Phe Leu Tyr Cys Met
        355                 360                 365

Ile Arg Asn Arg Asn Arg Gln Tyr
    370                 375
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<210> 19

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> SP forward primer

<400> 19

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<210> 20

<211> 12

<212> DNA

<213> Artificial Sequence

<220>

<223> SP reverse pirmer

<400> 20

aggcagaatg cg 12

<210> 21

<211> 13
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<220>
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<400> 21
gcgtgtctgc tca 13

<210> 22
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<220>
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<400> 22
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<210> 23
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<220>
<223> gp64 forward pirmer

<400> 23
atcaacaagc taa 13

<210> 24
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> gp64 reverse pirmer

<400> 24
ttaatattgt cta 13

REFERENCES CITED IN THE DESCRIPTION

Patent documents cited in the description

- US7527967B [0003] [0026] [0039]
- US20050208066A [0003]
- TWI368656 [0004]
- TW1368656 [0026] [0039]
- WO62162139A [0054]

Non-patent literature cited in the description

- **ZHOU et al.**Virology, 2006, vol. 352, 2427-437 [0003]
- **PREMANAND et al.**PLoS ONE, 2013, vol. 8, 2e55536- [0004]
- **SPENGER et al.**Eur. J. Biochem., 2002, vol. 269, 4458-4467 [0006]
- **WU et al.**A pH-Sensitive Heparin-Binding Sequence from Baculovirus gp64 Protein Is Important for Binding to Mammalian Cells but Not to Sf9 Insect CellsJournal of Virology, 2012, vol. 86, 1484-491 [0031]

Patentkrav

1. Rekombinant baculovirus, der på dets kappe viser et fusionsprotein, hvilket fusionsprotein omfatter:

(i) et heterologt antigen og

(ii) et C-terminalt område af baculovirus GP64-kappeprotein, der omfatter mindst 100 aminosyrerester i længden og mangler en B12D5-bindingsepitop bestående af aminosyresekvensen ifølge SEQ ID NO: 2 eller 3 placeret inde i det centrale basiske område af GP64-proteinet;

hvor det heterologe antigen er placeret ved N-terminus af fusionsproteinet, der vises på kappen af det rekombinant baculovirus.

2. Rekombinant baculovirus ifølge krav 1, hvis genom omfatter et transgen, der koder for et fusionsprotein, omfattende:

(a) et signalpeptid;

(b) det heterologe antigen og

(c) det C-terminale område af baculovirus GP64-kappeproteinet;

hvor antigenet er placeret ved signalpeptidets C-terminus.

3. Vektor, der omfatter et transgen, der koder for et fusionsprotein, hvilket fusionsprotein omfatter:

(a) et signalpeptid placeret ved fusionsproteinets N-terminus;

(b) et antigen og

(c) et C-terminalt område af baculovirus GP64-kappeprotein, der omfatter mindst 100 aminosyrerester i længden og mangler en B12D5-bindingsepitop bestående af aminosyresekvensen ifølge SEQ ID NO: 2 eller 3 placeret inde i det centrale basiske område af GP64-proteinet;

hvor antigenet er placeret ved signalpeptidets C-terminus.

4. Vektor ifølge krav 3, der er et rekombinant baculovirus.

5. Rekombinant baculovirus, der på dets kappe viser et fusionsprotein, hvilket fusionsprotein omfatter:

(i) et heterologt antigen og

(ii) et C-terminalt område af baculovirus GP64-kappeprotein, der omfatter mindst 100

aminosyrerester i længden og mangler en B12D5-bindingsepitop placeret inde i GP64-proteinets centrale basiske område bestående af aminosyresekvensen ifølge SEQ ID NO: 4;

hvor det heterologe antigen er placeret ved N-terminus af fusionsproteinet, der vises på kappen af det rekombinante baculovirus.

6. Vektor omfattende et transgen, der koder for et fusionsprotein, hvilket fusionsprotein omfatter:

(a) et signalpeptid, der er placeret ved fusionsproteinets N-terminus;

(b) et antigen og

(c) et C-terminalt område af baculovirus GP64-kappeprotein, der omfatter mindst 100 aminosyrerester i længden og mangler en B12D5 bindingsepitop placeret inde i GP64 proteinet centrale basiske område bestående af aminosyresekvensen ifølge SEQ ID NO: 4;

hvor antigenet er placeret ved signalpeptidets C-terminus.

7. Rekombinant baculovirus ifølge krav 1, hvor det C-terminale område af GP64-proteinet omfatter aminosyrer fra 327 til 512 ifølge SEQ ID NO: 1.

8. Rekombinant baculovirus ifølge krav 1, hvor det C-terminale område af GP64-proteinet har et N-terminus, der starter fra mellem aminosyrerester 292 og 328 ifølge SEQ ID NO: 1.

9. Rekombinant baculovirus ifølge krav 2, hvor signalpeptidet omfatter en aminosyresekvens udvalgt fra gruppen bestående af SEQ ID NO: 5, 6, 7, 8, 9, 10, 11 og 12.

10. Insektcelle, der omfatter det rekombinante baculovirus ifølge krav 1.

11. Rekombinant baculovirus ifølge krav 1, hvor antigenet er mindst ét udvalgt fra gruppen bestående af et patogent protein, et cancercelleprotein og et immuncheckpunktsprotein.

12. Rekombinant baculovirus ifølge krav 11, hvor:

(i) patogenet er mindst ét udvalgt fra gruppen bestående af humant papillomavirus, reproduktivt og respiratorisk syndromvirus hos svin, humant immundefektvirus-1, Denguevirus, hepatitis C-virus, hepatitis B-virus, porcint circovirus 2, klassisk svinefebervirus, mund- og klovsygevirus, Newcastle disease-virus, overførbar gastroenteritis, epidemisk diarrevirus hos svin, influenzavirus, pseudorabies virus, parvovirus, vesikulær-virus hos svin, poxvirus,

rotavirus, Mycoplasma pneumonia, herpesvirus, infektiøs bronchitis og infektiøs bursitisvirus;

(ii) canceren er mindst én udvalgt fra gruppen bestående af ikke-småcellet lungecancer, brystkarcinom, melanom, lymfomer, colonkarcinom, hepatocellulært karcinom og en hvilken som helst kombination deraf; og

(iii) immuncheckpunktet er mindst ét udvalgt fra gruppen bestående af PD-1, PD-L1, PD-L2 og CTLA-4.

13. Rekombinant baculovirus ifølge krav 1, hvor antigenet er mindst ét udvalgt fra gruppen bestående af klassisk svinefebervirus kappe-glycoprotein E2, epidemisk diarrevirus hos svin S1-protein, programmeret celledødsprotein 1 og et tumorassocieret antigen.

14. Vektor ifølge krav 3, hvor antigenet er mindst ét, der er udvalgt fra gruppen bestående af et patogent protein, et cancercelleprotein og et immuncheckpunktsprotein.

15. Vektor ifølge krav 3 eller 6 eller et rekombinant baculovirus ifølge krav 1 eller krav 5 til anvendelse i behandling ved fremkaldelse af et antigenspecifikt immunogent respons hos et individ med behov derfor.

DRAWINGS

FIG. 1A

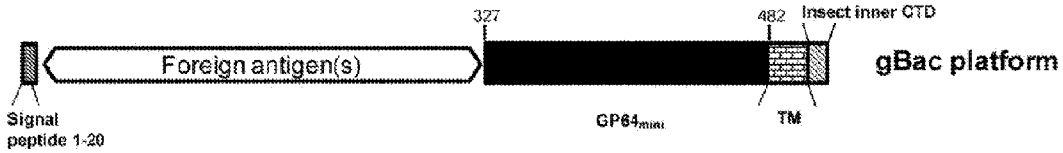


FIG. 1B

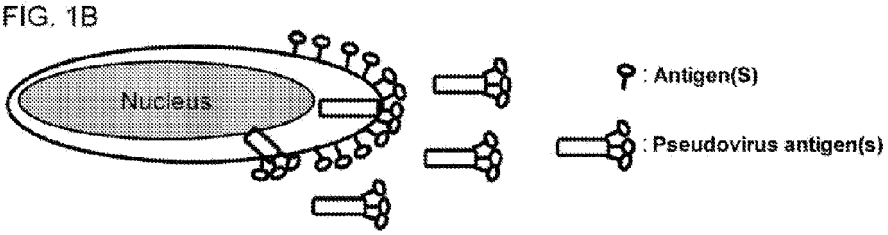


FIG. 2A

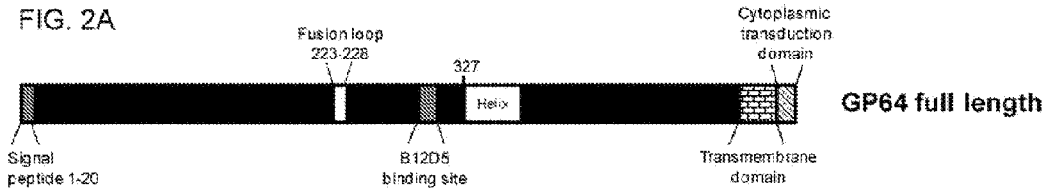
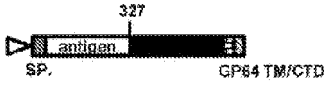


FIG. 2B

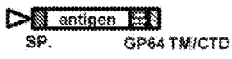


Reber-gBac

FIG. 2C

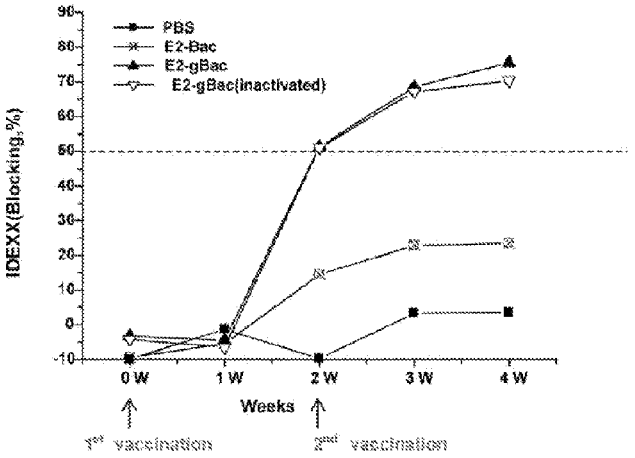
Antigen-gp64 (US7,527,967 B2)

FIG. 2D



Antigen-Bac (TW I368656)

FIG. 3



SN titer	IDEXX(Blocking,%)
<2	18±1
2	24±2
4	30±2
8	36±2
16	43±2
32	49±2
64	56±2
128	62±2
256	68±2
512	75±2
1024	80±1

FIG. 4

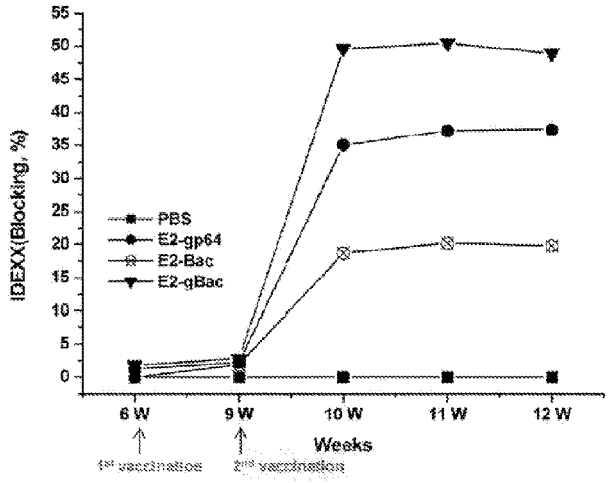


FIG. 5

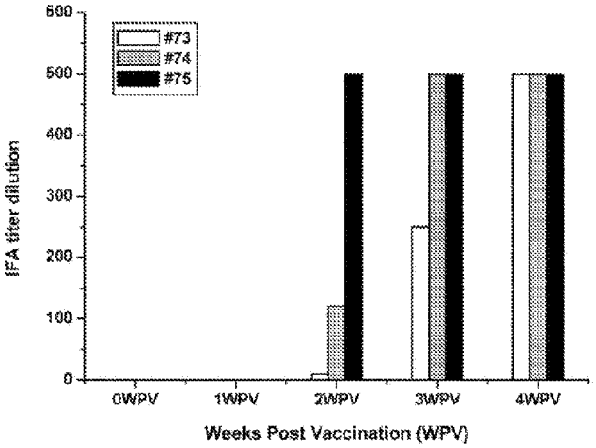


FIG. 6A

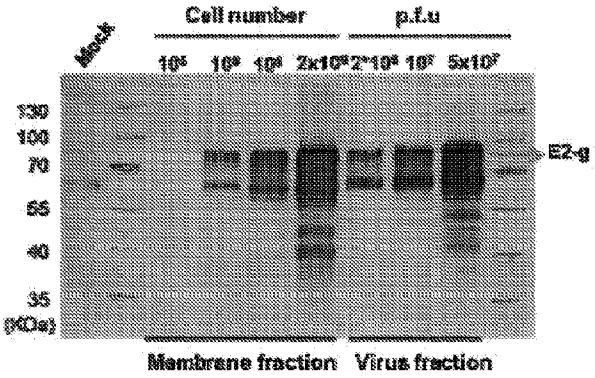


FIG. 6B

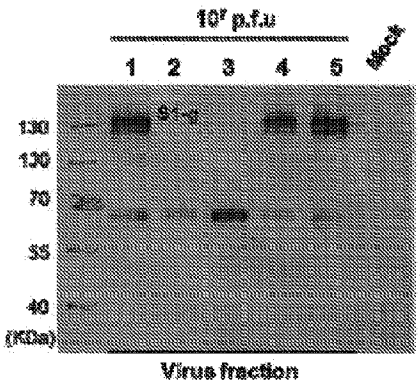


FIG. 6C

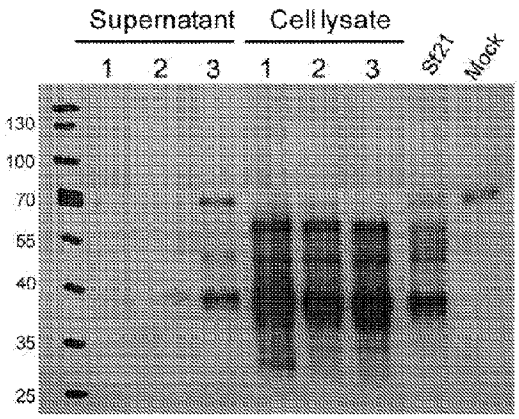


FIG. 6D