

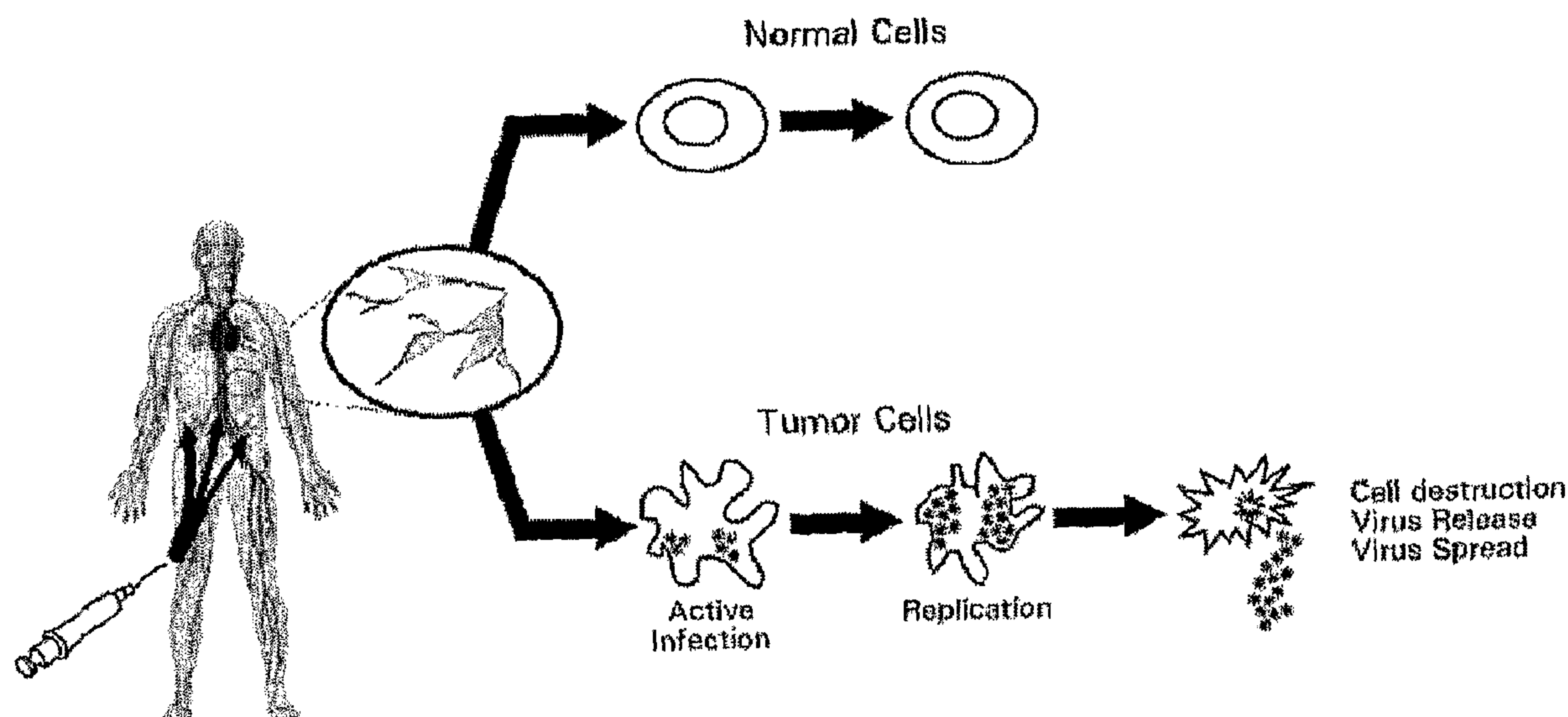


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(54) Title: SENECA VALLEY VIRUS BASED COMPOSITIONS AND METHODS FOR TREATING DISEASE



(57) **Abrégé/Abstract:**

[0384] The present invention relates to a novel RNA picornavirus that is called Seneca Valley virus ("SVV"). The invention provides isolated SW nucleic acids and proteins encoded by these nucleic acids. Further, the invention provides antibodies that are raised against the SVV proteins. Because SVV has the ability to selectively kill some types of tumors, the invention provides methods of using SVV and SVV polypeptides to treat cancer. Because SVV specifically targets certain tumors, the invention provides methods of using SW nucleic acids and proteins to detect cancer. Additionally, due to the information provided by the tumor-specific mechanisms of SVV, the invention provides methods of making new oncolytic virus derivatives and of altering viruses to have tumor-specific tropisms.

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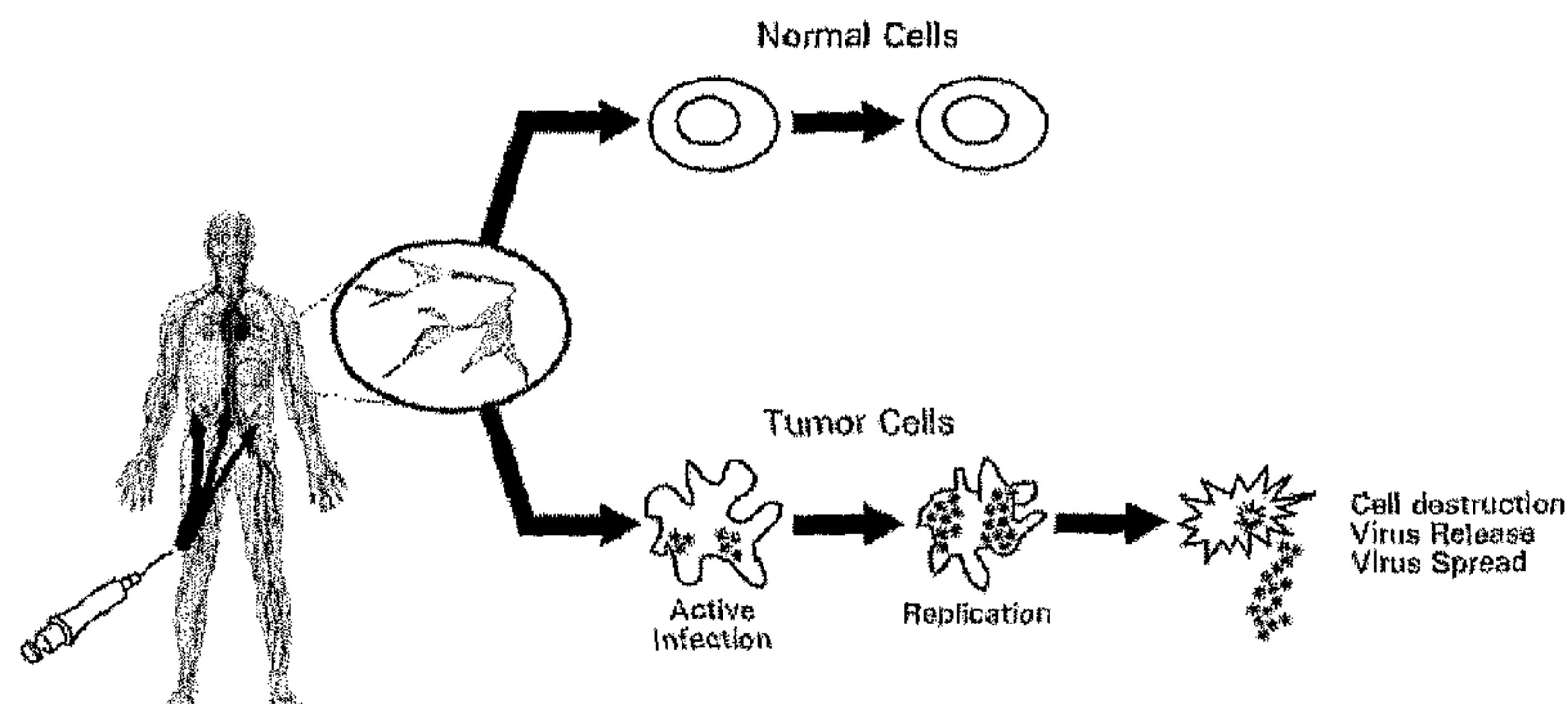
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(54) Title: SENECA VALLEY VIRUS BASED COMPOSITIONS AND METHODS FOR TREATING DISEASE



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**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
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Seneca Valley Virus Based Compositions and Methods for Treating Disease

[0001] This application claims priority to: U.S. Serial No. 60/664,442, which was filed on March 23, 2005; U.S. Serial No. 60/726,313, which was filed on October 13, 2005; and U.S. Serial No. 11/335,891, which was filed on January 19, 2006. These applications are hereby incorporated by reference in their entirety.

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[0003] All patent applications, published patent applications, issued and granted patents, texts, and literature references cited in this specification are hereby incorporated herein by reference in their entirety to more fully describe the state of the art to which the present invention pertains.

BACKGROUND OF THE INVENTION

[0004] Virotherapy holds great promise for treating cancer. Oncolytic viruses, which aim to specifically infect and kill cancer cells, whether native and/or engineered, may be more efficacious and less toxic than alternative treatments, such as chemotherapy and radiation. In addition, oncolytic virus therapy that uses replication competent viruses is the only therapy known that can amplify the therapeutic at the pharmacologically desired site.

[0005] A key aspect of cancer therapy is to achieve a high rate of killing of cancer cells versus normal cells. Accomplishing this goal has been difficult for many reasons, including the wide array of cell types involved, the systemic dissemination of cancer cells due to metastases, and the narrow biological differences between normal and cancer cells. While progress has been made, much still needs to be done to improve upon current cancer therapies.

[0006] In the past, surgeons have tried to remove tumors surgically without substantially harming the patient. Even complete removal of a primary tumor does not ensure survival since earlier metastases to unknown sites in the body are left undetected. There is also some research suggesting that surgical intervention may enhance the growth of distant metastases due to removal of tumor cells producing angiogenesis inhibitors. Finally, in many cases, the tumor grows back at the original site after surgical removal. Radiation

aims to selectively destroy the most rapidly proliferating cells at the expense of the others. However, tumor cells can escape radiation therapy either by becoming resistant or by being in a non-dividing state during treatment. In addition, radiation is not always selective in that many normal cells are actively dividing and killed by the treatment (cells in bone marrow, gastrointestinal cells, hair follicles, etc.).

[0007] Like radiation, chemotherapy is not completely selective and thus destroys many normal cells, and does not kill all tumor cells due to drug resistance and/or division state of the cell. Thus, chemotherapy and radiation therapies exploit a small differential sensitivity that exists between normal and cancer cells, giving them a narrow therapeutic index. A small therapeutic index is clearly an undesirable property of any modality to treat cancer. Therefore, novel cancer therapeutic approaches overcoming these limitations are desired.

[0008] One such novel approach is oncolytic virus therapy. Initially, replication-defective viruses carrying cytotoxic transgenes were utilized in attempts to treat cancer. However, they were found to be inefficient in transduction of tumors, inadequate spread within the tumor mass and not adequately selective toward cancers. To overcome this limitation, viruses were either modified to replicate selectively in tumor cells or viruses were discovered to have natural tumor-selective properties. These oncolytic viruses thus had the properties to replicate, spread, and kill tumor cells selectively through a tumor mass by locally injecting the virus or by systemically delivering the virus (Figure 1).

[0009] Despite the early promise of this newly defined class of anti-cancer therapeutics, several limitations remain that may limit their use as a cancer therapeutic. Therefore, there is an ongoing need for novel oncolytic viruses that can be utilized for cancer therapy.

SUMMARY OF THE INVENTION

[0010] A novel RNA picornavirus has been discovered (hereafter referred to as Seneca Valley virus ("SVV")) whose native properties include the ability to selectively kill some types of tumors. As demonstrated below in the examples, SVV selectively kills tumor lines with neurotropic properties, in most cases with a greater than 10,000 fold difference in the amount of virus necessary to kill 50% of tumor cells versus normal cells (*i.e.*, the EC₅₀ value). This result also translates *in vivo*, where tumor explants in mice are selectively

eliminated. Further, *in vivo* results indicate that SVV is not toxic to normal cells, in that up to 1×10^{14} vp/kg (vector or virus particles per kilogram) systemically administered causes no mortality and no visible clinical symptoms in immune deficient or immune competent mice.

[0011] SVV elicits efficacy at doses as low as 1×10^8 vp/kg; therefore, a very high therapeutic index of $>100,000$ is achieved. Efficacy is very robust in that 100% of large pre-established tumors in mice can be completely eradicated (*see* Example 11). This efficacy may be mediated with a single systemic injection of SVV without any adjunct therapy. Furthermore, SVV injected mice show neither clinical symptoms nor recurrence of tumors for at least 200 days following injection. SVV can also be purified to high titer and can be produced at $>200,000$ virus particles per cell in permissive cell lines. SVV-based viral therapy therefore shows considerable promise as a safe, effective and new line of treatment for selected types of cancers. Further, SVV has a small and easily manipulatable genome, simple and fast lifecycle, and a well-understood biology of replication, and thus is amenable to modification. These properties, at least in part, allow for methods that generate modified SVVs that have new cell or tissue specific tropisms, such that SVV-based therapy can be directed to new tumor types resistant to infection by the original SVV isolate.

[0012] Accordingly, the present invention provides an isolated nucleic acid comprising a nucleic acid sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 168, or a contiguous portion of any one of these sequences that is at least 50 nucleotides in length, or 95% identical to a contiguous portion of any one of these sequences that is at least 10, 15 or 20 nucleotides in length. The isolated nucleic acids of the invention can be RNA or DNA.

[0013] For all aspects of the invention, an isolated nucleic acid can comprise a nucleic acid sequence having at least about 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a contiguous portion of any one of the SVV nucleic acid SEQ ID NO sequences herein, wherein the contiguous portion is at least about 20, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 750, 1000, 1250, 1500, 2000 or 2500 nucleotides in length, for example. The SVV nucleic acid SEQ ID NO sequences include, for example, SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 168.

[0014] For all aspects of the invention, an isolated protein or peptide can comprise an amino acid sequence having at least about 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%,

96%, 97%, 98%, or 99% sequence identity to a contiguous portion of any one of the SVV amino acid SEQ ID NO sequences herein, wherein the contiguous portion is at least at least about 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, or 350 amino acids in length, for example. The SVV amino acid SEQ ID NO sequences include, for example, SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 169.

[0015] In another aspect, the invention provides an isolated nucleic acid comprising a nucleic acid sequence having at least 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:168, or to a contiguous portion of SEQ ID NO:168 that is at least 20, 50, 100, or 200 nucleotides in length. The isolated nucleic acids can comprise specific portions of SEQ ID NO:168, including but not limited to: the 5' untranslated region (UTR) of SVV spanning nucleotides 1-666 of SEQ ID NO:168; the coding sequence for the SVV polyprotein spanning nucleotides 667-7209 of SEQ ID NO:168; the coding sequence for the leader peptide of SVV spanning nucleotides 667-903 of SEQ ID NO:168; the coding sequence for the SVV VP4 protein spanning nucleotides 904-1116 of SEQ ID NO:168; the coding sequence for the SVV VP2 protein spanning nucleotides 1117-1968 of SEQ ID NO:168; the coding sequence for the SVV VP3 protein spanning nucleotides 1969-2685 of SEQ ID NO:168; the coding sequence for the SVV VP1 protein spanning nucleotides 2686-3477 of SEQ ID NO:168; the coding sequence for the SVV 2A protein spanning nucleotides 3478-3504 of SEQ ID NO:168; the coding sequence for the SVV 2B protein spanning nucleotides 3505-3888 of SEQ ID NO:168; the coding sequence for the SVV 2C protein spanning nucleotides 3889-4854 of SEQ ID NO:168; the coding sequence for the SVV 3A protein spanning nucleotides 4855-5124 of SEQ ID NO:168; the coding sequence for the SVV 3B protein spanning nucleotides 5125-5190 of SEQ ID NO:168; the coding sequence for the SVV 3C protein spanning nucleotides 5191-5823 of SEQ ID NO:168; the coding sequence for the SVV 3D protein spanning nucleotides 5824-7209 of SEQ ID NO:168; and the 3'UTR of SVV spanning nucleotides 7210-7280 of SEQ ID NO:168.

[0016] In one aspect, the invention provides methods for using the SVV 2A, SVV leader peptide, or other SVV proteins or peptide portions thereof, to shut off host cell protein translation. In one aspect, such SVV proteins can be used to shut off host cell protein translation by interfering or inhibiting with the cap binding protein complex in the host cell.

[0017] In another aspect, the invention provides methods for using SVV 2A or other SVV proteins or peptide portions thereof in order to cleave a peptide or protein.

[0018] In other aspects, the invention provides an isolated nucleic acid that hybridizes under conditions of high, moderate stringency or low stringency to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 168, or to a contiguous portion of any one of these sequences that is at least 50 nucleotides in length.

[0019] In another aspect, the invention provides a vector comprising a nucleic acid sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOS 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 168, or to a contiguous portion of any one of these sequences that is at least 50 nucleotides in length. Vector compositions can also comprise the nucleic acid regions of SEQ ID NO:168 that code for SVV proteins.

[0020] The present invention also provides an isolated polypeptide encoded by a nucleic acid having at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to a nucleic acid sequence comprising SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or to a contiguous portion of any one of these sequences that is at least 50 nucleotides in length. The invention also provides an isolated polypeptide encoded by a nucleic acid having at least 95%, 96%, 97%, 98%, or 99% sequence identity to a nucleic acid region of SEQ ID NO:168 that encodes a SVV protein.

[0021] In one aspect, the invention provides an isolated polypeptide comprising an amino acid sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 169, or to a contiguous portion of any one of these sequences that is at least 10 amino acids in length.

[0022] In another aspect, the invention provides an isolated polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, or 99% sequence identity to a contiguous portion of SEQ ID NO:169 that is at least 9, 10, 15, 20 or 50 amino acids in length. Exemplary contiguous portions of SEQ ID NO:169, include but are not limited to, regions that comprise a SVV protein, such as: the leader peptide spanning residues 1-79; VP4 spanning residues 80-150; VP2 spanning residues 151-434; VP3 spanning residues 435-673; VP1 spanning residues 674-937; 2A spanning residues 938-946; 2B spanning residues 947-1074; 2C spanning residues 1075-1396; 3A spanning residues 1397-1486; 3B spanning residues 1487-1508; 3C spanning residues 1509-1719; and 3D spanning residues 1720-2181.

[0023] In another aspect, the invention provides an isolated antibody which specifically binds a polypeptide comprising an amino acid sequence having at least 65%,

70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 169, or to a contiguous portion of any one of these sequences that is at least 9, 10, 15, or 20 amino acids in length. The isolated antibody can be generated such that it binds to any protein epitope or antigen of SEQ ID NOS:2 or 169. Further, the antibody can be a polyclonal antibody, a monoclonal antibody or a chimeric antibody.

[0024] In one aspect, the invention provides an isolated SVV or derivative or relative thereof, having a genomic sequence comprising a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO:1 or SEQ ID NO:168.

[0025] In another aspect, the invention provides an isolated virus having all the identifying characteristics and nucleic acid sequence of American Type Culture Collection (ATCC) Patent Deposit number PTA-5343. Some of the viruses of the present invention are directed to the PTA-5343 isolate, variants, homologues, relatives, derivatives and mutants of the PTA-5343 isolate, and variants, homologues, derivatives and mutants of other viruses that are modified in respect to sequences of SVV (both wild-type and mutant) that are determined to be responsible for its oncolytic properties.

[0026] The present invention further provides an isolated SVV comprising the following characteristics: a single stranded RNA genome (positive (+) sense strand) of ~7.5 or of ~7.3 kilobases (kb); a diameter of ~27 nanometers (nm); a capsid comprising at least 3 proteins that have approximate molecular weights of about 31 kDa, 36 kDa and 27 kDa; a buoyant density of approximately 1.34 g/mL on cesium chloride (CsCl) gradients; and replication competence in tumor cells. In this aspect, the 31 kDa capsid protein (VP1) can comprise an amino acid sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO:8 or residues 674-937 of SEQ ID NO:169; the 36 kDa capsid protein (VP2) can comprise an amino acid sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO:4 or residues 151-434 of SEQ ID NO:169; and the 27 kDa capsid protein (VP3) can comprise an amino acid sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO:6 or residues 435-673 of SEQ ID NO:169.

[0027] In another aspect, the invention provides an isolated SVV derivative or relative comprising the following characteristics: replication competence in tumor cells, tumor-cell tropism, and lack of cytolysis in normal cells. An SVV relative includes SVV-like picornaviruses, including viruses from the following USDA isolates: MN 88-36695, NC 88-

23626, IA 89-47552, NJ 90-10324, IL 92-48963, CA 131395; LA 1278; IL 66289; IL 94-9356; MN/GA 99-29256; MN 99197; and SC 363649. If an SVV-like picornavirus does not naturally have the characteristics of replication competence in tumor cells, tumor-cell tropism, and lack of cytolysis in non-tumor cells, then the SVV-like picornavirus can be mutated such that these characteristics are obtained. Such mutations can be designed by comparing the sequence of the SVV-like picornavirus to SVV, and making mutations into the SVV-like picornavirus such that its amino acid sequence is identical or substantially identical (in a particular region) to SVV. In another aspect, the virus is replication competent in tumor cell types having neuroendocrine properties.

[0028] In other aspects, the present invention provides: a pharmaceutical composition comprising an effective amount of a virus of the invention and a pharmaceutically acceptable carrier; a cell comprising a virus of the invention; a viral lysate containing antigens of a virus of the invention; and an isolated and purified viral antigen obtained from a virus of the invention.

[0029] In yet another aspect, the invention provides a method of purifying a virus of the invention, comprising: infecting a cell with the virus; harvesting cell lysate; subjecting cell lysate to at least one round of gradient centrifugation; and isolating the virus from the gradient.

[0030] In another aspect, the invention provides a method for treating cancer comprising administering an effective amount of a virus or derivative thereof, so as to treat the cancer, wherein the virus has a genomic sequence that comprises a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 168, or to a portion of SEQ ID NO:1 or SEQ ID NO:168. In one aspect, the invention provides a method for treating cancer comprising administering an effective amount of a virus or derivative thereof, so as to treat the cancer, wherein the virus has a genomic sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:1. The virus that has a genomic sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:1 can be, for example, a SVV mutant, a SVV-like picornavirus, or a cardiovirus. The SVV-like picornavirus can be, for example, a virus from one of the following isolates MN 88-36695, NC 88-23626, IA 89-47752, NJ 90-10324, IL 92-48963, CA 131395; LA 1278; IL 66289; IL 94-9356; MN/GA 99-29256; MN 99197; and SC 363649. The SVV-like picornaviruses can be wild-type or mutant.

[0031] In another aspect, the invention provides a method for treating cancer comprising administering an effective amount of a virus comprising a capsid encoding region that comprises a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NOS:3, 5, 7, nucleotides 904-3477 of SEQ ID NO:169, or to a contiguous portion thereof that is at least 75, 100, 200, or 500 nucleotides in length. The invention also provides a method for treating cancer comprising administering an effective amount of a virus comprising a capsid that comprises an amino acid sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:4, 6, 8, residues 80-937 of SEQ ID NO:169, or a contiguous portion thereof that is at least 25, 50, or 100 amino acids in length.

[0032] In one aspect, the present invention provides a method for inhibiting cancer progression comprising contacting a cancer cell with a virus or derivative thereof, wherein the virus or derivative thereof specifically binds to the cancerous cell, wherein the virus has a genomic sequence that comprises a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 168.

[0033] In another aspect, the invention provides a method for inhibiting cancer progression comprising contacting a cancer cell with a virus or derivative thereof, wherein the virus or derivative thereof specifically infects the cancerous cell, wherein the virus has a genomic sequence that comprises a sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 168, or to a contiguous portion of SEQ ID NO:168 that is at least 50, 100, 200, or 500 nucleotides in length.

[0034] In another aspect, the present invention provides a method for killing cancer cells comprising contacting a cancer cell with an effective amount of a virus or derivative thereof, wherein the virus has a genomic sequence that comprises a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 168.

[0035] In another aspect, the present invention provides a method for killing cancer cells comprising contacting a cancer cell with an effective amount of a virus or derivative thereof, wherein the virus has a genomic sequence that comprises a sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:168, or to a contiguous portion of SEQ ID NO:168 that is at least 50, 100, 200 or 500 nucleotides in length.

[0036] In these methods directed to cancer, the virus can be a picornavirus. The picornavirus can be a cardiovirus, erbovirus, aphthovirus, kobuvirus, hepatovirus, parechovirus, teschovirus, enterovirus, rhinovirus, SVV, or an SVV-like picornavirus. The cardiovirus can be selected from the group consisting of: vilyuisk human encephalomyelitis virus, Theiler's murine encephalomyelitis virus, and encephalomyocarditis virus. The SVV can be a virus having the ATCC deposit number PTA-5343 or a virus comprising a nucleic acid sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:1 or SEQ ID NO:168, or to a contiguous portion thereof that is at least 50, 100, 200, or 500 nucleotides in length. The SVV-like picornavirus can be a virus comprising a nucleic acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:168, or to a contiguous portion thereof that is at least 50, 100, 200, or 500 nucleotides in length. The SVV-like picornavirus can be, for example, a virus from one of the following isolates MN 88-36695, NC 88-23626, IA 89-47752, NJ 90-10324, IL 92-48963, CA 131395; LA 1278; IL 66289; IL 94-9356; MN/GA 99-29256; MN 99197; and SC 363649. The SVV-like picornaviruses can be wild-type or mutant.

[0037] The present invention also provides a method of killing an abnormally proliferative cell comprising contacting the cell with a virus of the invention. In one aspect, the abnormally proliferative cell is a tumor cell. In various aspects of this method, the tumor cell is selected from the group consisting of: human small cell lung cancer, human retinoblastoma, human neuroblastoma, human medulloblastoma, mouse neuroblastoma, Wilms' tumor, and human non-small cell lung cancer.

[0038] The present invention also provides a method of treating a neoplastic condition in a subject comprising administering to the subject an effective amount of a virus of the invention to the mammal. In one aspect, the neoplastic condition is a neuroendocrine cancer. In another aspect, the subject is a mammal. In another aspect, the mammal is a human.

[0039] The present invention also provides a method of producing a virus of the invention, comprising: culturing cells infected with the virus under conditions that allow for replication of the virus and recovering the virus from the cells or the supernatant. In one aspect of this method, the cells are PER.C6 cells. In another aspect of this method, the cells are H446 cells. In the various aspects of this method, the cells may produce over 200,000 virus particles per cell.

[0040] In another aspect, the present invention provides a method for detecting a virus of the invention, comprising: isolating RNA from test material suspected to contain the virus of the invention; labeling RNA corresponding to at least 15 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:168; probing the test material with the labeled RNA; and detecting the binding of the labeled RNA with the RNA isolated from the test material, wherein binding indicates the presence of the virus. In another aspect, the present invention provides a nucleic acid probe comprising a nucleotide sequence corresponding to at least 15 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:168, or its complement.

[0041] The present invention also provides a method for making an oncolytic virus, the method comprising: (a) comparing a SVV genomic sequence with a test virus genomic sequence; (b) identifying at least a first amino acid difference between a polypeptide encoded by the SVV genomic sequence and a polypeptide encoded by the test virus genomic sequence; (c) mutating the test virus genomic sequence such that the polypeptide encoded by the test virus genomic sequence has at least one less amino acid difference to the polypeptide encoded by the SVV genomic sequence; (d) transfecting the mutated test virus genomic sequence into a tumor cell; and (e) determining whether the tumor cell is lytically infected by the mutated test virus genomic sequence. In one aspect, the amino acid(s) mutated in the test virus are amino acids in a structural region, such as in the capsid encoding region. In another aspect, the amino acids mutated in the test virus are amino acids in a non-structural region.

[0042] In one aspect of the method for making an oncolytic virus, the SVV genomic sequence is obtained from the isolated SVV having the ATCC deposit number PTA-5343 or from a virus comprising a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 168, or a contiguous portion thereof. In one aspect, the SVV genomic sequence is obtained from the isolated SVV having the ATCC deposit number PTA-5343 or from a virus comprising a sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 168, or a contiguous portion thereof that is at least 50, 100, 200, or 500 nucleotides in length. In another aspect of this method, the step of mutating the test virus genomic sequence comprises mutating a cDNA having the test virus genomic sequence. In another aspect of this method, the step of transfecting the mutated test virus genomic sequence comprises transfecting RNA, wherein the RNA is generated from the cDNA having the mutated test virus genomic sequence.

[0043] In another aspect of the method for making an oncolytic virus, the test virus is a picornavirus. The test picornavirus can be a teschovirus, enterovirus, rhinovirus, cardiovirus, erbovirus, apthovirus, kobuvirus, hepatovirus, parechovirus or teschovirus. In another aspect, the test virus is a cardiovirus. In another aspect, the test virus is a SVV-like picornavirus. The SVV-like picornavirus can be, for example, a virus from one of the following isolates: MN 88-36695, NC 88-23626, IA 89-47552, NJ 90-10324, IL 92-48963, CA 131395; LA 1278; IL 66289; IL 94-9356; MN/GA 99-29256; MN 99197; and SC 363649. In another aspect, the amino acid differences identified in the methods for making an oncolytic virus are between a SVV capsid protein and a test virus capsid protein sequence. In another aspect for making an oncolytic virus, the test virus genomic sequence is selected from the group consisting of: Vilyuisk human encephalomyelitis virus, Theiler's murine encephalomyelitis virus, and encephalomyocarditis virus. In another aspect, the test virus genomic sequence is selected from an encephalomyocarditis virus. In yet another aspect, the encephalomyocarditis virus, the SVV-like picornavirus, or any other test virus can be selected from an isolate having a nucleic acid sequence comprising at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to SVV of ATCC deposit number PTA-5343 or SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 168, or a contiguous portion thereof that is at least 50, 100, 200, or 500 nucleotides in length.

[0044] In another aspect of the method for making an oncolytic cardiovirus, the amino acid difference between the test virus and SVV is in a capsid protein region of SVV, wherein the amino acid difference is aligned within SVV SEQ ID NO:4, 6, 8, residues 80-937 of SEQ ID NO:169, residues 80-150 of SEQ ID NO:169, residues 151-434 of SEQ ID NO:169, residues 435-673 of SEQ ID NO:169, or residues 674-937 of SEQ ID NO:169.

[0045] The present invention also provides a method for making a mutant virus having an altered cell-type tropism, the method comprising: (a) creating a library of viral mutants comprising a plurality of nucleic acid sequences; (b) transfecting the library of viral mutants into a permissive cell, such that a plurality of mutant viruses is produced; (c) isolating the plurality of mutant viruses; (d) incubating a non-permissive cell with the isolated plurality of mutant viruses; and (e) recovering a mutant virus that was produced in the non-permissive cell, thereby making a mutant virus having an altered tropism. In one aspect, this method further comprises the steps of: (f) incubating the recovered mutant virus in the non-permissive cell; and (g) recovering a mutant virus that that was produced in the non-

permissive cell. In another aspect, the method further comprises iteratively repeating steps (f) and (g). In another aspect, the library of viral mutants is created from a parental sequence comprising SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 168, or a contiguous portion thereof.

[0046] In one aspect of the method for making a mutant virus having an altered cell-type tropism, the incubating is conducted in a multi-well high-throughput platform wherein the platform comprises a different non-permissive cell-type in each well. In this aspect, the method can further comprise screening the platform to identify which wells contain a mutant virus that kills the cells. In another aspect, the screening is conducted by analyzing light absorbance in each well.

[0047] In another aspect of the method for making a mutant virus having an altered cell-type tropism, the non-permissive cell is a tumor cell.

[0048] In another aspect of the method for making a mutant virus having an altered cell-type tropism, the step of creating the library of viral mutants comprises: (i) providing a polynucleotide having a sequence identical to a portion of a genomic sequence of a virus; (ii) mutating the polynucleotide in order to generate a plurality of different mutant polynucleotide sequences; and (iii) ligating the plurality of mutated polynucleotides into a vector having the genomic sequence of the virus except for the portion of the genomic sequence of the virus that the polynucleotide in step (i) contains, thereby creating the library of viral mutants. In one aspect, the genomic sequence of a virus is from a picornavirus. In another aspect, the genomic sequence of a virus comprises a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 168, or a contiguous portion thereof. In another aspect, the genomic sequence of a virus comprises a sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 168, or a contiguous portion thereof that is at least 50, 100, 200, or 500 nucleotides in length. In one aspect, the virus that comprises a sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a contiguous portion of SEQ ID NO:168 that is at least 50, 100, 200, or 500 nucleotides in length is a SVV-like picornavirus. In another aspect, in the step of creating the library of viral mutants, the mutating of step (ii) is conducted by random insertion of nucleotides into the polynucleotide. In one aspect, the random insertion of nucleotides is conducted by trinucleotide-mutagenesis (TRIM). In another aspect, at least a portion of the nucleotides inserted into the polynucleotide encodes an epitope tag. In another aspect, in the

step of creating the library of viral mutants, the mutating of step (ii) is conducted in a capsid encoding region of the polynucleotide.

[0049] The present invention also provides a method for making a mutant virus having an altered cell-type tropism, the method comprising: (a) creating a library of mutant polynucleotide sequences of a virus, wherein the creating comprises: providing a polynucleotide encoding a capsid region of the virus; mutating the polynucleotide in order to generate a plurality of different mutant capsid-encoding polynucleotide sequences; and ligating the plurality of mutated capsid-encoding polynucleotides into a vector having the genomic sequence of the virus except for the capsid-encoding region, thereby creating the library of mutant polynucleotide sequences of the virus; (b) transfecting the library of mutant polynucleotide sequences into a permissive cell, such that a plurality of mutant viruses is produced; (c) isolating the plurality of mutant viruses; (d) incubating a non-permissive cell with the isolated plurality of mutant viruses; and (e) recovering a mutant virus that that was produced in the non-permissive cell, thereby making a mutant virus having an altered tropism. In one aspect, the method further comprises the steps of: (f) incubating the recovered mutant virus in the non-permissive cell; and (g) recovering a mutant virus that that was produced in the non-permissive cell. In another aspect, the method further comprises iteratively repeating steps (f) and (g). In another aspect, the mutating is conducted by random insertion of nucleotides into the capsid-encoding polynucleotide. In another aspect, at least a portion of the nucleotides randomly inserted into the capsid-encoding polynucleotide encodes an epitope tag. In another aspect, the random insertion of nucleotides is conducted by TRIM. In another aspect, the plurality of different mutant capsid-encoding polynucleotide sequences comprises greater than 10^8 or 10^9 different capsid-encoding polynucleotide sequences. The library of mutant polynucleotide sequences can be from, for example, a cardiovirus or an SVV-like picornavirus.

[0050] In one aspect, a method for making a mutant SVV having an altered cell-type tropism comprises: (a) creating a cDNA library of SVV mutants; (b) generating SVV RNA from the cDNA library of SVV mutants; (c) transfecting the SVV RNA into a permissive cell, such that a plurality of mutant SVV is produced; (d) isolating the plurality of mutant SVV; (e) incubating a non-permissive tumor cell with the isolated plurality of mutant SVV; and (f) recovering a mutant SVV that lytically infects the non-permissive tumor cell, thereby making a mutant SVV having an altered tropism. In another aspect, the method further comprises the

steps of: (g) incubating the recovered mutant SVV in the non-permissive cell; and (h) recovering a mutant SVV that lytically infects the non-permissive tumor cell. In another aspect, the method further comprises iteratively repeating steps (g) and (h). In one aspect, the incubating is conducted in a multi-well high-throughput platform wherein the platform comprises a different non-permissive tumor cell-type in each well. In another aspect, the method further comprises screening the platform to identify which wells contain a mutant SVV that lytically infects the cells. In another aspect, the screening is conducted by analyzing light absorbance in each well. In one aspect, the cDNA library of SVV mutants comprises a plurality of mutant SVV capsid polynucleotide sequences. In another aspect, the plurality of mutant SVV capsid polynucleotide sequences is generated by random insertion of nucleotides. In another aspect, at least a portion of the sequence of the nucleotides randomly inserted encodes an epitope tag. In another aspect, the random insertion of nucleotides is conducted by TRIM. In another aspect, the cDNA library of SVV mutants is generated from a SVV of ATCC deposit number PTA-5343. In another aspect, the cDNA library of SVV mutants is generated from a SVV comprising a sequence having at least 99%, 95%, 90%, 85%, 80%, 75%, 70%, or 65% sequence identity to SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 168, or to a contiguous portion thereof that is at least 50, 100, 200, or 500 nucleotides in length. In one aspect, the cDNA library of SVV mutants is generated from an SVV comprising a sequence having at least 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:168, or to a contiguous portion thereof that is at least 50, 100, 200, or 500 nucleotides in length. In another aspect, the non-permissive tumor cell is a tumor cell-line or a tumor cell-type isolated from a patient.

[0051] The present invention also provides a method for making a mutant virus having a tumor cell-type tropism *in vivo*, the method comprising: (a) creating a library of viral mutants comprising a plurality of nucleic acid sequences; (b) transfecting the library of viral mutants into a permissive cell, such that a plurality of mutant viruses is produced; (c) isolating the plurality of mutant viruses; (d) administering the isolated plurality of mutant viruses to a mammal with a tumor, wherein the mammal is not a natural host of the unmutated form of the mutant virus; and (e) recovering a virus that replicated in the tumor, thereby making a mutant virus having a tumor cell-type tropism *in vivo*. In one aspect, the step of creating a library of viral mutants comprises: providing a polynucleotide encoding a capsid region of a virus; mutating the polynucleotide in order to generate a plurality of different mutant capsid-encoding polynucleotide sequences; and ligating the plurality of

mutated capsid-encoding polynucleotides into a vector having the genomic sequence of the virus except for the capsid-encoding region, thereby creating the library of viral mutants. In another aspect, the virus recovered in step (e) lytically infects cells of the tumor. In another aspect for a method for making a mutant virus having a tumor cell-type tropism *in vivo*, the tumor is a xenograft, a syngeneic tumor, an orthotopic tumor or a transgenic tumor. In another aspect, the mammal is a mouse.

[0052] For all the methods of the present invention, the virus can be a picornavirus. The picornavirus can be a cardiovirus, erbovirus, aphthovirus, kobuvirus, hepatovirus, parechovirus, teschovirus, entrovirus, rhinovirus, or a virus belonging to the genus to which SVV belongs. The virus can be a cardiovirus. The virus can be an SVV-like picornavirus. The virus can be SVV. The SVV can be a SVV having the ATCC Patent Deposit No. PTA-5343 or a SVV comprising a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO:1, 3, 6, 7, 9, 11, 13, 15, 17, 19, 21, 168, or a contiguous portion thereof. Further, the cardiovirus can be selected from the group consisting of: vilyuisk human encephalomyelitis virus, Theiler's murine encephalomyelitis virus, and encephalomyocarditis virus. In one aspect, the SVV-like picornavirus is selected from the group of isolates consisting of: MN 88-36695, NC 88-23626, IA 89-47552, NJ 90-10324, IL 92-48963, CA 131395; LA 1278; IL 66289; IL 94-9356; MN/GA 99-29256; MN 99197; and SC 363649. In another aspect, the present invention encompasses any virus that is selected from an isolate having at least 99%, 95%, 90%, 85%, 80%, 75%, 70%, or 65% sequence identity to SVV of ATCC deposit number PTA-5343 or SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 168, or a contiguous portion thereof or is otherwise considered related to SVV to by sequence homology.

[0053] In another aspect, the present invention encompasses any virus having at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% nucleic acid sequence identity to SVV of ATCC deposit number PTA-5343, to SEQ ID NO:168, or to a contiguous portion of SEQ ID NOS: 1 or 168 that is at least 100, 200, 300, 400, 500, 750, 1000, 1500, or 2000 nucleotides in length.

[0054] The present invention also provides an oncolytic virus made by any of the methods for making a mutant virus disclosed herein. In one aspect, the present invention provides a method for treating a patient with an oncolytic virus, the method comprising: (a) inactivating an oncolytic virus made by any of the methods for making a mutant virus

disclosed herein, such that the oncolytic virus is non-infectious and the tropism of the oncolytic virus is unaffected; and (b) administering the irradiated oncolytic virus to a patient afflicted with a tumor. In another aspect, the method for treating a patient further comprises attaching a toxin to the inactivated oncolytic virus.

[0055] In another aspect, the present invention provides a method for treating a patient with a tumor with SVV, the method comprising: (a) inactivating a SVV such that the virus is non-infectious and the tropism is unaffected; and (b) administering the inactivated SVV in a patient afflicted with a tumor. In another aspect, the method for treating a patient with a tumor with SVV further comprises attaching a toxin to the inactivated SVV.

[0056] In another aspect, the present invention provides a SVV composition comprising an inactivated SVV or attenuated SVV. In another aspect, the present invention provides a SVV comprising an epitope tag incorporated in the capsid region.

[0057] The present invention also provides a method for treating a patient with a tumor with SVV, the method comprising: (a) creating a mutant SVV comprising an epitope tag encoded in the capsid; (b) attaching a toxin to the epitope tag; and (c) administering the mutant SVV with the attached toxin to a patient afflicted with a tumor. In one aspect, the creating comprises: inserting an oligonucleotide encoding an epitope tag into a capsid-encoding region polynucleotide of SVV. In one aspect, the mutant SVV does not have an altered cell-type tropism. In another aspect, the method further comprises inactivating the mutant SVV such that the mutant SVV is not infectious or cannot replicate.

[0058] The present invention also provides a method for detecting a tumor cell in a sample comprising: (a) isolating a tumor sample from a patient; (b) incubating the tumor sample with an epitope-tagged SVV; and (c) screening the tumor sample for bound SVV by detecting the epitope tag.

[0059] In one aspect, the invention provides a method for detecting a tumor cell *in vivo* comprising: (a) administering to a patient an inactivated epitope-tagged SVV, wherein a label is conjugated to the epitope-tag; and (b) detecting the label in the patient. In the methods for detecting a tumor cell of the present invention, the SVV can be a mutant SVV generated by the methods disclosed herein.

[0060] In one aspect, the invention provides a method for detecting a tumor cell in a sample comprising: (a) isolating a cell sample from a subject; (b) incubating the cell sample

with SVV (or an SVV-like picornavirus); (c) incubating the cell sample from step (b) with an antibody specific to SVV (or an antibody specific to an SVV-like picornavirus); and (d) screening the cell sample for bound antibody, wherein bound antibody indicates that the sample contains a tumor cell.

[0061] In one aspect, the invention provides a method for determining whether a subject is candidate for SVV therapy, the method comprising: (a) isolating a cell from the subject; (b) incubating the cell with SVV; (c) incubating the sample from step (b) with an anti-SVV antibody; and (d) detecting for the presence of the anti-SVV antibody on or in the cell, wherein a positive detection indicates that the subject is a candidate for SVV therapy.

[0062] Screening a cell sample for bound antibody or detecting for the presence of an anti-SVV antibody can be conducted by adding a secondary antibody that can bind to the constant regions or non-epitope binding regions of the anti-SVV antibody, wherein the secondary antibody is conjugated or labeled with a detectable marker. The detectable marker can be, for example, a fluorophore such as fluorescein. When a secondary antibody is labeled with a detectable marker, the detectable marker can be detected, for example, by fluorescent microscopy. The cell from the subject can be from a tissue biopsy from the subject. The tissue biopsy can be from a tumor in the subject or from a region in the subject that is suspected to contain tumor cells. SVV directly labeled with fluorophore can also be used in identification of tumor cells.

[0063] Further, the methods for treating neoplastic conditions, for detecting neoplastic conditions and for producing SVV, apply to wild-type SVV, mutant (including modified or variant) SVV, relatives of SVV, SVV-like picornaviruses, and other tumor-specific viruses of the invention.

[0064] The viruses of the present invention, and the compositions thereof, can be used in the manufacture of a medicament for treating the diseases mentioned herein. Further, the viruses and composition thereof of the invention can be used for the treatment of the diseases mentioned herein. Thus, in one aspect of the present invention, the present invention provides the use of SVV (or mutants, derivatives, relatives, and compositions thereof) for the treatment of cancer or in the manufacture of a medicament for treating cancer.

[0065] SVV and SVV-like viruses for gene therapy: Replication defective SVV expressing gene(s) of interest can be used to deliver genes to correct genetic disorders. SVV

and SVV-like viruses can also be used as delivery vehicle for siRNA to prevent any specific gene expression. Replication defective viruses can be grown in complementing cell lines and/or in the presence of a helper virus to provide for missing functions in the recombinant virus.

[0066] IRES of picornaviruses known to play a role in expression of genes in a tissue specific manner. IRES of SVV and SVV-like viruses can be used to replace IRES of other picornaviruses. This strategy can be used to generate viruses with altered tissue tropism. In one aspect, the invention provides an IRES of SVV or an IRES from an SVV-related virus for the purpose of expressing two genes from a single promoter in a tissue specific manner.

[0067] Self-cleavage properties of 2A protease of SVV can be used to express more than one gene in equal amounts using single promoter and transcription termination signal sequences. In one aspect, the invention provides a self-cleaving 2A peptide of SVV or of an SVV-related virus for the purpose of expressing of two or more proteins in equal amounts under the control of single promoter and a single poly(A) signal. In another aspect, the invention provides the use of an SVV or an SVV-related virus 3C protease to cleave polypeptides for production of proteins from a eukaryotic cell. In another aspect, the invention provides for the use of an SVV or an SVV-like virus leader peptide to cause shut off of cell protein synthesis in tumor cells or another cell type of interest.

[0068] Virus like particles of SVV can be generated and used as vaccines and identify a particular cell type in a mixed population of cells.

Deposit Information

[0069] The following material has been deposited with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia, 20110-2209, U.S.A., under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. All restrictions on the availability of the deposited material will be irrevocably removed upon the granting of a patent. Material: Seneca Valley Virus (SVV). ATCC Patent Deposit Number: PTA-5343. Date of Deposit: July 25, 2003.

BRIEF DESCRIPTION OF THE DRAWINGS

[0070] **Figure 1** shows a schematic of virotherapy using oncolytic viruses. Oncolytic viruses have the properties to replicate, spread and kill tumor cells selectively through a tumor mass by locally injecting the virus or by systemically delivering the virus.

[0071] **Figure 2** shows purified SVV stained with uranyl acetate and examined by transmission electron microscopy. Spherical virus particles are about 27 nm in diameter.

[0072] **Figure 3** is an electron micrograph of an SVV-infected PER.C6 cell that has a large crystalline inclusion and large vesicular bodies.

[0073] **Figure 4A** shows an analysis of SVV RNA. SVV genomic RNA is extracted using guanidium thiocyanate and a phenol extraction method using Trizol (Invitrogen Corp., Carlsbad, CA). RNA is resolved through a 1.25% denaturing agarose gel. The band is visualized by ethidium bromide (EtBr) staining and photographed. In lane 2, a predominant band of SVV genomic RNA is observed, indicating that the size of the full-length SVV genome is about 7.5 kilobases.

[0074] **Figure 4B** is a schematic showing the genome structure and protein products generated from polyprotein processing for picornaviruses, including SVV.

[0075] **Figures 5A-5E** presents the nucleotide sequence of SVV (SEQ ID NO:1) and the encoded amino acid sequence (SEQ ID NO:2). The stop codon is depicted by a "*" at positions 5671-3. As a general note, in sequence disclosures that include positions where the exact nucleotide is being confirmed, these positions are represented by an "n". Therefore, in codons that possess an "n", the relevant amino acid is depicted by a "x".

[0076] **Figures 6A-6D** presents the nucleotide sequence (SEQ ID NO:1) of the majority of the full-length genome of SVV. The nucleotide sequence was derived from the SVV isolate having the ATCC Patent Deposit Number: PTA-5343. Date of Deposit: July 25, 2003.

[0077] **Figures 7A-7B** presents the amino acid sequence (SEQ ID NO:2) encoded by SEQ ID NO:1.

[0078] **Figure 8** presents the nucleotide sequence (SEQ ID NO:3) of the partial 1B or VP2 encoding region of SVV. This sequence is identical to nucleotides 4-429 of SEQ ID NO:1.

[0079] **Figure 9** presents the amino acid sequence (SEQ ID NO:4) of the partial SVV VP2 protein that is encoded by SEQ ID NO:3. The sequence listed in SEQ ID NO:4 is identical to amino acids 2-143 of SEQ ID NO:2.

[0080] **Figure 10** presents the nucleotide sequence (SEQ ID NO:5) of the 1C or VP3 encoding region of SVV. This sequence is identical to nucleotides 430-1146 of SEQ ID NO:1.

[0081] **Figure 11** presents the amino acid sequence (SEQ ID NO:6) of the SVV VP3 protein that is encoded by SEQ ID NO:5. The sequence listed in SEQ ID NO:6 is identical to amino acids 144-382 of SEQ ID NO:2.

[0082] **Figure 12** presents the nucleotide sequence (SEQ ID NO:7) of the 1D or VP1 encoding region of SVV. This sequence is identical to nucleotides 1147-1923 of SEQ ID NO:1.

[0083] **Figure 13** presents the amino acid sequence (SEQ ID NO:8) of the SVV VP1 protein that is encoded by SEQ ID NO:7. The sequence listed in SEQ ID NO:8 is identical to amino acids 383-641 of SEQ ID NO:2.

[0084] **Figure 14** presents the nucleotide sequence (SEQ ID NO:9) of the 2A encoding region of SVV. This sequence is identical to nucleotides 1924-1965 of SEQ ID NO:1.

[0085] **Figure 15** presents the amino acid sequence (SEQ ID NO:10) of the SVV 2A protein that is encoded by SEQ ID NO:9. The sequence listed in SEQ ID NO:10 is identical to amino acids 642-655 of SEQ ID NO:2.

[0086] **Figure 16** presents the nucleotide sequence (SEQ ID NO:11) of the 2B encoding region of SVV. This sequence is identical to nucleotides 1966-2349 of SEQ ID NO:1.

[0087] **Figure 17** presents the amino acid sequence (SEQ ID NO:12) of the SVV 2B protein that is encoded by SEQ ID NO:11. The sequence listed in SEQ ID NO:12 is identical to amino acids 656-783 of SEQ ID NO:2.

[0088] **Figure 18** presents the nucleotide sequence (SEQ ID NO:13) of the 2C encoding region of SVV. This sequence is identical to nucleotides 2350-3315 of SEQ ID NO:1.

[0089] **Figure 19** presents the amino acid sequence (SEQ ID NO:14) of the SVV 2C protein that is encoded by SEQ ID NO:13. The sequence listed in SEQ ID NO:14 is identical to amino acids 784-1105 of SEQ ID NO:2.

[0090] **Figure 20** presents the nucleotide sequence (SEQ ID NO:15) of the 3A encoding region of SVV. This sequence is identical to nucleotides 3316-3585 of SEQ ID NO:1.

[0091] **Figure 21** presents the amino acid sequence (SEQ ID NO:16) of the SVV 3A protein that is encoded by SEQ ID NO:15. The sequence listed in SEQ ID NO:16 is identical to amino acids 1106-1195 of SEQ ID NO:2.

[0092] **Figure 22** presents the nucleotide sequence (SEQ ID NO:17) of the 3B encoding region of SVV. This sequence is identical to nucleotides 3586-3651 of SEQ ID NO:1.

[0093] **Figure 23** presents the amino acid sequence (SEQ ID NO:18) of the SVV 3B protein that is encoded by SEQ ID NO:17. The sequence listed in SEQ ID NO:18 is identical to amino acids 1196-1217 of SEQ ID NO:2.

[0094] **Figure 24** presents the nucleotide sequence (SEQ ID NO:19) of the 3C encoding region of SVV. This sequence is identical to nucleotides 3652-4284 of SEQ ID NO:1.

[0095] **Figure 25** presents the amino acid sequence (SEQ ID NO:20) of the SVV 3C protein that is encoded by SEQ ID NO:19. The sequence listed in SEQ ID NO:20 is identical to amino acids 1218-1428 of SEQ ID NO:2.

[0096] **Figure 26** presents the nucleotide sequence (SEQ ID NO:21) of the 3D encoding region of SVV. This sequence is identical to nucleotides 4285-5673 of SEQ ID NO:1.

[0097] **Figure 27** presents the amino acid sequence (SEQ ID NO:22) of the SVV 3D protein that is encoded by SEQ ID NO:21. The sequence listed in SEQ ID NO:22 is identical to amino acids 1429-1890 of SEQ ID NO:2.

[0098] **Figures 28A-28H** present an amino acid sequence alignment between SVV SEQ ID NO:2 and various members of the *Cardiovirus* genus, such as Encephalomyocarditis virus (EMCV; species *Encephalomyocarditis virus*), Theiler's murine encephalomyocarditis

virus (TMEV; species *Theilovirus*), a rat TMEV-like agent (TLV; species *Theilovirus*), and Vilyuisk human encephalomyelitis virus (VHEV; species *Theilovirus*). The specific sequences of the various Cardioviruses are presented in: SEQ ID NOS: 23 (EMCV-R), 24 (EMCV-PV21), 25 (EMCV-B), 26 (EMCV-Da), 27 (EMCV-Db), 28 (EMCV-PV2), 29 (EMCV-Mengo), 30 (TMEV/DA), 31 (TMEV/GDVII), 32 (TMEV/BeAn8386), 33 (TLV-NGS910) and 34 (VHEV/Siberia-55).

[0099] Number positions in Figure 28 do not correspond to the numbering of the sequence listings. The “/” symbol indicates cleavage sites where the polyprotein is cleaved into its final functional products. For example, the alignment between positions 1 and 157 is in the 1A (VP4) region. The alignment between positions: 159 and 428 is in the 1B (VP2) region; 430 and 668 is in the 1C (VP3) region; 670 and 967 is in the 1D (VP1) region; 969 and 1111 is in the 2A region; 1112 and 1276 is in the 2B region; 1278 and 1609 is in the 2C region; 1611 and 1700 is in the 3A region; 1702 and 1723 is in the 3B region; 1725 and 1946 is in the 3C region; 1948 and 2410 is in the 3D region. The alignment also shows regions of potential conservation or similarity between the viral sequences as can be determined by standard sequence analysis programs. The alignments were generated using BioEdit 5.0.9 and Clustal W 1.81.

[0100] **Figure 29** lists the final polypeptide products of SVV with respect to SEQ ID NO:2. Some conserved motifs are bolded and underlined: 2A/2B “cleavage” (NPGP (SEQ ID NO:111)); 2C ATP-binding (GxxGxGKS/T (SEQ ID NO:112) and hyhyhyxxD); 3B (VPg)/RNA attachment residue (Y); 3C (pro) active site residues (H, C, H); 3D (pol) motifs (KDEL/IR (SEQ ID NO:113), PSG, YGDD (SEQ ID NO:114), FLKR (SEQ ID NO:115)).

[0101] **Figure 30** lists the picornavirus species that were used in sequence analyses with SEQ ID NOS:1 and 2 to determine the phylogenetic relationship between SVV and these picornaviruses (see Example 4, Part I).

[0102] **Figure 31** shows the phylogenetic relationship between SVV (SEQ ID NO:4) and other picornaviruses in view of VP2 sequence analyses. The figure shows a bootstrapped neighbor-joining tree (see Example 4, Part I).

[0103] **Figure 32** shows a bootstrapped neighbor-joining tree for VP3 between SVV (SEQ ID NO:6) and other picornaviruses (see Example 4, Part I).

- [0104] **Figure 33** shows a bootstrapped neighbor-joining tree for VP1 between SVV (SEQ ID NO:8) and other picornaviruses (see Example 4, Part I).
- [0105] **Figure 34** shows a bootstrapped neighbor-joining tree for P1 (*i.e.*, 1A, 1B, 1C and 1D) between SVV (*i.e.*, partial P1 - amino acids 2-641 of SEQ ID NO:2) and other picornaviruses (see Example 4, Part I).
- [0106] **Figure 35** shows a bootstrapped neighbor-joining tree for 2C between SVV (SEQ ID NO:14) and other picornaviruses (see Example 4, Part I).
- [0107] **Figure 36** shows a bootstrapped neighbor-joining tree for 3C (pro) between SVV (SEQ ID NO:20) and other picornaviruses (see Example 4, Part I).
- [0108] **Figure 37** shows a bootstrapped neighbor-joining tree for 3D (pol) between SVV (SEQ ID NO:22) and other picornaviruses (see Example 4, Part I).
- [0109] **Figure 38** presents the actual amino acid percent identities of VP2 between SVV (SEQ ID NO:4) and other picornaviruses (see Example 4, Part I).
- [0110] **Figure 39** presents the actual amino acid percent identities of VP3 between SVV (SEQ ID NO:6) and other picornaviruses (see Example 4, Part I).
- [0111] **Figure 40** presents the actual amino acid percent identities of VP1 between SVV (SEQ ID NO:8) and other picornaviruses (see Example 4, Part I).
- [0112] **Figure 41** presents the actual amino acid percent identities of P1 between SVV (partial capsid or P1 - amino acids 2-641 of SEQ ID NO:2) and other picornaviruses (see Example 4, Part I).
- [0113] **Figure 42** presents the actual amino acid percent identities of 2C between SVV (SEQ ID NO:14) and other picornaviruses (see Example 4, Part I).
- [0114] **Figure 43** presents the actual amino acid percent identities of 3C (pro) between SVV (SEQ ID NO:20) and other picornaviruses (see Example 4, Part I).
- [0115] **Figure 44** presents the actual amino acid percent identities of 3D (pol) between SVV (SEQ ID NO:22) and other picornaviruses (see Example 4, Part I).
- [0116] **Figure 45** shows the VP2 (~36 kDa), VP1 (~31 kDa) and VP3 (~27 kDa) proteins of SVV as analyzed by SDS-PAGE. Purified SVV was subjected to SDS-PAGE and proteins were visualized by silver stain. Lane "MWt" is molecular weight markers; lane

“SVV” contains structural proteins of SVV. The sizes of three molecular weight markers and the names of viral proteins are also given.

[0117] **Figures 46A-46B** show the amounts of SVV in blood and tumor following systemic administration (Example 7). H446 tumor bearing nude mice were treated with SVV at a dose of 1×10^{12} vp/kg by tail vein injection. The mice were bled at 0, 1, 3, 6, 24, 48, 72 hours and at 7 days post-injection, and the plasma was separated from the blood immediately after collection, diluted in infection medium, and used to infect PER.C6 cells. The tumors were harvested at 6, 24, 48, 72 hours and at 7 days post-injection. The tumors were cut into small sections and suspended in one mL of medium and CVL was made.

[0118] **Figures 46C-46D** presents data relating to SVV clearance *in vivo*. The figures show that SVV exhibits a substantially longer resident time in the blood compared to similar doses of i.v. adenovirus (Example 7), and therefore SVV has a slower clearance rate than adenovirus *in vivo*. Following a single intravenous (i.v.) dose, SVV remains present in the blood for up to 6 hours (Figure 46C; Figure 46C is a duplication of Figure 46A for comparison purposes to Figure 46D), whereas adenovirus is cleared or depleted from the blood in about an hour (Figure 46D).

[0119] **Figure 47** shows immunohistochemistry and hematoxylin and eosin (H&E) staining of H446 xenograft sections (Example 7). H446 tumor bearing nude mice were treated with Hank's balanced salt solution (HBSS) or SVV at a dose of 1×10^{12} vp/kg by tail vein injection. The mice were sacrificed at 3 days post-injection and the tumors were collected. The virus proteins in the tumor cells are visualized by immunohistochemistry using SVV-specific mouse antibodies (upper panels). The general morphology of H446 tumor cells collected from HBSS or SVV treated mice were stained by H&E stain (lower panels).

[0120] **Figure 48** shows SVV mediated cytotoxicity in primary human hepatocytes (Example 9). Primary human hepatocytes plated in collagen coated 12-well plates were infected with SVV at 1, 10 and 100 and 1000 particles per cell (ppc). Three days after infection, the cell associated lactate dehydrogenase (LDH) and LDH in the culture supernatant were measured separately. Percent cytotoxicity was determined as a ration of LDH units in supernatant over maximal cellular LDH plus supernatant LDH.

[0121] **Figure 49** shows virus production by SVV in selected cell lines. To assess the replicative abilities of SVV, selected normal cells and tumor cells were infected with SVV at one virus particle per cell (ppc) (Example 9). After 72 hours, cells were harvested and CVL was assayed for titer on PER.C6 cells. For each cell line, the efficiency of SVV replication was expressed as plaque forming units per milliliter (pfu/ml).

[0122] **Figure 50** shows toxicity in nude and CD1 mice according to body weights (Example 10). The mean body weight of mice in each treatment group were measured different days post virus administration. Mice were injected with a single dose of SVV or PBS by tail vein on day 1.

[0123] **Figure 51** shows efficacy in a H446 xenograft model. H446 tumors are established in nude mice and the mice are sorted into groups (n=10) and treated via tail vein injection with either HBSS or six different doses of SVV (Example 11). On study day 20, five mice from the HBSS group that bear large tumors (mean tumor volume = 2000 mm³) were injected with 1×10^{11} vp/kg (indicated by an arrow). Data is expressed as mean tumor volume + standard deviation (SD).

[0124] **Figure 52** shows a picture of H446 xenograft nude mice that have been untreated or treated with SVV (Example 11). The efficacy of SVV is very robust in that 100% of large pre-established tumors were completely eradicated. SVV-treated mice show neither clinical symptoms nor recurrence of tumors for at least 200 days following injection.

[0125] **Figure 53** presents data relating to SVV tumor specificity and efficacy *in vitro* (Example 11). The graphs show cell survival following incubation of either H446 human small cell lung carcinoma (SCLC) tumor cells (top graph) or normal human H460 cells (bottom graph). SVV specifically killed the tumor cells with an EC₅₀ of approximately 10^{-3} particles per cell. In contrast, normal human cells were not killed at any concentration of SVV.

[0126] **Figure 54** depicts a representative plasmid containing the complete genome of SVV (Example 15). The presence of the T7 promoter on the vector upstream of the SVV sequence allows for the *in vitro* transcription of the SVV sequence such that SVV RNA molecules can be generated.

[0127] **Figure 55** depicts a schematic for the construction of a full-length and functional genomic SVV plasmid and subsequent SVV virus production (Example 16). To

obtain a functional genomic SVV clone, the complete genome of a SVV can be cloned into a vector with a T7 promoter. This can be accomplished by making cDNA clones of the virus, sequencing them and cloning contiguous pieces into one plasmid, resulting in the plasmid depicted “pSVV”. The plasmid with the full genome of SVV can then be reverse-transcribed to generate SVV RNA. The SVV RNA is then transfected into permissive mammalian cells and SVV virus particles can then be recovered and purified.

[0128] **Figure 56** depicts a schematic for the construction of a vector (“pSVV capsid”) containing the coding sequence (*i.e.*, coding regions for 1A-1D) for the SVV capsid (Example 16). The pSVV capsid can then be used to generate a library of SVV capsid mutants.

[0129] **Figure 57** shows one method of mutating the SVV capsid for the generation of a library of SVV capsid mutants (Example 16). The figure illustrates the insertion of an oligonucleotide sequence at random sites of the plasmid. The oligonucleotides can be random oligonucleotides, oligonucleotides with known sequences, or an oligonucleotide encoding an epitope tag. In the figure, the restriction enzyme CviJI randomly cleaves the pSVV capsid DNA. Linearized pSVV capsid DNA that has been cut at a single site is isolated and purified from a gel, and ligated with oligonucleotides.

[0130] **Figure 58** presents a scheme to generate a library of full-length SVV mutants comprising sequence mutations in the capsid encoding region (Example 16). For example, the capsid encoding region from a pSVV capsid mutant library (generated according to the scheme depicted in Figure 57, for example) is isolated by restriction digestion and gel purification. The vector containing the full-length SVV sequence is also digested such that the capsid encoding region is cut out. The capsid encoding region from the pSVV capsid mutant library is then ligated to the pSVV vector that is missing its wild-type capsid sequence, thereby generating a library of full-length SVV mutants (the “pSVVFL” vector) having a plurality of mutations in the capsid encoding region.

[0131] **Figure 59** presents a general method for producing the SVV virus particles comprising a library of capsid mutations (Example 16). The pSVVFL vector is reverse-transcribed to generate SVV RNA. The SVV RNA is transfected into permissive cells, wherein SVV mutant virus particles are produced. The virus particles lyse the cells and a population of SVV virus particles comprising a plurality of capsid variants, “SVV capsid library,” are isolated.

[0132] **Figure 60** shows a general method for screening SVV capsid mutants that can specifically infect tumor cells while being unable to infect non-tumor cells. The SVV capsid library is incubated with a tumor cell line or tissue of interest. After an initial incubation period, the cells are washed such that SVV virus particles that were unable to gain entry into the cells are eliminated. The cells are then maintained in culture until viral lysis is observed. Culture supernatant is then collected to isolate SVV capsid mutants that were able to lytically infect the tumor cell. These viruses can then be grown-up by infecting a known permissive cell-line prior to a counter-screen. A counter-screen is performed by incubating the SVV capsid mutant viruses that were able to infect the tumor cell with normal cells. Only those viruses that remain unbound in the supernatant are collected, thereby isolating mutant SVV viruses that have tumor-specificity. This process can be repeated to further refine the isolation of SVV tumor-specific viruses.

[0133] **Figure 61** shows a traditional method for testing whether virus mutants can bind and/or infect cell lines. Traditional methods require what are often inefficient methods for growing cell-lines, *i.e.* flasks, such that a mass-screen of a library of virus mutants in relation to a number of different cell-lines becomes burdensome.

[0134] **Figure 62** shows a high-throughput method of the invention for screening virus mutants that have the ability to specifically infect different cell-lines (Example 16). In this figure, a number of different tumor cell-lines are grown in a 384 well-plate. To each well, a sample of a virus is added (for example, a sample of a SVV capsid library). From those wells which show cytopathic effects, the media is collected such that any viruses in the media can be amplified by infecting permissive cell lines (for example, for SVV, H446 or PER.C6) in flasks or large tissue culture plates. The viruses are grown such that the RNA can be isolated and the sequence analyzed to determine the encoded peptide sequence inserted by the oligonucleotide-insertion mutagenesis of the capsid. The peptide itself can then be tested to determine whether it can bind to a tumor cell-type specifically.

[0135] **Figure 63** shows another high-throughput screening schematic (Example 16). Tumor and normal cell lines are grown in multi-well plates. Viruses are added to each well to test whether the cells are killed by virus-mediated lysis. Cytopathic effects can be quickly assayed by reading the light-absorbance in each well. Viruses from the wells showing cytopathic effects are grown up and tested in further *in vitro* (re-testing of tumor and normal cell lines) and *in vivo* models (testing whether the virus can kill explanted tumors in mice).

[0136] **Figure 64** shows that SVV capsid mutants (SEQ ID NOS: 45-48, respectively, in order of appearance) having new tumor-specific tropisms can be analyzed to generate tumor-selective peptides. Those SVV capsid mutants that enable the specific infection of a tumor cell line are sequenced to determine the peptide encoded by the oligonucleotide insertion. An amino acid consensus sequence can thereby be determined from the successful capsid mutants. Peptides having the consensus sequence can then be tested to determine whether they can bind specifically to the tumor cell-type in question. Tumor-selective peptides can then be attached to toxins or drugs in order to serve as tumor-specific targeting vehicles.

[0137] **Figure 65** illustrates that an SVV capsid library can be first tested *in vivo*. Mice (including normal, athymic, nude, CD-1 transgenics, etc.) can be explanted with a specific tumor. These mice are then injected with a SVV derivative library, such as a SVV capsid library. At certain time points, tumor cells are recovered from the mice, such that in those mice that display the elimination of a tumor, viruses will be isolated from initial tumor samples and grown-up in permissive cell lines.

[0138] **Figure 66** shows a clinical testing program for the SVV derivatives of the present invention.

[0139] **Figure 67** illustrates that SVV derivatives (with new tumor tropisms) encoding epitope tags in their capsid can be used for a variety of purposes. They can be used as a screening reagent to detect whether a specific tumor cell is present in tissue samples by assaying for the presence of the epitope. Alternatively, toxins or other therapeutics can be attached to the epitope tag, and the virus then administered to patients. Further, wild-type or derivative SVV can be irradiated or inactivated such that the virus particle itself is used as a therapeutic device. Either the virus particle induces cellular apoptosis due to the presence of apoptosis-inducing peptides, or the particle can have a toxin or some other therapeutic attached such that the virus is used a specific-targeting delivery device.

[0140] **Figure 68** shows the basic life-cycle of the picornavirus.

[0141] **Figure 69** shows a comparison of the polypeptide lengths of SVV compared to other picornaviruses.

- [0142] **Figure 70** lists an amino acid comparison of the picornavirus 2A-like NPG/P proteins (SEQ ID NOS: 49-110, respectively, in order of appearance). The sequence for SVV is listed at residues 635-656 of SEQ ID NO:2.
- [0143] **Figure 71** lists the amino acid sequence (SEQ ID NO:23) for EMCV-R.
- [0144] **Figure 72** lists the amino acid sequence (SEQ ID NO:24) for EMCV-PV21 (Accession CAA52361).
- [0145] **Figure 73** lists the amino acid sequence (SEQ ID NO:25) for EMCV-B (Accession P17593).
- [0146] **Figure 74** lists the amino acid sequence (SEQ ID NO:26) for EMCV-Da (Accession P17594).
- [0147] **Figure 75** lists the amino acid sequence (SEQ ID NO:27) for EMCV-Db.
- [0148] **Figure 76** lists the amino acid sequence (SEQ ID NO:28) for EMCV-PV2 (Accession CAA60776).
- [0149] **Figure 77** lists the amino acid sequence (SEQ ID NO:29) for EMCV-mengo (Accession AAA46547).
- [0150] **Figure 78** lists the amino acid sequence (SEQ ID NO:30) for TMEV/DA (Accession AAA47928).
- [0151] **Figure 79** lists the amino acid sequence (SEQ ID NO:31) for TMEV/GDVII (Accession AAA47929).
- [0152] **Figure 80** lists the amino acid sequence (SEQ ID NO:32) for TMEV/BeAn8386 (Accession AAA47930).
- [0153] **Figure 81** lists the amino acid sequence (SEQ ID NO:33) for TLV-NGS910 (Accession BAC58035).
- [0154] **Figure 82** lists the amino acid sequence (SEQ ID NO:34) for VHEV/Siberia-55 (Accession AAA47931).
- [0155] **Figures 83A-83H** present the full-length genomic sequence of SVV (SEQ ID NO:168) and the encoded polyprotein amino acid sequence (SEQ ID NO:169), where this full-length genomic sequence was obtained from SVV viruses grown from the SVV isolate having ATCC Patent Deposit Number PTA-5343. Specific features of the SVV genomic

sequence, such as the specific coding regions for proteins cleaved from the polyprotein sequence are described herein.

[0156] **Figures 84A-84D** present the full-length genomic sequence of SVV (SEQ ID NO:168). The sequence was obtained from SVV grown from the SVV isolate having ATCC Patent Deposit Number PTA-5343.

[0157] **Figures 85A-85B** present the amino acid sequence of the full-length polyprotein of SVV (SEQ ID NO:169) encoded by the nucleotides 667-7209 of SEQ ID NO:168.

[0158] **Figure 86** provides a phylogenetic analysis or epidemiology of SVV with respect to the full-length genome and polyprotein sequence of SVV from SEQ ID NOS:168 and 169. SVV is a unique virus, phylogenetically similar to cardioviruses, but in a separate tree. The SVV-like picornaviruses are most likely in the same tree or genus as SVV due to the high level of sequence identity between SVV and the SVV-like picornaviruses (see Figures 87-89) and due to the ability of antibodies raised against SVV-like picornaviruses to bind SVV (and vice versa) (see Example 4, Part III, Serum Studies).

[0159] **Figures 87A-87D** show a nucleic acid sequence comparison between SVV and some SVV-like picornaviruses in the areas of the P1 structural region and 2A. In particular, the comparison is in the VP2(partial)-VP3-VP1-2A(partial) regions. The listed SVV sequence is SEQ ID NO:170; the listed sequence for isolate IA 89-47752 is SEQ ID NO:171; the listed sequence for isolate CA 131395 is SEQ ID NO:172; the listed sequence for isolate NC 88-23626 is SEQ ID NO:173; the listed sequence for isolate MN 88-36695 is SEQ ID NO:174; the listed sequence for isolate NJ 90-10324 is SEQ ID NO:175; the listed sequence for isolate IL 92-48963 is SEQ ID NO:176; the listed sequence for isolate LA 1278 (97-1278) is SEQ ID NO:177; and the listed consensus sequence is SEQ ID NO:178.

[0160] **Figure 88** shows a nucleic acid sequence comparison between SVV and isolates IA 89-47752 and CA 131395 in the 2C coding region (partial). The listed SVV sequence is SEQ ID NO:179; the listed sequence for isolate IA 89-47752 is SEQ ID NO:180; the listed sequence for isolate CA 131395 is SEQ ID NO:181; and the listed consensus sequence is SEQ ID NO:182.

[0161] **Figures 89A-89B** show a nucleic acid sequence comparison between SVV and isolates NC 88-23626, MN 88-36695, IA 89-47752, NJ 90-10324, IL 92-48963, LA 97-

1278, and CA 131395 in the 3D polymerase coding region (partial) and 3' UTR region. The listed sequences are SVV (SEQ ID NO:183), NC 88-23626 (SEQ ID NO:184), MN 88-36695 (SEQ ID NO:185), IA 89-47752 (SEQ ID NO:186), NJ 90-10324 (SEQ ID NO:187), IL 92-48963 (SEQ ID NO:188), LA 97-1278 (SEQ ID NO:189), CA 131395 (SEQ ID NO:190), and consensus sequence (SEQ ID NO:191).

[0162] **Figures 90A-90E** show that a single dose of SVV is efficacious in reducing the size and preventing the growth of explanted tumors in mice. **Figure 90A** shows that SVV can reduce the size and prevent the growth of explanted H446 human SCLC tumors ($ED_{50} = 0.0007$). **Figure 90B** shows that SVV can reduce the size and prevent the growth of explanted Y79 human retinoblastoma tumors ($ED_{50} = 0.0007$). **Figure 90C** shows that SVV can reduce the size and prevent the growth of explanted H69AR human SCLC-MDR (multi drug resistant) tumors ($ED_{50} = 0.05$). **Figure 90D** shows that SVV can reduce the size and prevent the growth of explanted H1299 human HSCLC tumors ($ED_{50} = 4.8$). **Figure 90E** shows that SVV can reduce the size and prevent the growth of explanted N1E-115 murine neuroblastoma tumors in A/J mice (normal immunocompetent mice) ($ED_{50} = 0.001$).

[0163] **Figure 91** show a molecular model of the EMCV and TMEV capsid structures in comparison with the sequence of SVV. A molecular model in conjunction with the use of algorithms for antigenic prediction allows for peptide sequences to be chosen for polyclonal antibody generation. β -sheets are shown in brown, α -helices are shown in green, and a 12-mer peptide sequence chosen for polyclonal generation is shown in yellow. The particular sequence (in the VP2 region) was chosen because it presents good surface exposure according to the model.

[0164] **Figures 92A-92D** show the specificity of polyclonal antibodies against SVV. **Figure 92A** is a negative control, and presents an immunofluorescence image of cells infected with SVV that are stained with non-specific anti-mouse sera and secondary antibody. **Figures 92B and 92C** show immunofluorescence images of cells infected with SVV that are stained with mouse anti-SVV sera diluted 1:50 and secondary antibody (anti-mouse Ig conjugated to fluorescein). **Figure 92D** shows that polyclonal anti-SVV antibodies can be used in viral binding assays; the image shows an immunofluorescence image of SVV concentrated in an outline around a cell because the cell was put on ice to prevent SVV internalization.

[0165] **Figure 93** shows the results of a neutralization assay of GP102 sera on SVV (see Example 18). The neutralization titer (calculated as the highest dilution that neutralizes the virus is 100%) is 1:100.

[0166] **Figure 94** shows the results of a neutralization assay of anti-SVV antisera on MN 88-36695 (see Example 18). The neutralization titer is 1:560.

[0167] **Figure 95A** and **Figure 95B** depict neighbor-joining trees. These trees were constructed using PHYLIP (Phylogeny Inference Package Computer Programs for Inferring Phylogenies) and show the relationship between SVV and seven SVV-like picornaviruses when comparing sequences from regions in P1 and partial 2A (Figure 95A) and in the 3' end of the genome (Figure 95B).

DETAILED DESCRIPTION OF THE INVENTION

[0168] The terms “virus,” “viral particle,” “virus particle,” and “virion” are used interchangeably.

[0169] The terms “vector particle” and “viral vector particle” are interchangeable and are to be understood broadly - for example - as meaning infectious viral particles that are formed when, *e.g.*, a viral vector of the invention is transduced or transfected into an appropriate cell or cell line for the generation of infectious particles.

[0170] The terms “derivative,” “mutant,” “variant” and “modified” are used interchangeably to generally indicate that a derivative, mutant, variant or modified virus can have a nucleic acid or amino acid sequence difference in respect to a template viral nucleic acid or amino acid sequence. For example, a SVV derivative, mutant, variant or modified SVV may refer to a SVV that has a nucleic acid or amino acid sequence difference with respect to the wild-type SVV nucleic acid or amino acid sequence of ATCC Patent Deposit Number PTA-5343.

[0171] An “SVV-like picornavirus” as used herein can have at least about 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SVV at the nucleotide level (see SEQ ID NO:168, Figure 84, and Figure 83 for the SVV full-length genomic sequence), where the sequence comparison is not limited to a whole-genome analysis, but can be focused on a particular region of the genome, such as the 5'UTR, structural encoding regions, non-structural encoding regions, 3'UTR, and portions thereof. The particular length of the

genome for sequence comparison that is adequate to determine relatedness/likeness to SVV is known to one skilled in the art, and the adequate length can vary with respect to the percentage of identity that is present. The length for sequence comparison can be, for example, at least 20, 50, 100, 200, 300, 400, 500, 750, 1000, 1500, 2000, or 2500 nucleotides. Where the length is shorter, one skilled in the art understands, for example, that the identity between sequences can be higher in order to consider the two sequences to be related. However, such guidance is qualified at least with respect to considerations of sequence conservation, in that certain regions of the genome are more conserved than others between related species. Additionally, if an antiserum generated from a virus can neutralize SVV infection of an SVV permissive cell line, then the virus is considered to be an SVV-like picornavirus. Additionally, if an antiserum generated from a virus can neutralize SVV infection of an SVV permissive cell line, and that antiserum can also bind to other viruses (for example, if the antiserum can be used in indirect immunofluorescence assays to detect virus), then the other viruses that can be bound by the antiserum are considered to be SVV-like picornaviruses. For purposes of the invention, SVV-like picornaviruses can include cardioviruses. Exemplary SVV permissive cells or cell lines include, but are not limited to, Y79, NCI-H446, N1E-115, NCI-H1770, NCI-H82, PER.C6®, NCI-H69AR, SK-NEP-1, IMR-32, NCI-H187, NCI-H209, HCC33, NCI-H1184, D283 Med, SK-N-AS, BEK PCB3E1, ST, NCI-H1299, DMS 153, NCI-H378, NCI-H295R, BEK, PPASMC, PCASMC, PAoSMC, NCI-H526, OVCAR-3, NCI-H207, ESK-4, SW-13, 293, Hs 578T, HS 1.Tes, and LOX IMVI.

[0172] As used herein, the terms “cancer,” “cancer cells,” “neoplastic cells,” “neoplasia,” “tumor,” and “tumor cells,” are used interchangeably, and refer to cells that exhibit relatively autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation. Neoplastic cells can be malignant or benign. According to the present invention, one type of preferred tumor cells are those with neurotropic properties.

[0173] The terms “identical” or percent “identity” in the context of two or more nucleic acid or protein sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm such as Protein-Protein BLAST (Protein-Protein BLAST of GenBank

databases (Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-410)) or by visual inspection. The BLAST algorithm is described in Altschul *et al.*, J. Mol. Biol., 215:403-410 (1990), and publicly available BLAST software is provided through the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/>).

[0174] For example, as used herein, the term "at least 90% identical to" refers to percent identities from 90 to 100 relative to the reference polypeptides (or polynucleotides). Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polypeptide length of 100 amino acids are compared, no more than 10% (*i.e.*, 10 out of 100) amino acids in the test polypeptide differs from that of the reference polypeptide. Similar comparisons can be made between a test and reference polynucleotide. Such differences can be represented as point mutations randomly distributed over the entire length of an amino acid sequence or they can be clustered in one or more locations of varying length up to the maximum allowable, *e.g.*, 10 out of 100 amino acid differences (90% identity). Differences are defined as nucleic acid or amino acid substitutions, insertions or deletions. At the level of identities above about 85-90%, the result should be independent of the program and gap parameters set; such high levels of identity can be assessed readily, often without relying on software.

[0175] The concepts of "high stringency," "intermediate stringency" or "low stringency" refer to nucleic acid hybridization conditions. High stringency conditions refers to conditions that require a greater identity between a target's nucleic acid sequence and a probe's nucleic acid sequence in order for annealing or hybridization to occur between the target and the probe. Low stringency conditions refer to conditions that require a lower identity between a target's nucleic acid sequence and a probe's nucleic acid sequence in order for annealing or hybridization to occur between the target and the probe. Stringency conditions can be controlled by the salt concentration of the buffer or by the temperature at which the hybridization is carried out, where higher salt concentrations result in less stringent conditions and where higher temperatures result in more stringent conditions. Although stringency conditions will vary based on the length and nucleic acid content of the sequences undergoing hybridization, representative conditions of high, intermediate and low stringency are described in the following exemplary conditions. A commonly used hybridization buffer is SSC (sodium chloride sodium citrate) with a 20X stock concentration corresponding to 0.3

M trisodium citrate and 3 M NaCl. For high stringency conditions, the working concentration of SSC can be 0.1X – 0.5X (1.5 – 7.5 mM trisodium citrate, 15 – 75 mM NaCl) with the hybridization temperature set at 65°C. Intermediate conditions typically utilize a 0.5X – 2X SSC concentration (7.5 – 30 mM trisodium citrate, 75 – 300 mM NaCl) at a temperature of 55 – 62°C. Hybridizations conducted under low stringency conditions can use a 2X – 5X SSC concentration (30 – 75 mM trisodium citrate, 300 – 750 mM NaCl) at a temperature of 50 – 55°C. Note that these conditions are merely exemplary and are not to be considered limitations.

Seneca Valley Virus (SVV):

[0176] SVV is a novel, heretofore undiscovered RNA virus, and with respect to previously characterized picornaviruses, SVV is most closely related to members from the genus *Cardiovirus* in the family *Picornaviridae* (see International Application No. PCT/US2004/031594). The results of sequence analyses between SVV and other cardioviruses are discussed in PCT/US2004/031594, which is hereby incorporated by reference in its entirety. Since the time of the sequence analysis of SVV described in PCT/US2004/031594, the Picornavirus Study Group has initiated discussion as to whether SVV will be a member of a new genus. Figure 86 presents a genetic relationship tree between members of the family *Picornaviridae*.

[0177] From initial sequence comparisons to known picornaviruses (see International Application No. PCT/US2004/031504), there were two phylogenetic classification options: (1) to include SVV as a new species in the genus *Cardiovirus*; or (2) assign SVV to a new genus. At that time and for the International application, SVV was designated to be a novel member of the genus *Cardiovirus*. After further analyses however, it has been found that several characteristics of SVV differ with that of cardioviruses. For example, some cardiovirus genomes contain an extended internal poly(C) tract in their 5' UTRs. SVV does not contain a poly(C) tract. From the additional 5' sequence information, the Internal Ribosome Entry Sequence (IRES) of SVV has been mapped and compared to other picornaviruses, and it has been determined that the SVV IRES is Type IV, whereas cardiovirus IRES's are Type II. The cardioviruses have a long (150 amino acid (aa)) 2A protease while SVV has a short (9 aa) 2A protease. The size of this protein as well as others (Leader peptide, 3A) differs significantly between SVV and cardioviruses. From the study of other picornaviruses, it is known that these proteins are likely involved in host cell interactions

including tropism and virulence. Lastly, it is now thought that the overall sequences differ too much in a number of genome regions and SVV should therefore be considered to form a new genus. Additionally, multiple unique picornaviruses have been discovered at the USDA that are more similar to SVV than SVV is to other cardioviruses. Therefore, it has been decided by the Executive Committee of the International Committee for the Taxonomy of Viruses (ICVT) based on recommendations made by the Picornavirus Study Group that SVV will make up a new species of picornavirus, named Seneca Valley virus. However, currently, SVV and these unique USDA picornaviruses (herein referred to as being members of the group of SVV-like picornaviruses) are currently unassigned to any genus.

[0178] Several of the SVV-like picornaviruses discovered at the USDA are about 95-98% identical to SVV at the nucleotide level (for example, see Figures 87-89). Antisera against one virus (MN 88-36695) neutralizes SVV, and this virus is reactive to other antisera that can neutralize SVV. The SVV-like picornaviruses were isolated from pigs, and thus, pigs are likely a permissive host for SVV and other SVV-like viruses. Examples of SVV-like picornaviruses isolated from pigs include, but are not limited to, the following USDA isolates MN 88-36695, NC 88-23626, IA 89-47552, NJ 90-10324, IL 92-48963, CA 131395; LA 1278; IL 66289; IL 94-9356; MN/GA 99-29256; MN 99197; and SC 363649. SVV-like picornaviruses may also include cardioviruses closely related to SVV (as determined by sequence analysis or by cross-reactivity to antibodies raised against SVV antigens). Thus, for purposes of the present invention, SVV can be considered: (1) to be closely related to (or to be a member of) the genus *Cardiovirus* of the family *Picornaviridae*, and (2) to be a member of a new genus of the family *Picornaviridae*, where members of the new genus can include SVV and SVV-like picornaviruses not classified to be members of other genera.

[0179] SVV, like cardioviruses, can be distinguished from other picornaviruses by special features of their genome organization, common pathological properties, and the dissociability of their virions at pHs between 5 and 7 in 0.1M NaCl (Scraba, D. *et al.*, "Cardioviruses (*Picornaviridae*)," in Encyclopedia of Virology, 2nd Edition, R.G. Webster and A. Granoff, Editors, 1999). The genome of SVV consists of one single-stranded positive (+) sense strand RNA molecule having a size of 7,310 nucleotides including a poly(A) tail of 30 nucleotides in length (see Figures 83A-83H; Figures 84A-84D; SEQ ID NO:168). As SVV is a picornavirus, it has a number of features that are conserved in all picornaviruses: (i) genomic RNA is infectious, and thus can be transfected into cells to bypass the virus-receptor

binding and entry steps in the viral life cycle; (ii) a long (about 600-1200 bp) untranslated region (UTR) at the 5' end of the genome (for SVV, nucleotides 1-666 of SEQ ID NO:168), and a shorter 3' untranslated region (about 50-100 bp; for SVV, nucleotides 7210-7280 of SEQ ID NO:168; (iii) the 5' UTR contains a clover-leaf secondary structure known as the internal ribosome entry site (IRES) (which can be, for example, from about nucleotide 300 to about nucleotide 366 of SEQ ID NO:168); cardioviruses have a Type II IRES and SVV has a Type IV IRES; (iv) the rest of the genome encodes a single polyprotein (for SVV, nucleotides 667-7209 of SEQ ID NO:168 encode the polyprotein (SEQ ID NO:169)) and (v) both ends of the genome are modified, the 5' end by a covalently attached small, basic protein, "Vpg," and the 3' end by polyadenylation (for SVV, nucleotides 7281-7310 of SEQ ID NO:168).

[0180] The invention provides the isolated SVV virus (ATCC Patent Deposit number PTA-5343) and the complete genomic content of SVV therefrom. At first, the largest SVV genomic fragment that was sequenced is an isolated SVV nucleic acid, derived from the PTA-5343 isolate, that comprises the majority of the SVV genomic sequence, and is listed in Figures 5A-5E and Figures 6A-6D, and has the designation of SEQ ID NO:1 herein. Translation of this nucleotide sequence shows that the majority of the single polyprotein of SVV is encoded by SEQ ID NO:1. The amino acid sequence encoded by nucleotides 1 to 5673 of SEQ ID NO:1 is listed in Figures 5A-E and Figures 7A-7B has the designation of SEQ ID NO:2 herein. The full-length genome or what appears to be the full-length genome has since been obtained, and is listed in Figures 83A-83H and SEQ ID NO:168. Nucleotides 667-7209 encode the full-length polyprotein of SVV, and the amino acid sequence of the polyprotein is listed in Figures 83A-83H and SEQ ID NO:169.

[0181] The invention provides isolated (or purified) portions of SEQ ID NO:1, including SEQ ID NOS:3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, and isolated portions of SEQ ID NO:168, including the 5'UTR region (1-666), coding region for the leader peptide (667-903), coding region for the VP4 protein (904-1116), coding region for the VP2 protein (1117-1968), coding region for the VP3 protein (1969-2685), coding region for the VP1 protein (2686-3474), coding region for the coding region for the 2A protein (3478-3504), coding region for the 2B protein (3505-3888), coding region for the 2C protein (3889-4854), coding region for the 3A protein (4855-5124), coding region for the 3B protein (5125-5190), coding region for the 3C protein (5191-5823), coding region for the 3D protein (5824-7209), and the 3'UTR region including the poly(A) tail (7210-7310). The invention also provides isolated

nucleic acids that are portions of the specified portions listed above. The invention also provides mutants or derivatives of such isolated portions. The isolated portions of SEQ ID NOS:1 and 168 can be subcloned into expression vectors such that polypeptides encoded by these portions can be isolated. Further encompassed by the invention are isolated nucleic acids that can hybridize to SEQ ID NO:1 or SEQ ID NO:168, or any portion thereof, under high, moderate or low stringency conditions. The following table lists the nucleotides of SEQ ID NO:168 that encode the SVV proteins. The invention provides isolated (or purified) SVV proteins or portions thereof. The table also lists the amino acid sequences of the SVV proteins with respect to the polyprotein sequence listed in SEQ ID NO:169.

[0182] Table A: SVV Genome and Protein Features

SVV feature	Location in SEQ ID NO:168	Location in SEQ ID NO:169
5'UTR	1-666	N/A (not allowed)
Leader	667-903 (coding sequence for Leader peptide)	1-79
VP4	904-1116 (coding sequence for VP4)	80-150
VP2	1117-1968 (coding sequence for VP2)	151-434
VP3	1969-2685 (coding sequence for VP3)	435-673
VP1	2686-3474 or 3477 (coding sequence for VP1)	674-936 or 937
2A	3478-3504 (coding sequence for 2A)	938-946
2B	3505-3888 (coding sequence for 2B)	947-1074
2C	3889-4854 (coding sequence for 2C)	1075-1396
3A	4855-5124 (coding sequence for 3A)	1397-1486
3B	5125-5190 (coding sequence for 3B)	1487-1508
3C	5191-5823 (coding sequence for 3C)	1509-1719
3D	5824-7209 (coding sequence for 3D)	1720-2181
3'UTR	7210-7310	N/A

[0183] The invention provides an isolated SVV leader sequence peptide with the amino acid sequence of residues 1-79 of SEQ ID NO:169, which is encoded by nucleotides 667-903 of SEQ ID NO:168.

[0184] The invention provides an isolated SVV VP4 (1A) protein with the amino acid sequence of residues 80-150 of SEQ ID NO:169, which is encoded by nucleotides 904-1116 of SEQ ID NO:168.

[0185] The invention provides an isolated SVV VP2 (1B) protein with the amino acid sequence of residues 151-434 of SEQ ID NO:169, which is encoded by nucleotides 1117-1968 of SEQ ID NO:168. The invention also provides an isolated partial SVV VP2 (1B)

protein with the amino acid sequence of SEQ ID NO:4, as listed in Figure 9 (which corresponds to amino acids 2-143 of SEQ ID NO:2). The amino acid sequence of the partial SVV VP2 protein is encoded by the nucleic acid sequence of SEQ ID NO:3, as listed in Figure 8 (which corresponds to nucleotides 4-429 of SEQ ID NO:1).

[0186] The invention provides an isolated SVV VP3 (1C) protein with the amino acid sequence of residues 435-673 of SEQ ID NO:169, which is encoded by nucleotides 1969-2685 of SEQ ID NO:168. The invention also provides an isolated SVV VP3 (1C) protein with the amino acid sequence of SEQ ID NO:6, as listed in Figure 11 (which corresponds to amino acids 144-382 of SEQ ID NO:2). The amino acid sequence of the SVV VP3 protein is encoded by the nucleic acid sequence of SEQ ID NO:5, as listed in Figure 10 (which corresponds to nucleotides 430-1146 of SEQ ID NO:1).

[0187] The invention provides an isolated SVV VP1 (1D) protein with the amino acid sequence of residues 674-937 of SEQ ID NO:169, which is encoded by nucleotides 2686-3477 of SEQ ID NO:168. The invention also provides an isolated SVV VP1 (1D) protein with the amino acid sequence of SEQ ID NO:8, as listed in Figure 13 (which corresponds to amino acids 383-641 of SEQ ID NO:2). The amino acid sequence of the SVV VP1 protein is encoded by the nucleic acid sequence of SEQ ID NO:7, as listed in Figure 12 (which corresponds to nucleotides 1147-1923 of SEQ ID NO:1).

[0188] The invention provides an isolated SVV 2A protein with the amino acid sequence of residues 938-946 of SEQ ID NO:169, which is encoded by nucleotides 3478-3504 of SEQ ID NO:168. The invention also provides an isolated SVV 2A protein with the amino acid sequence of SEQ ID NO:10, as listed in Figure 15 (which corresponds to amino acids 642-655 of SEQ ID NO:2). The amino acid sequence of the SVV 2A protein is encoded by the nucleic acid sequence of SEQ ID NO:9, as listed in Figure 14 (which corresponds to nucleotides 1924-1965 of SEQ ID NO:1).

[0189] The invention provides an isolated SVV 2B protein with the amino acid sequence of residues 947-1074 of SEQ ID NO:169, which is encoded by nucleotides 3505-3888 of SEQ ID NO:168. The present invention also provides an isolated SVV 2B protein with the amino acid sequence of SEQ ID NO:12, as listed in Figure 17 (which corresponds to amino acids 656-783 of SEQ ID NO:2). The amino acid sequence of the SVV 2B protein is encoded by the nucleic acid sequence of SEQ ID NO:11, as listed in Figure 16 (which corresponds to nucleotides 1966-2349 of SEQ ID NO:1).

[0190] The invention provides an isolated SVV 2C protein with the amino acid sequence of residues 1075-1396 of SEQ ID NO:169, which is encoded by nucleotides 3889-4854 of SEQ ID NO:168. The invention also provides an isolated SVV 2C protein with the amino acid sequence of SEQ ID NO:14, as listed in Figure 19 (which corresponds to amino acids 784-1105 of SEQ ID NO:2). The amino acid sequence of the SVV 2B protein is encoded by the nucleic acid sequence of SEQ ID NO:13, as listed in Figure 18 (which corresponds to nucleotides 2350-3315 of SEQ ID NO:1).

[0191] The invention provides an isolated SVV 3A protein with the amino acid sequence of residues 1397-1486 of SEQ ID NO:169, which is encoded by nucleotides 4855-5124 of SEQ ID NO:168. The invention also provides an isolated SVV 3A protein with the amino acid sequence of SEQ ID NO:16, as listed in Figure 21 (which corresponds to amino acids 1106-1195 of SEQ ID NO:2). The amino acid sequence of the SVV 3A protein is encoded by the nucleic acid sequence of SEQ ID NO:15, as listed in Figure 20 (which corresponds to nucleotides 3316-3585 of SEQ ID NO:1).

[0192] The invention provides an isolated SVV 3B (VPg) protein with the amino acid sequence of residues 1487-1508 of SEQ ID NO:169, which is encoded by nucleotides 5125-5190 of SEQ ID NO:168. The invention also provides an isolated SVV 3B protein with the amino acid sequence of SEQ ID NO:18, as listed in Figure 23 (which corresponds to amino acids 1196-1217 of SEQ ID NO:2). The amino acid sequence of the SVV 3B protein is encoded by the nucleic acid sequence of SEQ ID NO:17, as listed in Figure 22 (which corresponds to nucleotides 3586-3651 of SEQ ID NO:1).

[0193] The invention provides an isolated SVV 3C ("pro" or "protease") protein with the amino acid sequence of residues 1509-1719 of SEQ ID NO:169, which is encoded by nucleotides 5191-5823 of SEQ ID NO:168. The invention also provides an isolated SVV 3C protein with the amino acid sequence of SEQ ID NO:20, as listed in Figure 25 (which corresponds to amino acids 1218-1428 of SEQ ID NO:2). The amino acid sequence of the SVV 3C protein is encoded by the nucleic acid sequence of SEQ ID NO:19, as listed in Figure 24 (which corresponds to nucleotides 3652-4284 of SEQ ID NO:1).

[0194] The invention provides an isolated SVV 3D ("pol" or "polymerase") protein with the amino acid sequence of residues 1720-2181 of SEQ ID NO:169, which is encoded by nucleotides 5824-7209 of SEQ ID NO:168. The invention also provides an isolated SVV 3D protein with the amino acid sequence of SEQ ID NO:22, as listed in Figure 27 (which

corresponds to amino acids 1429-1890 of SEQ ID NO:2). The amino acid sequence of the SVV 3C protein is encoded by the nucleic acid sequence of SEQ ID NO:19, as listed in Figure 24 (which corresponds to nucleotides 4285-5673 of SEQ ID NO:1; nucleotides 5671-5673, "tga," code for a stop-codon, which is depicted in the amino acid sequence listings as an asterisk "*").

[0195] The nucleic acids of the present invention include both RNA and DNA forms, and implicitly, the complementary sequences of the provided listings.

[0196] Thus, the isolated SVV nucleic acid depicted by SEQ ID NO:168 has a length of 7,310 nucleotides that encodes a polyprotein with the amino acid sequence depicted by SEQ ID NO:169. The isolated SVV nucleic acid depicted by SEQ ID NO:1 has a length of 5,752 nucleotides that encodes a polypeptide with the amino acid sequence depicted by SEQ ID NO:2. The SVV genomic sequence is translated as a single polyprotein that is cleaved into various downstream "translation products." The present invention encompasses all nucleic acid fragments of SEQ ID NO: 168 and SEQ ID NO:1, and all polypeptides encoded by such fragments.

[0197] The full-length SVV polyprotein amino acid sequence is depicted by SEQ ID NO:169 and is encoded by nucleotides 667-7209 of SEQ ID NO:168. The majority of the full-length SVV polyprotein amino acid sequence is encoded by nucleotides 1-5673 of SEQ ID NO:1. The polyprotein is cleaved into three precursor proteins, P1, P2 and P3 (see Figure 4B). P1, P2 and P3 are further cleaved into smaller products. The cleavage products of the structural region P1 (1ABCD; or the capsid region) are 1ABC, VP0, VP4, VP2, VP3 and VP1. The cleavage products of the non-structural protein P2 (2ABC) are 2A, 2BC, 2B and 2C. The cleavage products of the non-structural region P3 polyprotein (3ABCD) are 3AB, 3CD, 3A, 3C, 3D, 3C', and 3D'.

[0198] In certain embodiments, the invention provides isolated nucleic acids that comprise: (i) the coding sequence of 1ABCD or the capsid region (nucleotides 904-3477 of SEQ ID NO:168); (ii) the coding sequence of 1ABC (nucleotides 904-2685 of SEQ ID NO:168); (iii) the coding sequence of VP0 (nucleotides 904-1968 of SEQ ID NO:168); (iv) the coding sequence of 2ABC (nucleotides 3478-4854 of SEQ ID NO:168; nucleotides 1924-3315 of SEQ ID NO:1); (v) the coding sequence of 2BC (nucleotides 3505-4854 of SEQ ID NO:168; nucleotides 1966-3315 of SEQ ID NO:1); (iii) the coding sequence of 3ABCD (nucleotides 4855-7209 of SEQ ID NO:168; nucleotides 3316-5673 of SEQ ID NO:1); (iv)

the coding sequence of 3AB (nucleotides 4855-5190 of SEQ ID NO:168; nucleotides 3316-3651 of SEQ ID NO:1); and (v) the coding sequence of 3CD (nucleotides 5191-7209 of SEQ ID NO:168; nucleotides 3652-5673 of SEQ ID NO:1). The invention also provides isolated proteins or peptides encoded by the coding sequences described above, including fragments thereof.

[0199] The basic capsid structure of picornaviruses consists of a densely packed icosahedral arrangement of 60 protomers, each consisting of 4 polypeptides, VP1, VP2, VP3 and VP4, all of which are derived from the cleavage of the original protomer, VP0. The SVV virus particle is about 27 nm in diameter (*see* Figure 2), which is consistent with the size of other picornavirus particles, which are about 27-30 nm in diameter.

[0200] The kinetics of picornavirus replication is rapid, the cycle being completed in about 5-10 hours (typically by about 8 hours) (*see* Figure 68 for a schematic of the picornavirus replication cycle). Upon receptor binding, the genomic RNA is released from the particle into the cytoplasm. Genomic RNA is then translated directly by polysomes, but in about 30 minutes after infection, cellular protein synthesis declines sharply, almost to zero. This phenomenon is called "shutoff," and is a primary cause of cytopathic effects (cpe). Shutoff appears to be due to cleavage of the host cell's 220 kDa cap-binding complex (CBC) that is involved in binding the m7G cap structure at the 5' end of all eukaryotic mRNA during initiation of translation. The cleavage of the CBC appears to be caused by the 2A protease.

[0201] The 5' UTR contains the IRES. Normally, eukaryotic translation is initiated when ribosomes bind to the 5' methylated cap and then scans along the mRNA to find the first AUG initiation codon. The IRES overcomes this process and allows Picornavirus RNA's to continue to be translated after degradation of CBC. In one embodiment, the invention provides for an isolated nucleic acid comprising the SVV IRES, wherein the IRES is contained within the 5'UTR. In one embodiment, the SVV IRES can be from nucleotides 300-366 of SEQ ID NO:168. The 5'UTR of SVV is present at nucleotides 1-666 of SEQ ID NO:168.

[0202] The virus polyprotein is initially cleaved by the 2A protease into polyproteins P1, P2 and P3 (*see* Figure 4B). Further cleavage events are then carried out by 3C, the main picornavirus protease. One of the cleavage products made by 3C is the virus RNA-dependent RNA polymerase (3D), which copies the genomic RNA to produce a negative (-) sense

strand. The (-) sense strand forms the template for the (+) strand (genomic) RNA synthesis. Some of the (+) strands are translated to produce additional viral proteins and some (+) strands are packaged into capsids to form new virus particles.

[0203] The (+) strand RNA genome is believed to be packaged into preformed capsids, although the molecular interactions between the genome and the capsid are not clear. Empty capsids are common in all picornavirus infections. The capsid is assembled by cleavage of the P1 polyprotein precursor into a protomer consisting of VP0, VP3, and VP1, which join together enclosing the genome. Maturation and infectivity of the virus particle relies on an internal autocatalytic cleavage of VP0 into VP2 and VP4. Release of newly formed virus particles occurs when the cell lyses.

[0204] The present invention also provides an isolated virus having all the identifying characteristics and nucleic acid sequence of ATCC Patent Deposit number PTA-5343. Viruses of the present invention can be directed to the PTA-5343 isolate, variants, homologues, derivatives and mutants of the PTA-5343 isolate, and variants, homologues, derivatives and mutants of other picornaviruses that are modified in respect to sequences of SVV (both wild-type as disclosed herein and mutant) that are determined to be responsible for its oncolytic properties.

[0205] The present invention further provides antibodies that are specific against: the isolated SVV having the ATTC Patent Deposit number PTA-5343, and epitopes from the isolated SVV proteins having the amino acid sequences SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 169 (including entire polyprotein, VP4, VP2, VP3, VP1, 2A, 2B, 2C, 3A, 3B, 3C, 3D, and portions thereof; *see* Table A *supra* for amino acids in SEQ ID NO:169 that make up these proteins). The invention also includes antibodies that are specific against epitopes from the proteins that are encoded by fragments or portions of SEQ ID NO:1 or SEQ ID NO:168.

[0206] Comparative analyses of the RNA sequences from a variety of cardiovirus isolates have shown >45% nucleotide identity between genomes. Cardioviruses can be subclassified into the EMC-like viruses ("EMCV" - such as, Mengo, B, R; and also MM, ME, Columbia-SK), the Theiler's-like viruses ("TMEV" - such as, BeAn, DA and GD VII strains), and the Vilyuisk viruses.

[0207] In analyzing the SVV sequence to other viruses, it appears that SVV is a cardiovirus (see Example 4 and Figures referenced therein). If EMCV and TMEV are taken as the standard cardioviruses, SVV is clearly not a typical cardiovirus. However, even these two viruses have their differences, notably in the 5' UTR (Pevear et al., 1987, J. Virol., 61: 1507-1516). Phylogenetically SVV clusters with EMCV and TMEV in much of its polyprotein (P1, 2C, 3C^{pro} and 3D^{pol} regions; see Figures 31-37), indicating that SVV is most likely a cardiovirus.

[0208] SVV is phylogenetically similar to cardioviruses, but it has now been determined to be in a separate tree (see Figure 86). SVV can be in a separate genus because: (1) SVV IRES is Type IV (cardiovirus IRES are Type II); (2) multiple unique viruses ("SVV-like picornaviruses") are more similar to SVV than SVV is to other cardioviruses (see Example 18 and Figures 87-89); and antibodies that can neutralize SVV infection of permissive cell lines or were raised against SVV are able to bind to the SVV-like picornaviruses. Thus, an SVV-like picornavirus can be used in any of the present methods, including the methods to treat cancer, where it is determined that the SVV-like picornavirus is naturally oncolytic or is made to be oncolytic (for example, by designing mutations in the SVV-like picornavirus genome based on the SVV sequence). In one embodiment, MN 88-36696 is used in the present methods to treat cancer.

Methods for Treating Cancer:

[0209] The present invention provides methods for cancer therapy using viruses modified in view of the oncolytic properties of SVV, including picornaviruses (including SVV-like picornaviruses), derivatives, variants, mutants or homologues thereof. The present invention shows that wild-type SVV (*i.e.*, ATTC Patent Deposit number PTA-5343) has the ability to selectively kill some types of tumors. For example, SVV can selectively kill tumor cells that have neurotropic or neuroendocrine properties, including small cell lung cancer (SCLC) and neuroblastomas. Other examples of neuroendocrine tumors that are contemplated to be treated by the viruses of the present invention include, but are not limited to: adrenal pheochromocytomas, gastrinomas (causing Zollinger-Ellison syndrome), glucagonomas, insulinomas, medullary carcinomas (including medullary thyroid carcinoma), multiple endocrine neoplasia syndromes, pancreatic endocrine tumors, paragangliomas, VIPomas (vasoactive intestinal polypeptide tumor), islet cell tumors, and pheochromocytoma.

[0210] In one embodiment, the invention provides methods for treating or reducing neuroendocrine tumors by administering to a subject SVV or an SVV-like picornavirus, where the neuroendocrine tumor expresses (or overexpresses) one or more neuroendocrine tumor markers, including but not limited to, NTR (Neurotensin receptor), ATOH (, GL11, Myc, GRP receptors, GRP, Neuronal enolase (neuron specific enolase (NSE)), carcinoembryonic antigen (CEA), chromogranin A, NCAM, IgF2, BCL-2, sonic hedgehog pathway, and a chemokine receptor.

[0211] Also encompassed in the present invention are the four types of neuroendocrine lung tumors. The most serious type, small cell lung cancer (SCLC), is among the most rapidly growing and spreading of all cancers. Large cell neuroendocrine carcinoma is a rare cancer that, with the exception of the size of the cells forming the cancer, is very similar to SCLC in its prognosis and in how patients are treated. Carcinoid tumors, also known as carcinoids, comprise the other 2 types of lung neuroendocrine cancer. These two types are typical carcinoid and atypical carcinoid.

[0212] Not being bound by theory, the ability of SVV to specifically kill tumor cells may include, but is not limited to: selective replication, cell protein synthesis shut-off, apoptosis, lysis via tumor-selective cell entry, tumor-selective translation, tumor-selective proteolysis, tumor-selective RNA replication, and combinations thereof.

[0213] SVV has many advantageous characteristics over other oncolytic viruses, including modified adenoviruses, for example: (i) SVV has a very high selectivity for cancers with neural properties, including SCLC, Wilms' tumor, retinoblastoma, and neuroblastoma - for example, SVV shows a greater than 10,000-fold selectivity toward neuroendocrine tumor cells; (ii) SVV has been shown to have a 1,000 fold better cell-killing specificity than chemotherapy treatments; (iii) SVV exhibits no overt toxicity in mice following systemic administration with as high as 10^{14} viral particles per kilogram; (iv) the efficacy of SVV is very robust in that 100% of large pre-established tumors can be eradicated in mice, with no recurrence of tumor growth; (v) SVV can be purified to high titer and can be produced at more than 200,000 particles per cell in permissive cell lines; (vi) SVV has a small size (the SVV virus particle is less than 30 nm in diameter) enabling better penetration and spread in tumors than other oncolytic viruses, (vii) SVV replicates quickly (less than 12 hours) and (viii) no modification of SVV is necessary for its use as a specific anti-tumor agent.

[0214] Further, initial studies (*see* Example 6) indicate some additional factors that make SVV an advantageous tool for oncolytic viral therapy: (i) human serum samples do not contain neutralizing antibodies directed against SVV; (ii) SVV is not inhibited by complement; and (iii) SVV does not produce hemagglutination of human erythrocytes. All of these factors contribute to the fact that SVV exhibits a longer circulation time *in vivo* than other oncolytic viruses (for example, *see* Example 7).

[0215] The present invention provides methods for selectively killing a neoplastic cell in a cell population that comprises contacting an effective amount of SVV with said cell population under conditions where the virus can transduce the neoplastic cells in the cell population, replicate and kill the neoplastic cells. Besides methods where SVV kills tumor cells *in vivo*, the present methods encompass embodiments where the tumors can be: (1) cultured *in vitro* when infected by SVV; (2) cultured in the presence of non-tumor cells; and (3) the cells are mammalian (both tumor and non-tumor cells), including where the cells are human cells. The *in vitro* culturing of cells and infection by SVV can have various applications. For example, *in vitro* infection be used as a method to produce large amounts of SVV, as method for determining or detecting whether neoplastic cells are present in a cell population, or as a method for screening whether a mutant SVV can specifically target and kill various tumor cell or tissue types.

[0216] The present invention further provides an *ex vivo* method of treating cancer wherein cells are isolated from a human cancer patient, cultured *in vitro*, infected with a SVV which selectively kills the cancer cells, and the non-tumor cells are introduced back to the patient. Alternatively, cells isolated from a patient can be infected with SVV and immediately introduced back to the patient as a method for administering SVV to a patient. In one embodiment, the cancer cells are of a hematopoietic origin. Optionally, the patient may receive treatment (*e.g.*, chemotherapy or radiation) to destroy the patient's tumor cell *in vivo* before the cultured cells are introduced back to the patient. In one embodiment, the treatment may be used to destroy the patient's bone marrow cells.

[0217] Polymer coated SVV can be used to target the SVV to any specific cell type. This coating strategy can also be used to overcome antibodies to SVV.

[0218] SVV possesses potent antitumor activity against tumor cell-types with neural characteristics. SVV does not exhibit cytolytic activity against tested normal human. Further SVV is not cytotoxic to primary human hepatocytes. Table 1 below summarizes initial

studies that have been conducted to determine the *in vitro* cytolytic potency of SVV against selected tumor cell types.

Table 1: SVV Cytolytic Potency Against Selected Tumor Cell-Types

Cell Line	Cell Type	EC ₅₀ (VP/cell)
H446	Human SCLC	0.0012
PER.C6	Human Embryonic Retinoblast	0.02
H69AR	SCLC-Multidrug Resistant	0.035
293	AD5 DNA Transformed Human Kidney	0.036
Y79	Human Retinoblastoma	0.00035
IMR32	Human Brain Neuroblastoma	0.035
D283	Med Human Brain Cerebellar Medulloblastoma	0.25
SK-N-AS	Human Brain Neuroblastoma	0.474
N1E-115	Mouse Neuroblastoma	0.0028
BEKPCB3E1	Bovine embryonic Kidney cells transformed with Ad5E1	0.99
H1299	Human non-SCLC	7.66
ST	Porcine Testis	5.9
DMS153	Human SCLC	9.2
BEK	Bovine Embryonic Kidney	17.55
M059K	Human Brain Malignant Glioblastoma	1,061
PK15	Porcine Kidney	1,144
FBRC	Fetal Bovine Retina	10,170
HCN-1A	Human Brain	23,708
H460	Human LCLC	>30,000 (inactive)
Neuro 2A	Mouse Neuroblastoma	>30,000 (inactive)
DMS79	Human SCLC	>30,000 (inactive)
H69	Human SCLC	>30,000 (inactive)
C8D30	Mouse Brain	>30,000 (inactive)
MRC-5	Human Fetal Lung Fibroblast	>30,000 (inactive)
HMVEC	Neonatal vascular endothelial cells	>30,000 (inactive)
HMVEC	Adult vascular endothelial cells	>30,000 (inactive)
A375-S2	Human Melanoma	>30,000 (inactive)
SK-MEL-28	Melanoma	>30,000 (inactive)
PC3	Human prostate cancer	>30,000 (inactive)
PC3M2AC6	Human prostate cancer	>30,000 (inactive)
LnCap	Human Prostate cancer	>30,000 (inactive)
DU145	Human prostate cancer	>30,000 (inactive)

[0219] Table 1-A below provides a list of cell lines that are permissive are non-permissive to SVV infection. The Table shows the cytolytic potency and selectivity of SVV.

Table 1-A: *In Vitro* Cytolytic Potency and Selectivity of SVV

Cell Line	Species	Stage	State	Organ	Type	Metastatic Site	EC50*
PERMISSIVE							
Y79	Human	Adult	Cancer	Eye, Retina	Retinoblastoma		0.00035, 0.0007
NCI-H446	Human	Adult	Metastatic Cancer	Lung	Variant Small Cell Lung Carcinoma (SCLC)	Pleural effusion	0.0012, 0.002, 0.0007
N1E-115	Murine	Adult	Cancer	Brain	Neuroblastoma		0.0028, 0.001
NCI-H1770	Human	Adult	Metastatic Cancer	Lung	Non-Small Cell Lung Carcinoma (NSCLC)	Lymph Node	0.00724
NCI-H82	Human	Adult	Metastatic Cancer	Lung	Variant Small Cell Lung Carcinoma (SCLC)	Pleural effusion	0.015
PER.C6®	Human	Fetal	Cancer	Eye, Retina	Retinoblast		0.02, 0.0049
NCI-H69AR	Human	Adult	Cancer	Lung	Small Cell Lung Carcinoma, multi-drug resistant (SCLC)		0.035, 0.05
SK-NEP-1	Human	Adult	Metastatic Cancer	Kidney	Wilms' Tumor	Pleural effusion	0.03
IMR-32	Human	Adult	Cancer	Brain	Neuroblastoma		0.035, 0.0059, 0.05
NCI-H187	Human	Adult	Metastatic Cancer	Lung	Classic Small Cell Lung Carcinoma (SCLC)	Pleural effusion	0.00343
NCI-H209	Human	Adult	Metastatic Cancer	Lung	Small Cell Lung Carcinoma (SCLC)	Bone Marrow	0.04
NCI-H1184	Human	Adult	Metastatic Cancer	Lung	Small Cell Lung Carcinoma (SCLC)	Lymph Node	0.155
D283 Med	Human	Adult	Metastatic Cancer	Brain, Cerebellum	Medulloblastoma	Peritoneum	0.25
SK-N-AS	Human	Adult	Metastatic Cancer	Brain	Neuroblastoma	Bone Marrow	0.474
BEK PCB3E1	Bovine	Fetal	Normal, Ad5 transformed	Kidney	Ad5E1 transformed		0.99
ST	Porcine	Fetal	Normal, immortalized	Testis			5.9
NCI-H1299	Human	Adult	Metastatic Cancer	Lung	Large Cell Lung Carcinoma	Lymph Node	7.66, 4.8
DMS 153	Human	Adult	Metastatic Cancer	Lung	Small Cell Lung Carcinoma (SCLC)	Liver	9.2
NCI-H295R	Human	Adult	Cancer	Adrenal Gland, Cortex	Adrenocortical Carcinoma		16.5
BEK	Bovine	Fetal	Normal, immortalized	Kidney			17.55
PPASMC	Porcine	Adult	Normal, Primary	Lung, Pulmonary Artery	Smooth Muscle Cells		18.4
PCASMC	Porcine	Adult	Normal, Primary	Heart, Coronary Artery	Smooth Muscle Cells		11.9
PAoSMC	Porcine	Adult	Normal, Primary	Heart, Aorta	Smooth Muscle Cells		88
NCI-H526	Human	Adult	Metastatic Cancer	Lung	Variant Small Cell Lung Carcinoma (SCLC)	Bone Marrow	46.4
OVCAR-3	Human	Adult	Cancer	Ovary	Adenocarcinoma		39
ESK-4	Porcine	Fetal	Normal, Immortalized	Kidney	Fibroblast		60
SW-13	Human	Adult	Cancer	Adrenal Gland, Cortex	Small Cell Adenocarcinoma		<100
293	Human	Fetal	Normal, Ad5 transformed	Kidney	Ad5 transformed		0.036, 184.8

Cell Line	Species	Stage	State	Organ	Type	Metastatic Site	EC50*
Hs 578T	Human	Adult	Cancer	Breast	Carcinoma		273
Hs 1.Tes	Human	Fetal	Normal, Immortalized	Testis			416
LOX IMVI	Human	Adult	Cancer	Skin	Melanoma		569
PK(15)	Porcine	Adult	Normal, Immortalized	Kidney			1144, 129
NON PERMISSIVE							
WI-38	Human	Fetal	Normal, Immortalized	Lung	Fibroblast		>10,000
IMR-90	Human	Fetal	Normal, Immortalized	Lung	Fibroblast		>10,000
MRC-5	Human	Fetal	Normal, Immortalized	Lung	Fibroblast		>10,000
HCN-1A	Human	Adult	Normal, Immortalized	Brain, Cortical Neuron			>10,000
HMVEC	Human	Adult (neonatal)	Normal, Primary	Skin	Microvascular Endothelial Cells		>10,000
HMVEC	Human	Adult	Normal, Primary	Skin	Microvascular Endothelial Cells		>10,000
HUVEC	Human	Adult	Normal, Primary	Umbilical Vein	Endothelial Cells		>10,000
HRE	Human	Adult	Normal, Primary	Kidney	Epithelial Cells		>10,000
HRCE	Human	Adult	Normal, Primary	Kidney	Cortical Epithelial Cells		>10,000
PHH	Human	Adult	Normal, Primary	Liver	Hepatocyte		>10,000
HCASMC-c	Human	Adult	Normal, Primary	Heart, Coronary Artery	Smooth Muscle Cells		>10,000
HCAEC	Human	Adult	Normal, Primary	Heart, Coronary Artery	Endothelial Cells		>10,000
HAEC	Human	Adult	Normal, Primary	Heart, Aorta	Endothelial Cells		>10,000
HAoSMC-c	Human	Adult	Normal, Primary	Heart, Aorta	Smooth Muscle Cells		>10,000
NHA	Human	Adult	Normal, Primary	Brain	Astrocytes		1713
HPASMC	Human	Adult	Normal, Primary	Lung	Smooth Muscle Cells		>10,000
PBMC	Human	Adult	Normal, Primary	Peripheral Blood	Mononuclear Cells		>10,000
SF-295	Human	Adult	Cancer	Brain	Glioblastoma		>10,000
U251	Human	Adult	Cancer	Brain	Glioblastoma		>10,000
SF-539	Human	Adult	Cancer	Brain	Glioblastoma		>10,000
SNB-19	Human	Adult	Cancer	Brain	Glioblastoma		>10,000
SF-268	Human	Adult	Cancer	Brain	Glioblastoma		3103
U-118MG	Human	Adult	Cancer	Brain	Glioblastoma, Astrocytoma		>10,000
SNB-75	Human	Adult	Cancer	Brain	Astrocytoma		>10,000
M059K	Human	Adult	Cancer	Brain, Glial Cell	Malignant Glioblastoma		1061
KK	Human	Adult	Cancer	Brain, Glial Cell	Glioblastoma		>10,000
HCC-2998	Human	Adult	Cancer	Colon	Carcinoma		>10,000
KM12	Human	Adult	Cancer	Colon	Carcinoma		>10,000
HT-29	Human	Adult	Cancer	Colon	Adenocarcinoma		>10,000
HCT 116	Human	Adult	Cancer	Colon	Carcinoma		>10,000
HCT-15	Human	Adult	Cancer	Colon	Carcinoma		>10,000
COLO 205	Human	Adult	Metastatic Cancer	Colon	Adenocarcinoma	Ascites	>10,000
SW620	Human	Adult	Metastatic Cancer	Colon	Colorectal Carcinoma	Lymph Node	6503 , >10,000
PC3M-2AC6	Human	Adult	Cancer	Prostate			>10,000
PC3M-2AC6 + 2-AP	Human	Adult	Cancer	Prostate			ND

Cell Line	Species	Stage	State	Organ	Type	Metastatic Site	EC50*
PC-3	Human	Adult	Metastatic Cancer	Prostate	Adenocarcinoma	Bone	>10,000
LNCaP.FGC	Human	Adult	Metastatic Cancer	Prostate	Adenocarcinoma	Lymph Node	>10,000
DU 145	Human	Adult	Metastatic Cancer	Prostate	Adenocarcinoma	Brain	>10,000
Hep3B	Human	Adult	Cancer	Liver	Hepatocellular Carcinoma		>10,000
Hep G2	Human	Adult	Cancer	Liver	Hepatocellular Carcinoma		>10,000
786-O	Human	Adult	Cancer	Kidney	Clear Cell Adenocarcinoma		>10,000
TK-10	Human	Adult	Cancer	Kidney	Carcinoma		>10,000
RXF 393	Human	Adult	Cancer	Kidney	Carcinoma		>10,000
UO-31	Human	Adult	Cancer	Kidney	Carcinoma		>10,000
SN12C	Human	Adult	Cancer	Kidney	Carcinoma		>10,000
A-498	Human	Adult	Cancer	Kidney	Carcinoma		>10,000
ACHN	Human	Adult	Cancer	Kidney	Carcinoma		>10,000
SW839	Human	Adult	Cancer	Kidney	Renal Clear Cell Adenocarcinoma		>10,000
Caki-1	Human	Adult	Metastatic Cancer	Kidney	Clear Cell Adenocarcinoma	Skin	>10,000
5637	Human	Adult	Cancer	Bladder	Carcinoma		>10,000
NCI-H1339	Human	Adult	Cancer	Lung			>10,000
NCI-H1514	Human	Adult	Cancer	Lung			>10,000
A549	Human	Adult	Cancer	Lung	Carcinoma		>10,000
S8	Human	Adult	Cancer	Lung	Carcinoma		>10,000
NCI-H727	Human	Adult	Cancer	Lung	Carcinoid		>10,000
NCI-H835	Human	Adult	Cancer	Lung	Carcinoid		>10,000
UMC-11	Human	Adult	Cancer	Lung	Carcinoid		>10,000
DMS 114	Human	Adult	Cancer	Lung	Small Cell Lung Carcinoma (SCLC)		>10,000
DMS 53	Human	Adult	Cancer	Lung	Small Cell Lung Carcinoma (SCLC)		>10,000
NCI-H69	Human	Adult	Cancer	Lung	Small Cell Lung Carcinoma (SCLC)		>10,000
NCI-H2195	Human	Adult	Metastatic Cancer	Lung	Small Cell Lung Carcinoma (SCLC)	Bone Marrow	>10,000
DMS 79	Human	Adult	Metastatic Cancer	Lung	Small Cell Lung Carcinoma (SCLC)	Pleural effusion	>10,000
NCI-H146	Human	Adult	Metastatic Cancer	Lung	Classic Small Cell Lung Carcinoma (SCLC)	Bone Marrow	>10,000
NCI-H1618	Human	Adult	Metastatic Cancer	Lung	Classic Small Cell Lung Carcinoma (SCLC)	Bone Marrow	>10,000
NCI-H345	Human	Adult	Metastatic Cancer	Lung	Classic Small Cell Lung Carcinoma (SCLC)	Bone Marrow	>10,000
HOP-62	Human	Adult	Cancer	Lung	Non-Small Cell Lung Carcinoma (NSCLC)		>10,000
EKVX	Human	Adult	Cancer	Lung	Non-Small Cell Lung Carcinoma (NSCLC)		>10,000
HOP-92	Human	Adult	Cancer	Lung	Non-Small Cell Lung Carcinoma (NSCLC)		>10,000
NCI-H522	Human	Adult	Cancer	Lung	Non-Small Cell Lung Carcinoma (NSCLC)		>10,000
NCI-H23	Human	Adult	Cancer	Lung	Non-Small Cell Lung Carcinoma (NSCLC)		>10,000
NCI-H322M	Human	Adult	Cancer	Lung	Non-Small Cell Lung Carcinoma (NSCLC)		>10,000
NCI-H226	Human	Adult	Metastatic Cancer	Lung	Squamous Cell Carcinoma, Mesothelioma (NSCLC)	Pleural effusion	>10,000
NCI-H460	Human	Adult	Metastatic Cancer	Lung	Large Cell Lung Carcinoma	Pleural effusion	>10,000

Cell Line	Species	Stage	State	Organ	Type	Metastatic Site	EC50*
HeLa, HeLa S3	Human	Adult	Cancer	Cervix	Adenocarcinoma		>10,000
CCRF-CEM	Human	Adult	Cancer	Peripheral Blood, T lymphoblast	Acute Lymphoblastic Leukemia (ALL)		>10,000
MOLT-4	Human	Adult	Cancer	Peripheral Blood, T lymphoblast	Acute Lymphoblastic Leukemia (ALL)		>10,000
RPMI 8226	Human	Adult	Cancer	Peripheral Blood, B lymphocyte	Plasmacytoma, Myeloma		>10,000
SR	Human	Adult	Metastatic Cancer	Lymphoblast	Large Cell Lymphoblastic Lymphoma	Pleural effusion	>10,000
HL-60(TB)	Human	Adult	Cancer	Peripheral Blood, Promyleoblast	Acute Promyelocytic Leukemia (APL)		>10,000
K-562	Human	Adult	Metastatic Cancer	Bone Marrow	Chronic Myelogenous Leukemia (CML)	Pleural effusion	>10,000
UACC-257	Human	Adult	Cancer	Skin	Melanoma		>10,000
M14	Human	Adult	Cancer	Skin	Melanoma		>10,000
UACC-62	Human	Adult	Cancer	Skin	Melanoma		6614
SK-MEL-2	Human	Adult	Cancer	Skin	Malignant Melanoma		>10,000
SK-MEL-28	Human	Adult	Cancer	Skin	Malignant Melanoma		>10,000
A375.S2	Human	Adult	Cancer	Skin	Malignant Melanoma		>10,000
SK-MEL-28	Human	Adult	Cancer	Skin	Malignant Melanoma		>10,000
SK-MEL-5	Human	Adult	Metastatic Cancer	Skin	Malignant Melanoma	Lymph Node	>10,000
MALME-3M	Human	Adult	Metastatic Cancer	Skin	Malignant Melanoma	Lung	>10,000
BT-549	Human	Adult	Cancer	Breast	Ductal Carcinoma		>10,000
NCI/ADR-RES	Human	Adult	Cancer	Breast	Carcinoma		>10,000
MCF7	Human	Adult	Metastatic Cancer	Breast	Adenocarcinoma	Pleural effusion	>10,000
MDA-MB-231	Human	Adult	Metastatic Cancer	Breast	Adenocarcinoma	Pleural effusion	>10,000
T-47D	Human	Adult	Metastatic Cancer	Breast	Ductal Carcinoma	Pleural effusion	>10,000
MDA-MB-435	Human	Adult	Metastatic Cancer	Breast	Ductal Adenocarcinoma	Pleural effusion	>10,000
IGR-OV1	Human	Adult	Cancer	Ovary	Carcinoma		>10,000
OVCAR-4	Human	Adult	Cancer	Ovary	Adenocarcinoma		>10,000
OVCAR-5	Human	Adult	Cancer	Ovary	Adenocarcinoma		>10,000
OVCAR-8	Human	Adult	Cancer	Ovary	Adenocarcinoma		>10,000
SK-OV-3	Human	Adult	Metastatic Cancer	Ovary	Adenocarcinoma	Ascites	>10,000
BxPC-3	Human	Adult	Cancer	Pancreas	Adenocarcinoma		>10,000
AsPC-1	Human	Adult	Metastatic Cancer	Pancreas	Adenocarcinoma	Ascites	>1000
NCI-H295	Human	Adult	Cancer	Adrenal Gland, Cortex	Adrenocortical Carcinoma		>10,000
TT	Human	Adult	Cancer	Thyroid	Medullary Carcinoma		>10,000
C8-D30	Murine	Adult	Normal	Brain, Cerebellum			>10,000
LLC1	Murine	Adult	Cancer	Lung	Lewis Lung Carcinoma		>10,000
RM-1	Murine	Adult	Cancer	Prostate			>10,000
MLTC-1	Murine	Adult	Cancer	Testis	Leydig Cell Tumor		>10,000
KLN 205	Murine	Adult	Cancer	Lung	Squamous Cell Carcinoma		>10,000
CMT-64	Murine	Adult	Cancer	Lung	Small Cell Lung Carcinoma (SCLC)		>10,000
CMT-93	Murine	Adult	Cancer	Rectum	Polyploid Carcinoma		>10,000
B16-F0	Murine	Adult	Cancer	Skin	Melanoma		>10,000
RM-2	Murine	Adult	Cancer	Prostate			>10,000

Cell Line	Species	Stage	State	Organ	Type	Metastatic Site	EC50*
RM-9	Murine	Adult	Cancer	Prostate			>10,000
Neuro-2A	Murine	Adult	Cancer	Brain	Neuroblastoma		>10,000
FBRC	Bovine	Fetal		Eye, Retina			>10,000
MDBK	Bovine	Adult	Normal, Immortalized	Kidney			>10,000
CSL 503	Ovine	Adult	Normal, Immortalized	Lung	Ad5E1 transformed		>10,000
OFRC	Ovine	Adult	Normal, Immortalized	Eye, Retina	Ad5E1 transformed		>10,000
PC-12	Rat	Adult	Cancer	Adrenal Gland	Pheochromocytoma		>10,000
Vero	Monkey	Adult	Normal, Immortalized	Kidney			>10,000
PAOEC	Porcine	Adult	Normal, Primary	Heart, Aorta	Endothelial Cells		>10,000
PCAEC	Porcine	Adult	Normal, Primary	Heart, Coronary Artery	Endothelial Cells		>10,000
PPAEC	Porcine	Adult	Normal, Primary	Lung, Pulmonary Artery	Endothelial Cells		>10,000
TBD							
NCI-H289	Human	Adult	Cancer	Lung			TBD
NCI-H1963	Human	Adult	Cancer	Lung	Small Cell Lung Carcinoma (SCLC)		TBD
NCI-H2227	Human	Adult	Cancer	Lung	Small Cell Lung Carcinoma (SCLC)		TBD
NCI-H378	Human	Adult	Metastatic Cancer	Lung	Classic Small Cell Lung Carcinoma (SCLC)	Pleural effusion	TBD
NCI-H2107	Human	Adult	Metastatic Cancer	Lung	Small Cell Lung Carcinoma (SCLC)	Bone Marrow	TBD
HCC970	Human	Adult	Metastatic Cancer	Lung	Small Cell Lung Carcinoma (SCLC)	Bone Marrow	TBD
HCC33	Human	Adult	Metastatic Cancer	Lung	Small Cell Lung Carcinoma (SCLC)	Pleural effusion	<1000/TBD
BON	Human	Adult	Cancer	Pancreas	Carcinoid		TBD
H1T-T15	Hamster	Adult	Normal, Immortalized	Pancreas	Islets of Langerhans, b-cell		TBD
							*EC50 determined after 3 days except where noted

[0220] Table 1-A lists the results of SVV permissivity experiments on 165 primary cells and cell lines, representing 22 tissues from 8 different species. The results indicate that virtually all adult normal are nonpermissive for SVV. Thirteen primary adult human cell cultures tested were nonpermissive. Of the twelve bovine, ovine, porcine and primate normal cell cultures tested, only three cell cultures were permissive, which were porcine smooth muscle cells. This result is consistent with the hypothesis that the natural host for SVV may be pigs. Besides the porcine smooth muscle cells, only neuroendocrine cancer cell lines or most fetal lines were permissive.

[0221] Murine studies (*see Examples*) show that SVV can specifically kill tumors with great efficacy and specificity *in vivo*. These *in vivo* studies show that SVV has a number of advantages over other oncolytic viruses. For example, one important factor affecting the

ability of an oncolytic tumor virus to eradicate established tumors is viral penetration. In studies with adenoviral vectors, Ad5 based vectors had no effect on SCLC tumor development in athymic mice. Based on immunohistochemical results, adenovirus did not appear to penetrate the established tumors. In contrast, SVV was able to eliminate H446 SCLC tumors in athymic nude mice following a single systemic administration. SVV has a small size (<30 nm in diameter) enabling better penetration and spread in tumor tissue than other viruses, and thus, the small size of SVV may contribute to its ability to successfully penetrate and eradicate established tumors.

[0222] Additional *in vivo* tests demonstrate the efficacy of a single intravenous dose of SVV in murine tumor models using athymic nude mice and immunocompetent mice. The tumor models tested were: (1) H446 (human SCLC); (2) Y79 (human retinoblastoma); (3) H69AR (human multi-drug resistant SCLC); (4) H1299 (human NSCLC); and (5) N1E-115 (murine neuroblastoma). The results of these tests are shown in Figures 90A-E and Example 11. The tests demonstrate efficacy of a single intravenous dose of SVV in all models and show an agreement between relative ranks of *in vitro* ED₅₀ and *in vivo* efficacy in human xenograft models. The results in the N1E-115 immunocompetent murine neuroblastoma model shows that SVV can be efficacious against tumors in subjects with normal immune systems.

[0223] Chemoresistance is a major issue facing any patient that receives chemotherapy as a facet of cancer therapy. Patients that become chemoresistant often, if not always, have a much poorer prognosis and may be left with no alternative therapy. It is well known that one of the major causes of chemoresistance is the expression, over expression, or increased activity of a family of proteins called Multiple Drug Resistant proteins (MRPs). Applicants have found that a sensitivity of certain tumor cells for SVV is also correlated with the chemoresistant state of cancer cells and MRP expression. H69 is a chemosensitive (adriamycin) cell line that is resistant to SVV *in vitro*, whereas H69AR is a chemoresistant cell line that overexpresses MRPs and is sensitive to SVV (*see* Table 1). Evidence indicates that overexpression of MRPs, including MDR, correlates with sensitivity of cells to SVV killing. Thus, in one embodiment, the present invention provides a method for treating cancer wherein SVV kills cells overexpressing an MRP.

[0224] The invention also provides methods for treating diseases that are a result of abnormal cells, such as abnormally proliferative cells. The method comprises contacting said

abnormal cells with SVV in a manner that results in the destruction of a portion or all of the abnormal cells. SVV can be used to treat a variety of diseases that are a result of abnormal cells. Examples of these diseases include, but are not limited to, cancers wherein the tumor cells display neuroendocrine features and neurofibromatosis.

[0225] Neuroendocrine tumors can be identified by a variety of methods. For example, neuroendocrine tumors produce and secrete a multitude of peptide hormones and amines. Some of these substances cause a specific clinical syndrome: carcinoid, Zollinger-Ellison, hyperglycemic, glucagonoma and WDHA syndrome. Specific markers for these syndromes are basal and/or stimulated levels of urinary 5-HIAA, serum or plasma gastrin, insulin, glucagon and vasoactive intestinal polypeptide, respectively. Some carcinoid tumors and about one third of endocrine pancreatic tumors do not present any clinical symptoms and are called 'nonfunctioning' tumors. Therefore, general tumor markers such as chromogranin A, pancreatic polypeptide, serum neuron-specific enolase and subunits of glycoprotein hormones have been used for screening purposes in patients without distinct clinical hormone-related symptoms. Among these general tumor markers chromogranin A, although its precise function is not yet established, has been shown to be a very sensitive and specific serum marker for various types of neuroendocrine tumors. This is because it may also be elevated in many cases of less well-differentiated tumors of neuroendocrine origin that do not secrete known hormones. At the moment, chromogranin A is considered the best general neuroendocrine serum or plasma marker available both for diagnosis and therapeutic evaluation and is increased in 50-100% of patients with various neuroendocrine tumors. Chromogranin A serum or plasma levels reflect tumor load, and it may be an independent marker of prognosis in patients with midgut carcinoids.

[0226] The invention also provides a pharmaceutical composition comprising SVV and a pharmaceutically acceptable carrier. Such compositions, which can comprise an effective amount of SVV in a pharmaceutically acceptable carrier, are suitable for local or systemic administration to individuals in unit dosage forms, sterile parenteral solutions or suspensions, sterile non-parenteral solutions or oral solutions or suspensions, oil in water or water in oil emulsions, and the like. Formulations for parenteral and non-parenteral drug delivery are known in the art. Compositions also include lyophilized and/or reconstituted forms of SVV. Acceptable pharmaceutical carriers are, for example, saline solution, protamine sulfate (Elkins-Sinn, Inc., Cherry Hill, NJ), water, aqueous buffers, such as

phosphate buffers and Tris buffers, or Polybrene (Sigma Chemical, St. Louis, MO) and phosphate-buffered saline and sucrose. The selection of a suitable pharmaceutical carrier is deemed to be apparent to those skilled in the art from the teachings contained herein. These solutions are sterile and generally free particulate matter other than SVV. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. Excipients that enhance infection of cells by SVV may be included.

[0227] SVV is administered to a host or subject in an amount that is effective to inhibit, prevent or destroy the growth of the tumor cells through replication of the virus in the tumor cells. Methods that utilize SVV for cancer therapy include systemic, regional or local delivery of the virus at safe, developable, and tolerable doses to elicit therapeutically useful destruction of tumor cells. Even following systemic administration, the therapeutic index for SVV is at least 10, preferably at least 100 or more preferably at least 1000. In general, SVV is administered in an amount of between 1×10^8 and 1×10^{14} vp/kg. The exact dosage to be administered is dependent upon a variety of factors including the age, weight, and sex of the patient, and the size and severity of the tumor being treated. The viruses may be administered one or more times, which may be dependent upon the immune response potential of the host. Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. If necessary, the immune response may be diminished by employing a variety of immunosuppressants, so as to permit repetitive administration and/or enhance replication by reducing the immune response to the viruses. Anti-neoplastic viral therapy of the present invention may be combined with other anti-neoplastic protocols. Delivery can be achieved in a variety of ways, employing liposomes, direct injection, catheters, topical application, inhalation, etc. Further, a DNA copy of the SVV genomic RNA, or portions thereof, can also be a method of delivery, where the DNA is subsequently transcribed by cells to produce SVV virus particles or particular SVV polypeptides.

[0228] A therapeutically effective dose refers to that amount of the virus that results in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of viruses can be determined by standard procedures in cell cultures or

experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population of animals or cells; for viruses, the dose is in units of vp/kg) and the ED₅₀ (the dose - vp/kg - therapeutically effective in 50% of the population of animals or cells) or the EC₅₀ (the effective concentration - vp/cell (see Table 1 for example) - in 50% of the population of animals or cells). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD₅₀ and ED₅₀ or EC₅₀. Viruses which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of viruses lies preferably within a range of circulating concentrations that include the ED₅₀ or EC₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

[0229] In yet another aspect, a method for treating a host organism having a neoplastic condition is provided, comprising administering a therapeutically effective amount of a viral composition of the invention to said host organism. In one embodiment, the neoplastic tissue is abnormally proliferating, and the neoplastic tissue can be malignant tumor tissue. Preferably, the virus is distributed throughout the tissue or tumor mass due to its capacity for selective replication in the tumor tissue. Neoplastic conditions potentially amenable to treatment with the methods of the invention include those with neurotropic properties.

Methods for Producing the Viruses of the Present Invention:

[0230] Methods for producing the present viruses to very high titers and yields are additional aspects of the invention. As stated, SVV can be purified to high titer and can be produced at more than 200,000 particles per cell in permissive cell lines. Cells that are capable of producing high quantities of viruses include, but are not limited to, PER.C6 (Fallaux *et al.*, Human Gene Therapy, 9:1909-1917, 1998), H446 (ATCC# HTB-171) and the other cell lines listed in Table 1 where the EC₅₀ value is less than 10.

[0231] For example, the cultivation of picornaviruses can be conducted as follows. The virus of interest is plaque purified and a well-isolated plaque is picked and amplified in a permissive cell line, such as PER.C6. A crude virus lysate (CVL) from the infected cells can be made by multiple cycles of freeze and thaw, and used to infect large numbers of permissive cells. The permissive cells can be grown in various tissue culture flasks, for

example, 50x150 cm² flasks using various media, such as Dulbecco's modified Eagle medium (DMEM, Invitrogen, Carlsbad, CA)) containing 10% fetal bovine serum (Biowhitaker, Walkersville, MD) and 10 mM magnesium chloride (Sigma, St Louis, MO). The infected cells can be harvested between 12 and 48 hours after infection or when complete cytopathic effects (CPE) are noticed, and are collected by centrifugation at 1500 rpm for 10 minutes at 4°C. The cell pellet is resuspended in the cell culture supernatant and is subjected to multiple cycles of freeze and thaw. The resulting CVL is clarified by centrifugation at 1500 rpm for 10 minutes at 4°C. Virus can be purified by gradient centrifugation. For example, two rounds of CsCl gradients can suffice for SVV purification: a one-step gradient (density of CsCl 1.24 g/ml and 1.4 g/ml) followed by one continuous gradient centrifugation (density of CsCl 1.33 g/ml). The purified virus concentration is determined spectrophotometrically, assuming $1A_{260} = 9.5 \times 10^{12}$ particles (Scraba D.G., and Palmenberg, A.C. 1999. *Cardioviruses (Picornaviridae)*. In: Encyclopedia of Virology, Second edition, R.G. Webster and A Granoff Eds). Infectivity titers of purified virus are also determined by a standard plaque and/or tissue culture infective dose 50 (TCID₅₀) assay using PER.C6 or any other suitable cell type. The yield of SVV from PER.C6 cells are greater than 200, 000 particles per cell with particles to PFU ratio of about 100. The yields of SVV from other permissive cells (H446-ATCC# HTB-171) may be at least this high or higher. SVV can also be purified by column chromatography.

[0232] In addition, several steps in a commercially attractive large scale Good Manufacturing Processes (GMP) are applicable to the purification of SVV. The invention also contemplates methods for purifying SVV that are based on methods for purifying adenoviruses. These methods include isolating SVV based on its density, since SVV has a very similar density to adenovirus and can be co-purified with adenovirus.

Methods for Detecting and Studying Tumors:

[0233] The present invention provides methods for detecting tumor or neoplastic cells in a patient using the viruses of the present invention. Cellular samples can be obtained from a patient and screened by incubating the sample with an epitope-tagged SVV (or other tumor-specific viruses provided by the invention, *i.e.*, tumor-specific mutant cardioviruses), and then screening the sample for bound SVV by detecting the epitope tag. Alternatively, the sample can be screened by detecting whether the SVV causes any cellular lysis. If SVV does cause cellular lysis, or if SVV can bind specifically to cells in the sample, this would indicate

the possibility that the sample contains neoplastic or tumor cells known to be capable of being bound and/or infected by SVV.

[0234] Additionally, SVV can be used in a method for detecting a tumor cell *in vivo*. In such a method, epitope-tagged SVV can first be inactivated in a manner where SVV can still bind to tumor cells specifically but cannot replicate. Tumor cells that have bound SVV can be detected by assaying for the epitope tag. Detection of the epitope tag can be accomplished by antibodies that specifically bind the epitope, where the antibodies are either labeled (for example, fluorescently) or where the antibodies can then be detected by labeled secondary antibodies.

[0235] The present methods of detection encompass detecting any type of tumor or neoplastic cell that is specifically targeted by any virus of the present invention. Specific tumor types include, for example, neuroendocrine-type tumors, such as retinoblastomas, SCLC, neuroblastomas glioblastomas and medulloblastomas.

[0236] The present invention also provides the use of SVV as a tool to study tumor cells. SVV selectively destroys some tumor cell types, and has very little, if any, toxic effects on non-tumor cells. Because of these characteristics, SVV can be used to study tumors and possibly discover a new tumor specific gene and/or pathway. In other words, there is some characteristic of the tumor cells that allows replication of SVV, wherein normal cells do not exhibit said characteristic. Upon identification of a new tumor specific gene and/or pathway, therapeutic antibodies or small molecules can then be designed or screened to determine whether these reagents are anti-tumor agents.

[0237] The present invention also provides a method for identifying all types of cancers that respond to SVV. In one embodiment, the method for identifying SVV-responsive cells comprises obtaining cells, contacting said cells with SVV and detecting cell killing or detecting viral replication. Cell killing can be detected using various methods known to one skilled in the art (*e.g.*, MTS assay, *see* High-Throughput section herein). Methods of detecting virus replication are also known to one skilled in the art (*e.g.*, observance of CPE, plaque assay, DNA quantification methods, FACS to detect quantity of virus in the tumor cells, RT-PCR assays to detect viral RNA, etc.). In one embodiment, the cells are cancer cells. Examples of cancer cells include, but are not limited to, established tumor cell lines and tumor cells obtained from a mammal. In one embodiment, the mammal

is a human. In a further embodiment, the cells are cancer cells obtained from a human cancer patient.

[0238] The method for identifying SVV-responsive cancer cells may be used to discover tumor cell lines or tumor tissues that are permissive for SVV replication. Also, by determining the characteristics of permissive tumor cells, one may be able to identify characteristics of tumor cells that cause the cells to be selectively killed by SVV. The discovery of these characteristics could lead to new targets for cancer drugs. Also, the methods for identifying SVV responsive cancer cells could be used as a screen for human cancer patients who would benefit from treatment with SVV.

[0239] For example, antibodies against SVV or an SVV-like picornavirus (polyclonal, monoclonal, etc.) can be used in a viral binding assay to pre-screen patients prior to SVV or SVV-like picornavirus therapy. The pre-screening can be conducted generally as follows: (1) cells from a patient are isolated, the cells can be from a tumor biopsy for example, (2) the cells are stained with anti-SVV or anti-SVV-like picornavirus antibodies, (3) a secondary antibody conjugated with a marker (such as fluorescein or some other detectable dye or fluorophore) that is specific to the anti-SVV or anti-SVV-like picornavirus antibodies is added (for example, if the antibodies were raised in a rabbit, then the secondary antibody would be specific for rabbit immunoglobulins), and (4) detection for the marker is conducted - for example, fluorescence microscopy can be conducted where the marker is fluorescein. (Step 3 is optional if the anti-SVV or anti-SVV-like picornavirus antibodies are directly conjugated, *i.e.*, where the antibodies are monoclonal. If the antibodies are polyclonal, indirect immunofluorescence - use of a secondary antibody - is suggested.) If the patient's tumor cells are permissive for SVV or SVV-like picornavirus infection, then the patient is a candidate for SVV or SVV-like picornavirus therapy. In a virus binding assay, the patient's tumor cells can be determined to be permissive for SVV if the cells are positive for antibody staining. For example, Figures 92B-92C shows immunofluorescent images of cells permissive for SVV and have been infected with SVV.

[0240] In pre-screening patients with a viral binding assay, the cell sample from the patient can also be a tissue section of a tissue suspected to contain tumor cells. The tissue section can then be prepared into sections and incubated with SVV prior to histochemistry with anti-SVV or anti-SVV-like picornavirus antibodies.

[0241] The invention also provides methods of detecting SVV. In one embodiment, the detection assay is based on antibodies specific to SVV polypeptide epitopes. In another embodiment, the detection assay is based on the hybridization of nucleic acids. In one embodiment, RNA is isolated from SVV, labeled (*e.g.*, radioactive, chemiluminescence, fluorescence, etc.) to make a probe. RNA is then isolated from test material, bound to nitrocellulose (or a similar or functionally equivalent substrate), probed with the labeled SVV RNA, and the amount of bound probe detected. Also, the RNA of the virus may be directly or indirectly sequenced and a PCR assay developed based on the sequences. In one embodiment, the PCR assay is a real time PCR assay.

Methods for Making Viruses with Altered Tropism:

[0242] The present invention provides methods for constructing SVV mutants (or variants or derivatives) where these mutants have an altered cell-type tropism. SVV-like picornaviruses may also be mutated in order to provide a particular cell-type tropism. Specifically, SVV and SVV-like picornavirus mutants are selected for their ability to specifically bind and/or kill tumor or neoplastic cells that are known to be resistant to wild-type SVV or wild-type SVV-like picornavirus binding.

[0243] The native or wild-type SVV has a simple genome and structure that allow the modification of the native virus to target other cancer indications. These new derivatives have an expanded tropism toward non-neural cancers and still maintain the high therapeutic index found in the native SVV. One possible means of targeting is the inclusion of tissue-specific peptides or ligands onto the virus.

[0244] To select cancer-targeting viral candidates, the present invention provides methods to construct and screen an oncolytic virus library with a genetic insertion that encodes a random peptide sequence in the capsid region of the native SVV. A random peptide library with a diversity of 10^8 is believed to be sufficient and should yield peptides that specifically direct the virus to tumor tissue.

[0245] Various studies have shown that tumor cells exhibit different characteristics from normal cells such as: (1) tumor cells have more permeable cell membranes; (2) tumors have specific stromal cell types such as angiogenic endothelial cells which have previously been shown to express cell type specific receptors; and (3) tumor cells differentially express certain receptors, antigens and extracellular matrix proteins (Arap, W. *et al.*, Nat. Med., 2002, 8(2): 121-127; Kolonin, M. *et al.*, Curr. Opin. Chem. Biol., 2001, 5(3): 308-313; St. Croix, B.

et al., Science, 2000, 289(5482): 1997-1202). These studies demonstrated that tumor and normal tissues are distinct at the molecular level and targeted drug delivery and treatment of cancer is feasible. Specifically, several peptides selected by homing to blood vessels in mouse models have been used for targeted delivery of cytotoxic drugs (Arap, W. *et al.*, Science, 1998, 279(5349): 377-380), pro-apoptotic peptides (Ellerby, H.M. *et al.*, Nat. Med., 1999, 17(8): 768-774), metalloprotease inhibitor (Koivunen, E. *et al.*, Nat. Biotechnol., 1999, 17(8): 768-774), cytokine (Curnis, F. *et al.*, Nat. Biotechnol., 2000, 18(11): 1185-1190), fluorophores (Hong, F.D. and Clayman, G.L., Cancer Res., 2000, 60(23): 6551-6556) and genes (Trepel, M. *et al.*, Hum. Gene Ther., 2000, 11(14): 1971-1981). The tumor-targeting peptides have proven to increase the efficacy and lower the toxicity of the parental drugs.

[0246] A library of SVV derivatives can be generated by the insertion of a random peptide sequence into the capsid region of the virus. As shown in Figure 57, a vector is first generated that contains the SVV capsid region, *i.e.*, “pSVV capsid.” This capsid vector can then be mutagenized, for example, by cutting the vector with a restriction enzyme that cuts DNA at random positions, *i.e.*, CviJI (a blunt cutter). The vector is cut at numerous positions, and DNA that has been cut only once by CviJI can be isolated by gel-purification (*see* Figure 57). This isolated population of DNA contains a plurality of species that have been cut in the capsid region at different locations. This population is then incubated with oligonucleotides and ligase, such that a percentage of the oligonucleotides will be ligated into the capsid region of the vector at a number of different positions. In this manner, a library of mutant SVV capsids can be generated.

[0247] The oligonucleotides that are inserted into the capsid encoding region can be either random oligonucleotides, non-random oligonucleotides (*i.e.*, the sequence of the oligonucleotide is pre-determined), or semi-random (*i.e.*, a portion of the oligonucleotide is pre-determined and a portion has a random sequence). The non-random aspect of the contemplated oligonucleotides can comprise an epitope-encoding region. Contemplated epitopes include, but are not limited to, c-myc - a 10 amino acid segment of the human protooncogene myc (EQKLISEEDL (SEQ ID NO: 35); HA - haemagglutinin protein from human influenza hemagglutinin protein (YPYDVPDYA (SEQ ID NO: 36)); and His₆ (SEQ ID NO:116) - a sequence encoding for six consecutive histidines.

[0248] The library of mutant capsid polynucleotides (for example, “pSVV capsid library” in Figure 57) can then be digested with restriction enzymes such that only the mutant

capsid encoding region is excised. This mutant capsid encoding region is then ligated into a vector containing the full-length genomic sequence minus the capsid encoding region (*see* Figure 58, for example). This ligation generates a vector having a full-length genomic sequence, where the population (or library) of vectors comprise a plurality of mutant capsids. In Figure 58, this library of SVV mutants comprising different capsids is denoted as “pSVVFL capsid.” The pSVVFL capsid vector library is then linearized and *in vitro* transcribed in order to generate mutant SVV RNA (*see* Figure 59). The mutant SVV RNA is then transfected into a permissive cell line such that those SVV sequences that do not possess a debilitating mutation in its capsid are translated by the host cells to produce a plurality of mutant SVV particles. In Figure 59, the plurality of mutant SVV particles are denoted as a “SVV capsid library.”

[0249] The peptide encoded by the oligonucleotide inserted into the capsid encoding region can serve as a targeting moiety for specific viral infection. The viruses that target a specific type of cancer would selectively infect only those cancer cells that have a receptor to the peptide, replicate in those cells, kill those cells, and spread to only those same types of cells. This methodology enables the identification of novel tumor-targeting peptides and ligands, tumor-selective receptors, therapeutic SVV derivatives and other virus derivatives, including picornavirus derivatives.

[0250] *In vitro* and *in vivo* screening of SVV mutant libraries have several advantages over other technologies such as peptide bead libraries and phage display. Unlike these other technologies, the desirable candidate here, *i.e.* an SVV derivative that selectively binds to a cancer cell, will replicate *in situ*. This replication-based library approach has numerous advantages over prior methods of discovering new cell binding moieties, such as phage display. First, the screening of a SVV library is based on replication. Only the desired viral derivatives can replicate in the target tissue, in this case certain cancer cells. The screening/selection process will yield very specific viral candidates that have both the targeting peptide moiety and may be a cancer therapeutic itself. On the contrary, phage display screens will only result in binding events and yields only the targeting peptide candidates. Thus, SVV library screening offers a much faster and selective approach. Second, during *in vitro* or *in vivo* phage display screening, phages recovered from the target cells have to be amplified in bacteria, while SVV derivatives can be directly recovered and purified from infected cells (or from the culture supernatant of lytically infected cells). Third,

SVV has a smaller genome that renders easier manipulability; thus it is possible to randomly insert the genetic information into the capsid region to ensure an optimized insertion.

Therefore, construction and screening of the SVV library has a high possibility to produce highly effective viral derivatives. These derivatives are designed and screened to specifically infect cancers with non-neural properties.

[0251] The insertion of oligonucleotides into the capsid encoding region will result in the generation of some defective mutants. Mutants may be defective in the sense that the insertion of an oligonucleotide sequence can result in a stop codon, such that the viral polyprotein will not be produced. Also, mutants may be defective in the sense that the insertion of an oligonucleotide sequence may result in the alteration of the capsid structure such that capsid can no longer be assembled. To decrease the probability that the insertion of oligonucleotide sequences may result in stop codon or untenable capsid structure, random oligonucleotides can be designed such that they do not encode for stop codons or for certain amino acids using methods such as TRIM.

[0252] To determine whether there is an optimal insertion point in the capsid region for oligonucleotides, one can generate an RGD-SVV library (*see* Example 16). The polynucleotide encoding the SVV capsid is randomly cut, for example, with CviJI. The randomly linearized capsid polynucleotides are then ligated to oligonucleotides encoding at least the RGD amino acid sequence (arginine-glycine-aspartic acid). These RGD-capsid sequences are then ligated into SVV full-length sequence vectors that are missing the capsid sequence. RGD-SVV derivatives viruses are produced and tested for their ability to infect and replicate in certain integrin-expressing cell lines (as the RGD peptide has been shown to target entities to integrin receptors). The RGD-SVV derivatives that are successful in infecting the integrin-expressing cell lines are then analyzed to determine whether there is a predominant insertion site for the RGD oligonucleotide. This site can then be used for site-directed insertion of random, non-random or semi-random oligonucleotides.

[0253] Further, in comparing portions of the capsid encoding region between SVV and other picornaviruses (*see* Figure 28), there are various non-boxed regions between the viruses where the sequence similarity is at its lowest. These regions may be important in contributing to the different tropisms between the viruses. Thus, these regions may be candidate locations for oligonucleotide insertion mutagenesis of the SVV capsid (and for other viral capsids).

Inactivated SVV as a Tumor-Specific Therapeutic:

[0254] Since SVV and SVV-capsid derivatives can target specific tumor cell-types and/or tissues, the SVV particle itself can be used as a delivery vehicle for therapeutics. In such a method, the need for the oncolytic abilities of SVV becomes optional, as the delivered therapeutic can kill the targeted tumor cell.

[0255] For example, the wild-type SVV can be inactivated such that the virus no longer lyses infected cells, but where the virus can still specifically bind and enter targeted tumor cell-types. There are many standard methods known in the art to inactivate the replicative functions of viruses. For example, whole virus vaccines are inactivated by formalin or β -propiolactone such that the viruses cannot replicate. The wild-type SVV may itself contain peptides that cause the apoptosis of cells. Alternatively, SVV can be irradiated. However, irradiated viruses should first be tested to ensure that they are still capable of specifically targeting tumor cells, as certain irradiation conditions may cause protein, and thus capsid, alterations. Further, mutant SVVs can be generated where the packaging signal sequence is deleted. These SVV mutants are able to specifically bind and enter target cells, but replicated SVV genomic RNA will not be packaged and assembled into capsids. However, this method may prove to be useful as initial entry of these mutant SVVs will cause host-protein synthesis shut-off such that tumor-cell death is still achieved.

[0256] Derivative SVVs having mutant capsids can also be inactivated and used to kill cancer cells. Derivative SVVs with oligonucleotides encoding epitope tags inserted into the capsid region can be used as vehicles to deliver toxins to tumor cells. As described herein, derivative SVVs can be randomly mutagenized and screened for tumor-specific tropisms. Toxins can be attached to the epitope tags, such that the virus delivers the toxin to tumor cells. Alternatively, therapeutic antibodies that specifically bind to the epitope tag can be used, such that the virus delivers the therapeutic antibody to the tumor cell.

High-Throughput Screening:

[0257] The present invention encompasses high-throughput methods for screening viruses that have the ability to specifically infect different cell-lines. The specificity of infection can be detected by assaying for cytopathic effects. For example, a number of different tumor cell-lines can be grown in different wells of a multi-well plate that is amenable for high-throughput screening, for example a 384 well-plate. To each well, a sample of virus is added to test whether the cells are killed by virus-mediated lysis. From

those wells that show cytopathic effects, the media is collected such that any viruses in the media can be amplified by infecting permissive cell lines in flasks or large tissue culture plates. The viruses are grown such that the RNA can be isolated and the sequence analyzed to determine sequence mutations that may be responsible for providing a tumor cell-type specific tropism for a virus.

[0258] Various colorimetric and fluorometric methods can quickly assay cytopathic effects, including fluorescent-dye based assays, ATP-based assays, MTS assays and LDH assays. Fluorescent-dye based assays can include nucleic acid stains to detect dead-cell populations, as cell-impermeant nucleic acid stains can specifically detect dead-cell populations. If it is desired to simultaneously detect both live-cell and dead-cell populations, nucleic acid stains can be used in combination with intracellular esterase substrates, membrane-permeant nucleic acid stains, membrane potential-sensitive probes, organelle probes or other cell-permeant indicators to detect the live-cell population. For example, Invitrogen (Carlsbad, CA) offers various SYTOX™ nucleic acid stains that only penetrate cells with compromised plasma membranes. Ethidium bromide and propidium iodide can also be used to detect dead or dying cells. These stains are high-affinity nucleic acid stains that can be detected by any light-absorbance reader

[0259] For example, lysis can be based on the measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells into the supernatant. To detect the presence of LDH in cell culture supernatants, a substrate mixture can be added such that LDH will reduce the tetrazolium salt INT to formazan by a coupled enzymatic reaction. The formazan dye can then be detected by a light-absorbance reader. Alternatively, an MTS assay [3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfoophenyl)-2H-tetrazolium, inner salt] using phenazine methosulfate (PMS) as the electron coupling reagent can also be used to detect cytotoxicity. Promega (Madison, WI) offers a CellTiter 96® AQueous One Solution Cell Proliferation Assay kit where the solution reagent is added directly to culture wells, incubated for 1-4 hours and then absorbance is recorded at 490 nm. The quantity of formazan product as measured by the amount of 490 nm absorbance is directly proportional to the number of living cells in culture.

[0260] There are numerous high-throughput devices for reading light-absorbance. For example, SpectraMax Plus 384 Absorbance Platereader (Molecular Devices) can detect wavelengths from 190-1000 nm in 1 nm increments. The device can read 96-well

microplates in 5 seconds and 384-well microplates in 16 seconds for ultra fast sample throughput.

[0261] Virus replication can also be assayed as an indication of successful infection, and such detection methods can be used in a high-throughput manner. For example, real-time RT-PCR methods can be used to detect the presence of virus transcripts in cell-culture supernatants. Upon reverse-transcription of viral RNA into cDNA, the cDNA can be amplified and detected by PCR with the use of double-stranded DNA-binding dyes (for example, SYBR[®] Green, Qiagen GmbH, Germany). The amount of PCR product can then be directly measured using a fluorimeter.

[0262] Viruses from the wells showing cytopathic effects are grown up and tested in further *in vitro* (re-testing of tumor and normal cell lines) and *in vivo* models (testing whether the virus can kill explanted tumors in mice).

Antibodies:

[0263] The present invention is also directed to antibodies that specifically bind to the viruses of the present invention, including the proteins of the viruses. Antibodies of the present invention include naturally occurring as well as non-naturally occurring antibodies, including, for example, single chain antibodies, chimeric antibodies, bifunctional antibodies and humanized antibodies, as well as antigen-binding fragments thereof. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains (Huse *et al.*, Science 246:1275-1281, 1989). These and other methods of making, for example, chimeric, humanized, CDR-grafted, single chain, and bifunctional antibodies are well known to those skilled in the art (Winter and Harris, Immunol. Today 14:243-246, 1993; Ward *et al.*, Nature 341:544-546, 1989; Harlow and Lane, Antibodies: A laboratory manual, Cold Spring Harbor Laboratory Press, 1988); Hilyard *et al.*, Protein Engineering: A practical approach, IRL Press 1992; Borrabeck, Antibody Engineering, 2d ed., Oxford University Press 1995). Antibodies of the invention include intact molecules as well as fragments thereof, such as Fab, F(ab')₂, and Fv which are capable of binding to an epitopic determinant present in a polypeptide of the present invention.

[0264] Where a peptide portion of a SVV polypeptide of the invention (*i.e.*, any peptide fragment from SEQ ID NO:2 or SEQ ID NO:169) or peptide portion of another viral

polypeptide of the invention used as an immunogen for antibody generation is non-immunogenic, it can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH), or by expressing the peptide portion as a fusion protein. Various other carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art (for example, by Harlow and Lane, *supra*, 1988). Methods for raising polyclonal antibodies, for example, in a rabbit, goat, mouse or other mammal, are well known in the art (see, for example, Green *et al.*, "Production of Polyclonal Antisera," in Immunochemical Protocols, Manson, ed., Humana Press 1992, pages 1-5; Coligan *et al.*, "Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters," in Curr. Protocols Immunol. (1992), section 2.4.1).

[0265] Monoclonal antibodies also can be obtained using methods that are well known and routine in the art (Kohler and Milstein, Nature 256:495, 1975; Coligan *et al.*, *supra*, 1992, sections 2.5.1-2.6.7; Harlow and Lane, *supra*, 1988). For example, spleen cells from a mouse immunized with a virus, viral polypeptide or fragment thereof, can be fused to an appropriate myeloma cell line to produce hybridoma cells. Cloned hybridoma cell lines can be screened using, for example, labeled SVV polypeptide to identify clones that secrete monoclonal antibodies having the appropriate specificity, and hybridomas expressing antibodies having a desirable specificity and affinity can be isolated and utilized as a continuous source of the antibodies. Polyclonal antibodies similarly can be isolated, for example, from serum of an immunized animal. Such antibodies, in addition to being useful for performing a method of the invention, also are useful, for example, for preparing standardized kits. A recombinant phage that expresses, for example, a single chain antibody also provides an antibody that can be used for preparing standardized kits. Monoclonal antibodies, for example, can be isolated and purified from hybridoma cultures by a variety of well established techniques, including, for example, affinity chromatography with Protein-A SEPHAROSE gel, size exclusion chromatography, and ion exchange chromatography (Barnes *et al.*, in Meth. Mol. Biol. 10:79-104, Humana Press 1992); Coligan *et al.*, *supra*, 1992, see sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3).

[0266] An antigen-binding fragment of an antibody can be prepared by proteolytic hydrolysis of a particular antibody, or by expression of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic

cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a thiol-reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly (see, for example, Goldenberg, U.S. Pat. NOS. 4,036,945 and 4,331,647; Nisonhoff *et al.*, Arch. Biochem. Biophys. 89:230. 1960; Porter, Biochem. J. 73:119, 1959; Edelman *et al.*, Meth. Enzymol., 1:422 (Academic Press 1967); Coligan *et al.*, *supra*, 1992, see sections 2.8.1-2.8.10 and 2.10.1-2.10.4).

[0267] Another example of an antigen binding fragment of an antibody is a peptide coding for a single complementarity determining region (CDR). CDR peptides can be obtained by constructing polynucleotides encoding the CDR of an antibody of interest. Such polynucleotides can be prepared, for example, using the polymerase chain reaction to synthesize a variable region encoded by RNA obtained from antibody-producing cells (for example, Larrick *et al.*, Methods: A Companion to Methods in Enzymology 2:106, 1991).

[0268] The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

[0269] There are many different labels and methods of labeling antibodies known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the antibody, or alternatively to the antigen, or will be able to ascertain such, using routine experimentation.

[0270] As various changes can be made in the above methods and compositions without departing from the scope and spirit of the invention as described, it is intended that all subject matter contained in the above description, shown in the accompanying drawings, or defined in the appended claims be interpreted as illustrative, and not in a limiting sense.

EXAMPLES

[0271] The examples described below are provided to illustrate the present invention and are not included for the purpose of limiting the invention.

Example 1

Amplification and Purification of Virus

[0272] Cultivation of SVV in PER.C6 cells: SVV is plaque purified once and a well isolated plaque is picked and amplified in PER.C6 cells (Fallaux et al., 1998). A crude virus lysate (CVL) from SVV infected PER.C6 cells is made by three cycles of freeze and thaw and used to infect PER.C6 cells. PER.C6 cells are grown in 50 x 150 cm² T.C. flasks using Dulbecco's modified Eagle medium (DMEM, Invitrogen, Carlsbad, CA, USA)) containing 10% fetal bovine serum (Biowhitaker, Walkersville, MD, USA) and 10 mM magnesium chloride (Sigma, St Louis, MO, USA). The infected cells harvested 30 hr after infection when complete CPE is noticed and are collected by centrifugation at 1500 rpm for 10 minutes at 4°C. The cell pellet is resuspended in the cell culture supernatant (30 ml) and is subjected to three cycles of freeze and thaw. The resulting CVL is clarified by centrifugation at 1500 rpm for 10 minutes at 4°C. Virus is purified by two rounds of CsCl gradients: a one-step gradient (density of CsCl 1.24 g/ml and 1.4 g/ml) followed by one continuous gradient centrifugation (density of CsCl 1.33 g/ml). The purified virus concentration is determined spectrophotometrically, assuming $1A_{260} = 9.5 \times 10^{12}$ particles (Scraba D.G., and Palmenberg, A.C. 1999. *Cardioviruses (Picornaviridae)*. In: Encyclopedia of Virology, Second edition, R.G. Webster and A Granoff Eds). Titers of purified virus are also determined by a standard plaque assay using PER.C6 cells. The yield of SVV from PER.C6 cells are greater than 200,000 particles per cell with particles to PFU ratio of about 100. The yields of SVV from other permissive cells (H446-ATCC# HTB-171) may be at least this high or higher.

Example 2

Electron Microscopy

[0273] SVV is mounted onto formvar carbon-coated grids using the direct application method, stained with uranyl acetate, and examined in a transmission electron microscope. Representative micrographs of the virus are taken at high magnification. For the transmission electron microscope, ultra-thin sections of SVV-infected PER.C6 cells are cut from the embedded blocks, and the resulting sections are examined in the transmission electron microscope.

[0274] The purified SVV particles are spherical and about 27 nm in diameter, appearing singly or in small aggregates on the grid. A representative picture of SVV is shown in Figure 2. In some places, broken viral particles and empty capsids with stain penetration are also seen. Ultrastructural studies of infected PER.C6 cells revealed crystalline inclusions in the cytoplasm. A representative picture of PER.C6 cells infected with SVV is shown in Figure 3. The virus infected cells revealed a few large vesicular bodies (empty vesicles).

Example 3

Nucleic Acid Isolation of SVV

[0275] RNA Isolation: SVV genomic RNA was extracted using guanidium thiocyanate and a phenol extraction method using Trizol (Invitrogen). Isolation was performed according to the supplier's recommendations. Briefly, 250 µl of the purified SVV was mixed with 3 volumes TRIZOL and 240 µl of chloroform. The aqueous phase containing RNA was precipitated with 600 µl isopropanol. The RNA pellet was washed twice with 70% ethanol, dried and dissolved in DEPC-treated water. The quantity of RNA extracted was estimated by optical density measurements at 260 nm. An aliquot of RNA was resolved through a 1.25% denaturing agarose gel (Cambrex Bio Sciences Rockland Inc., Rockland, ME USA) and the band was visualized by ethidium bromide staining and photographed (Figure 4).

[0276] cDNA synthesis: cDNA of the SVV genome was synthesized by RT-PCR. Synthesis of cDNA was performed under standard conditions using 1 µg of RNA, AMV reverse transcriptase, and random 14-mer oligonucleotide or oligo-dT. Fragments of the cDNA were amplified, cloned into plasmids and the clones are sequenced

Example 4**SVV Sequence Analysis and Epidemiology:****[0277] Part I: SVV SEQ ID NO:1**

[0278] The nucleotide sequence of SVV SEQ ID NO:1 was analyzed to determine its evolutionary relationship to other viruses. The translated product (SEQ ID NO:2) for this ORF was picornavirus-like and reached from the middle of VP2 to the termination codon at the end of the 3D polymerase and was 1890 amino acids in length (Fig. 5A-5E and 7A-7B). The 3' untranslated region (UTR), nucleotides 5671-5734, which follows the ORF is 64 nucleotides (nt) in length, including the termination codon and excluding the poly(A) tail of which 18 residues are provided (Fig. 5E).

[0279] Preliminary comparisons (not shown) of three partial genome segments of SVV had revealed that SVV was most closely related members of the genus *Cardiovirus* (family *Picornaviridae*). Therefore an alignment of the polyprotein sequences of SVV, encephalomyocarditis virus (EMCV; species *Encephalomyocarditis virus*, Theiler's murine encephalomyelitis virus (TMEV; species *Theilovirus*), Vilyuisk human encephalomyelitis virus (VHEV; species *Theilovirus*) and a rat TMEV-like agent (TLV; species *Theilovirus*) was constructed (Fig. 28). From this alignment, the SVV polyprotein processing was compared to the polyprotein processing of the most closely related members of the *Cardiovirus* genus. Cleavage sites between the individual polypeptides is demarcated by the "/" character in Fig. 28.

[0280] In picornaviruses, most polyprotein cleavages are carried out by one or more virus-encoded proteases, although in cardio-, aphtho-, erbo- and teschoviruses the cleavage between P1-2A and 2B is carried out by a poorly understood *cis*-acting mechanism related to the 2A sequence itself and critically involving the sequence "NPG/P", where "/" represents the break between the 2A and 2B polypeptides (Donnelly et al., 1997, *J. Gen. Virol.* 78: 13-21). One of the parechoviruses, Ljungan virus, has this sequence (NPGP) present upstream of a typical parechovirus 2A and is either an additional 2A or is the C-terminal end of the P1 capsid region. In all nine currently recognised picornavirus genera, 3C^{pro} carries out all but the *cis*-acting self-cleaving reactions (i.e. 2A cleaves at its N-terminus in entero- and rhinoviruses and L cleaves at its C-terminus in aphthoviruses and erboviruses). The post-assembly cleavage of the capsid polypeptide VP0 to VP4 and VP2 is not carried out by 3C^{pro},

but by an unknown mechanism which may involve the virus RNA. The VP0 cleavage does not occur in parechoviruses and kobuviruses. The normal cardiovirus 3C^{pro} cleavage site has either a glutamine (Q) or glutamate (E) at the -1 position and glycine (G), serine (S), adenine (A) or asparagine (N) at the +1 position (Table 2). The cleavages of the SVV polyprotein conform to this pattern except for the VP3/VP1 site which is histidine (H)/serine (S) (Table 2); however, H/S is probably present as the cleavage site between 3A and 3B^{VPg} in at least one strain of equine rhinitis A virus (ERAV; genus *Aphthovirus*) (Wutz et al., 1996, *J. Gen. Virol.* 77:1719-1730).

Table 2. Cleavage sites of SVV and cardioviruses

Between		SVV	EMCV	TMEV	Rat TLV	VHEV
L	VP4	Not known	LQ/GN (SEQ ID NO: 125)	PQ/GN (SEQ ID NO: 138)	PQ/GN (SEQ ID NO: 152)	PQ/GN (SEQ ID NO: 163)
VP4	VP2	Not known	LA/DQ (SEQ ID NO: 126)	LL/DQ (SEQ ID NO: 139)	LL/DQ (SEQ ID NO: 153)	LL/DE (SEQ ID NO: 164)
				LM/DQ (SEQ ID NO: 140)		
VP2	VP3	EQ/GP (SEQ ID NO: 117)	RQ/SP (SEQ ID NO: 127)	AQ/SP (SEQ ID NO: 141)	PQ/SP (SEQ ID NO: 154)	PQ/SP (SEQ ID NO: 165)
VP3	VP1	FH/ST (SEQ ID NO: 118)	PQ/GV (SEQ ID NO: 128)	PQ/GV (SEQ ID NO: 142)	PQ/GV (SEQ ID NO: 155)	PQ/GV (SEQ ID NO: 166)
				PQ/GI (SEQ ID NO: 143)		
				PQ/GS (SEQ ID NO: 144)		
VP1	2A	KQ/KM (SEQ ID NO: 119)	LE/SP (SEQ ID NO: 129)	LE/NP (SEQ ID NO: 145)	LQ/NP (SEQ ID NO: 156)	LE/NP (SEQ ID NO: 167)
2A	2B	NPG/P* (SEQ ID NO: 111)	NPG/P* (SEQ ID NO: 130)	NPG/P* (SEQ ID NO: 146)	NPG/P* (SEQ ID NO: 157)	Nk
2B	2C	MQ/GP (SEQ ID NO: 120)	QQ/SP (SEQ ID NO: 131)	PQ/GP (SEQ ID NO: 147)	AQ/SP (SEQ ID NO: 158)	Nk
2C	3A	LQ/SP (SEQ ID NO: 121)	AQ/GP (SEQ ID NO: 132)	AQ/SP (SEQ ID NO: 148)	AQ/SP (SEQ ID NO: 159)	Nk
			AQ/AP (SEQ ID NO: 133)			
3A	3B	SE/NA	EQ/GP	EQ/AA	EQ/AA	Nk

3B	3C	(SEQ ID NO: 122) MQ/QP (SEQ ID NO: 123)	(SEQ ID NO: 134) IQ/GP (SEQ ID NO: 135) VQ/GP (SEQ ID NO: 136)	(SEQ ID NO: 149) IQ/GG (SEQ ID NO: 150)	(SEQ ID NO: 160) IQ/GG (SEQ ID NO: 161)	Nk
3C	3D	MQ/GL (SEQ ID NO: 124)	PQ/GA (SEQ ID NO: 137)	PQ/GA (SEQ ID NO: 151)	PQ/GA (SEQ ID NO: 162)	Nk

*, the break between 2A and 2B is not a cleavage event.

[0281] Primary cleavages (P1/P2 and P2/P3) of SVV: These primary cleavage events are predicted to occur in a similar fashion to cardio-, aphtho-, erbo- and teschoviruses, involving separation of P1-2A from 2B by a novel mechanism involving the sequence NPG/P (SEQ ID NO:111) and a traditional cleavage event by 3C^{pro} between 2BC and P3 (Table 2).

[0282] P1 cleavages: Cleavages within the SVV P1 capsid coding region were relatively easy to predict by alignment with sequence with EMCV and TMEV (Table 2).

[0283] P2 cleavages: The 2C protein is involved in RNA synthesis. The 2C polypeptide of SVV contains NTP-binding motifs GxxGxGKS/T (SEQ ID NO:112) (domain A) and hyhyhyxxD (in which hy is any hydrophobic residue; domain B) present in putative helicases and all picornavirus 2Cs (Fig. 29).

[0284] P3 cleavages: Prediction of the P3 cleavage sites was also relatively straightforward. Little is known about the function of the 3A polypeptide. However, all picornavirus 3A proteins contain a putative transmembrane alpha-helix. Primary sequence identity is low in this protein between SVV and cardioviruses (See Fig. 28 between positions 1612 to 1701).

[0285] The genome-linked polypeptide, VPg, which is encoded by the 3B region, shares few amino acids in common with the other cardioviruses, however, the third residue is a tyrosine, consistent with its linkage to the 5' end of the virus genome (Rothberg et al., 1978). See Fig. 28 between positions 1703 and 1724.

[0286] The three-dimensional structure of four picornavirus 3C cysteine proteases have been solved and the active-site residues identified (HAV, Allaire et al., 1994, Nature, 369: 72-76; Bergmann et al., 1997, J. Virol., 71: 2436-2448; PV-1, Mosimann et al., 1997, J. Mol. Biol., 273: 1032-1047; HRV-14, Matthews et al., 1994, Cell, 77: 761-771; and HRV-2, Matthews et al., 1999, Proc. Natl. Acad. Sci. USA, 96: 11000-11007). The cysteine bolded in

Fig. 29 is the nucleophile, while the first bolded histidine is the general base and the specificity for glutamine residues is defined mainly by the second bolded histidine; all three residues are conserved in the SVV sequence (Fig. 29) and all other known picornaviruses (Fig. 28; for 3C sequence comparison see between positions 1726 and 1946).

[0287] The 3D polypeptide is the major component of the RNA-dependent RNA polymerase and SVV contains motifs conserved in picorna-like virus RNA-dependent RNA polymerases, i.e. KDEL/IR (SEQ ID NO:113), PSG, YGDD (SEQ ID NO:114) and FLKR (SEQ ID NO:115) (Fig. 3; Fig. 28 between positions 1948 and 2410).

[0288] Myristoylation of the N-terminus of P1: In most picornaviruses the P1 precursor polypeptide is covalently bound by its N-terminal glycine residue (when present the N-terminal methionine is removed) to a molecule of myristic acid via an amide linkage (Chow et al., 1987, *Nature*, 327: 482-486). Consequently the cleavage products VP0 and VP4 which contain the P1 N-terminus are also myristoylated. This myristoylation is carried out by myristoyl transferase which recognises an eight amino acid signal beginning with glycine. In picornaviruses, a five residue consensus sequence motif, G-x-x-x-T/S, has been identified (Palmenberg, 1989, In *Molecular Aspects of Picornavirus Infection and Detection*, pp. 211-241, Ed. Semler & Ehrenfeld, Washington D.C., Amer. Soc. for Micro.). Parechoviruses (*Human parechovirus* and *Ljungan virus*) as well as not having a maturation cleavage of VP0 are apparently not myristoylated, however, there appears to be some type of molecule blocking the N-terminus of VP0 for these viruses.

Comparisons of the individual SVV polypeptides with the public sequence databases

[0289] Each of the SVV polypeptides (SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22) were compared to the public protein sequence databases using the FASTA online program at the European Bioinformatics Institute (EBI; <http://www.ebi.ac.uk/>). The results (best matches) of these comparisons are shown in Table 3. The capsid polypeptides (VP2, VP3 and VP1) taken as a whole, along with 2C, 3C^{pro} and 3D^{pol} are most closely related to members of the cardiovirus genus, however, the short predicted 2A sequence is closer to that of Ljungan virus (genus *Parechovirus*). A more detailed comparison of the SVV 2A nucleotide sequence with similar sequences is shown in Fig. 28 (see also Fig. 70 for 2A-like NPG/P protein comparison).

Table 3. Database matches of individual predicted polypeptides of Seneca Valley virus

SVV polypeptide	Length (aa)	% identity	% identity ungapped	aa overlap	Organism	Matched protein
L (Leader)	No data	-	-	-	-	-
VP4 (1A)	No data	-	-	-	-	-
VP2 (1B)	>142	42.857	44.037	112	TMEV WW	VP2
		~ 51	-	~80	EMCV BEL-2887A/91	VP2
VP3 (1C)	239	44.068	46.637	236	EMCV ATCC VR-129B	VP3
VP1 (1D)	259	31.086	36.404	267	EMCV M100/1/02	VP1
2A	14	71.429	71.429	14	Ljungan virus 174F	2A1
2B	128	39.286	41.509	56	<i>Ureaplasma urealyticum</i>	Multiple banded antigen
2C	322	38.602	40.190	329	EMCV PV21	2C
3A	90	37.838	41.791	74	<i>Chlorobium tepidum</i> TLS*	Enolase 2†
3B ^{VPg}	22	No matches	-	-	-	-
3C ^{pro}	211	37.089	38.537	213	EMCV-R	3C protease
3D ^{pol}	462	58.009	58.515	462	EMCV-PV21	3D polymerase

* a photosynthetic, anaerobic, green-sulfur bacterium

† 2-phosphoglycerate dehydratase 2) (2-phospho-D-glycerate hydro-lyase 2

[0290] The significance of the matches of SVV 2B with *Ureaplasma urealyticum* multiple banded antigen or 3A with *Chlorobium tepidum* endolase 2 is not clear, however, these relationships maybe worthy of further investigation.

Phylogenetic comparison of SVV polypeptides with other picornaviruses

[0291] Those SVV polypeptides which could be aligned with the cardioviruses (VP2, VP3, VP1, 2C, 3C and 3D) were compared with the same proteins of representative members of each of the picornavirus species (Table 4). The programs BioEdit v5.0.9 (Hall, 1999, Nucl. Acids. Symp. Ser., 41: 95-98) and Clustal X v1.83 (Thompson et al., 1997, Nucl. Acids Res., 25:4876-4882) were used to make the alignments and to construct distance matrices and unrooted Neighbor-joining trees according to the algorithm of Saitou and Nei (Saitou and Nei, 1987, Mol. Biol. Evol., 4: 406-425). Confidence limits on branches were accessed by bootstrap resampling (1000 pseudo-replicates). The trees were drawn using TreeView 1.6.6 (Page, 1996) (Figs. 31 to 37). The distance matrices used to construct the trees used values corrected for multiple substitutions, while Figures 38-44 show the actual percentage amino acid identities. Table 4 shows the current classification of the family *Picornaviridae* and the representative virus sequences used in these comparisons.

Table 4. The taxonomic classification of the picornaviruses used in the comparisons with SVV.

Genus	Species	Representative virus	Abbrev.	Acc. No.
Enterovirus	<i>Poliovirus</i>	Poliovirus 1	PV-1	V01149
	<i>Human enterovirus A</i>	Coxsackievirus A16	CV-A16	U05876
	<i>Human enterovirus B</i>	Coxsackievirus B5	CV-B5	X67706
	<i>Human enterovirus C</i>	Coxsackievirus A21	CV-A21	D00538
	<i>Human enterovirus D</i>	Enterovirus 70	EV-70	D00820
	<i>Simian enterovirus A</i>	Simian enterovirus A1	SEV-A	AF201894
	<i>Bovine enterovirus</i>	Bovine enterovirus 1	BEV-1	D00214
	<i>Porcine enterovirus B</i>	Porcine enterovirus 9	PEV-9	AF363453
	<i>Not yet designated</i>	Simian virus 2*	SV2	AY064708
New genus?	<i>Porcine enterovirus A</i>	Porcine enterovirus 8*	PEV-8	AF406813
Rhinovirus	<i>Human rhinovirus A</i>	Human rhinovirus 2	HRV-2	X02316
	<i>Human rhinovirus B</i>	Human rhinovirus 14	HRV-14	K02121
Cardiovirus	<i>Encephalomyocarditis virus</i>	Encephalomyocarditis virus	EMCV	M81861
	<i>Theilovirus</i>	Theiler's murine encephalomyelitis virus	TMEV	M20562
Aphthovirus	<i>Foot-and-mouth disease virus</i>	Foot-and-mouth disease virus O	FMDV-O	X00871
	<i>Equine rhinitis A virus</i>	Equine rhinitis A virus	ERAV	X96870
Hepatovirus	<i>Hepatitis A virus</i>	Hepatitis A virus	HAV	M14707
	<i>Avian encephalomyelitis-like viruses</i>	Avian encephalomyelitis virus	AEV	AJ225173
Parechovirus	<i>Human parechovirus</i>	Human parechovirus 1	HPeV-1	L02971
	<i>Ljungan virus</i>	Ljungan virus	LV	AF327920
Kobuvirus	<i>Aichi virus</i>	Aichi virus	AiV	AB040749
	<i>Bovine kobuvirus</i>	Bovine kobuvirus	BKV	AB084788
<i>Erbovirus</i>	<i>Equine rhinitis B virus</i>	Equine rhinitis B virus 1	ERBV-1	X96871
<i>Teschovirus</i>	<i>Porcine teschovirus</i>	Porcine teschovirus I	PTV-1	AJ011380

* the current taxonomic status of SV2 and PEV-8 places them in the enterovirus genus, however, it has been suggested that they may be reclassified in a new genus (Krumbholz et al., 2002; Oberste et al., 2003).

[0292] The trees of the individual capsid proteins (Figs. 31 to 33) are not all representative of the tree produced when the data from all tree polypeptides is combined (Fig. 34). This is probably the result of difficulties in aligning the capsid polypeptides, particularly when they are not full length as is the case for VP2 (Fig. 31). However, the P1, 2C, 3C^{pro} and 3D^{pol} trees are all in agreement and show that SVV clusters with EMCV and TMEV.

Seneca Valley virus as a member of the cardiovirus genus

[0293] Clearly the 3D^{pol} of SVV is related to the cardioviruses, almost as closely as EMCV and TMEV are to each other (Fig. 37; Fig. 44). In the other polypeptides which are generally considered as being relatively conserved in picornaviruses, 2C and 3C, SVV is also most closely related to the cardioviruses although it is not as closely related to EMCV and TMEV as they are to each other (Fig. 42 and Fig. 43, respectively). In the outer capsid proteins (taken as a whole), SVV is also most closely related to the cardioviruses and has approximately the same relationship as the two aphthovirus species, *Foot-and-mouth disease virus* and *Equine rhinitis A virus* (~33%). SVV diverges greatly from the cardioviruses in the 2B and 3A polypeptides and has no detectable relationship with any known picornavirus.

However, this is not without precedent; avian encephalomyelitis virus differs considerably from hepatitis A virus (HAV) in 2A, 2B and 3A (Marvil et al., 1999, *J. Gen. Virol.*, 80:653-662) but is tentatively classified within the genus *Hepatovirus* along with HAV.

[0294] Seneca Valley virus is clearly not a typical cardiovirus if EMCV and TMEV are taken as the standard. However, even these two viruses have their differences, notably in the 5' UTR (Pevear et al., 1987, *J. Gen. Virol.*, 61: 1507-1516). However, phylogenetically SVV clusters with EMCV and TMEV in much of its polyprotein (P1, 2C, 3C^{pro} and 3D^{pol} regions). Ultimately, the taxonomic position of SVV within the Picornaviridae will be decided by the Executive Committee (EC) of the International Committee for the Taxonomy of Viruses (ICTV) following recommendations by the *Picornaviridae* Study Group and supporting published material. There are two options: i) include SVV as a new species in the cardiovirus genus; or ii) assign SVV to a new genus.

[0295] **Part II: SVV SEQ ID NO:168**

[0296] The full-length genome of SVV (Figures 83A-83H; SEQ ID NO:168; Example 15) allowed further epidemiological studies. The results of the further epidemiological studies are shown in Figure 86, where SVV is shown to be genetically related to cardioviruses such as EMCV and TMEV, but in a separate tree.

[0297] The features of the SVV full-length genome with respect to its untranslated and coding regions are listed at Table A *supra*. The features of the full-length SVV in comparison to EMCV and TMEV-GDVII are listed in the table below.

Feature	SVV nt length	SVV aa length	EMCV [M81861] nt length	EMCV [M81861] aa length	TMEV-GDVII [M20562] nt length	TMEV-GDVII [M20562] aa length
5' UTR	666	-	833	-	1068	-
Leader	237	79	201	67	228	76
VP4	213	71	210	70	213	71
VP2	852	284	768	256	801	267
VP3	717	239	693	231	696	232
VP1	792	264	831	277	828	276
2A	27	9	429	143	426	142
2B	384	128	450	150	381	127
2C	966	322	975	325	978	326
3A	270	90	264	88	264	88
3B	66	22	60	20	60	20
3D	1386	462	1380	460	1383	461
3' UTR	71	-	126	-	128	-

[0298] The cleavage sites of SVV (based on full-length sequence, see also bolded amino acids between at protein boundaries in Figures 83A-83H) are compared to the cleavage sites of other cardioviruses in the table below.

Between		SVV	EMCV	TMEV	Rat TLV	VHEV
L	VP4	LQ/GN (SEQ ID NO:192)	LQ/GN (SEQ ID NO:192)	PQ/GN (SEQ ID NO:193)	PQ/GN (SEQ ID NO:193)	PQ/GN (SEQ ID NO:193)
VP4	VP2	LK/DH (SEQ ID NO:194)	LA/DQ (SEQ ID NO:195)	LL/DQ (SEQ ID NO:196) LM/DQ (SEQ ID NO:197)	LL/DQ (SEQ ID NO:196)	LL/DE (SEQ ID NO:198)
VP2	VP3	EQ/GP (SEQ ID NO:117)	RQ/SP (SEQ ID NO:199)	AQ/SP (SEQ ID NO:200)	PQ/SP (SEQ ID NO:201)	PQ/SP (SEQ ID NO:201)
VP3	VP1	FH/ST (SEQ ID NO:118)	PQ/GV (SEQ ID NO:202)	PQ/GV (SEQ ID NO:202) PQ/GI (SEQ ID NO:203) PQ/GS (SEQ ID NO:204)	PQ/GV (SEQ ID NO:202)	PQ/GV (SEQ ID NO:202)
VP1	2A	MQ/SG (SEQ ID NO:205)	LE/SP (SEQ ID NO:206)	LE/NP (SEQ ID NO:207)	LQ/NP (SEQ ID NO:208)	LE/NP (SEQ ID NO:207)
2A	2B	NPG/P* (SEQ ID NO:111)	NPG/P* (SEQ ID NO:111)	NPG/P* (SEQ ID NO:111)	NPG/P* (SEQ ID NO:111)	unknown
2B	2C	MQ/GP (SEQ ID NO:120)	QQ/SP (SEQ ID NO:209)	PQ/GP (SEQ ID NO:210)	AQ/SP (SEQ ID NO:200)	unknown
2C	3A	LQ/SP (SEQ ID NO:121)	AQ/GP (SEQ ID NO:211) AQ/AP (SEQ ID NO:212)	AQ/SP (SEQ ID NO:200)	AQ/SP (SEQ ID NO:200)	unknown
3A	3B	SE/NA (SEQ ID NO:122)	EQ/GP (SEQ ID NO:213)	EQ/AA (SEQ ID NO:214)	EQ/AA (SEQ ID NO:214)	unknown
3B	3C	MQ/QP (SEQ ID NO:123)	IQ/GP (SEQ ID NO:215) VQ/GP (SEQ ID NO:216)	IQ/GG (SEQ ID NO:217)	IQ/GG (SEQ ID NO:217)	unknown
3C	3D	MQ/GL (SEQ ID NO:124)	PQ/GA (SEQ ID NO:218)	PQ/GA (SEQ ID NO:218)	PQ/GA (SEQ ID NO:218)	unknown

* ribosome skipping sequence

[0299] Multiple unique viruses were discovered at the USDA that are more similar to SVV than SVV is to other cardioviruses. These USDA virus isolates, herein considered to be members of the group called "SVV-like picornaviruses," are: MN 88-36695, NC 88-23626, IA 89-47552, NJ 90-10324, IL 92-48963, CA 131395; LA 1278; IL 66289; IL 94-9356; MN/GA 99-29256; MN 99197; and SC 363649. These SVV-like picornaviruses and SVV are considered to comprise a new picornavirus genus.

[0300] Each of these SVV-like picornaviruses are unique, and are about 95%-98% identical to SVV at the nucleotide level (see Figures 87-89 for nucleotide sequence comparisons between SVV and these USDA isolates).

[0301] Part III: Serum Studies

[0302] Pigs are a permissive host for the USDA virus isolates identified above. The isolate MN 88-36695 was inoculated into a gnotobiotic pig and antisera generated (GP102). The antisera binds to all of the other USDA isolates listed above and to SVV. The antisera does not react with 24 common porcine virus pathogens indicating its specificity. Porcine sera was also tested for neutralizing antibodies to 1278 (Plum Island virus). Sera were collected in the US and 8/29 sera were positive with titers ranging from 1:57 to 1:36,500.

[0303] To test whether the pig is the natural source for SVV, serum samples from various animals were obtained and tested for their ability to act as neutralizing antibodies against SVV infection of permissive cells. The Serum Neutralization Assay is conducted as follows: (1) Dilute various serums 1:2 and 1:4; (2) Mix with 100 TCID₅₀ of virus (SVV; but any virus can be tested to determine whether a serum can neutralize its infection); (3) Incubate at 37°C for 1 hour; (4) Add to 1x10⁴ PER.C6 cells (or other permissive cell type); (5) Incubate at 37°C for 3 days; and (6) Measure CPE using MTS assay. The neutralization titer is defined as the highest dilution of sera that neutralizes SVV (or other virus in question) at 100%.

[0304] The serum neutralization results showed that there is a minimal or no presence of neutralizing antibodies in human and primate populations. In one experiment, 0/22 human sera contained neutralizing antibodies to SVV. In another experiment, only 1/28 human sera contained neutralizing antibodies. In a third experiment, 0/50 human sera from Amish farmers were neutralizing. In another experiment, 0/52 primate sera from four species were neutralizing.

[0305] The serum neutralization results showed that there is a prevalence of neutralizing antibodies in farm animal populations. In one experiment, 27/71 porcine sera from farms were neutralizing. In another experiment, 4/30 porcine sera from a disease-free farm were neutralizing. In another experiment, 10/50 bovine sera were neutralizing. In yet another experiment, 5/35 wild mouse sera were neutralizing. Because antibodies cross-reactive to SVV and/or SVV-like picornaviruses have been found in pigs, cows, and mice, these data indicate that SVV and/or SVV-like picornaviruses may be prevalent in a wide-variety of non-primate animals.

[0306] A crude viral lysate of MN 88-36695 was tested to assess its cytotoxicity ability on two cell lines permissive (NCI-H446; HEK293) for SVV and on two cell lines non-permissive (NCI-H460 and S8) for SVV. The cytotoxicity profile for MN 88-36695 was identical to SVV: the TCID₅₀ for NCI-H446 was 1.6×10^{-6} ; the TCID₅₀ for HEK293 was 1.3×10^{-2} ; and NCI-H460 and S8 were non-permissive for MN 88-36695. This data indicates that SVV-like picornaviruses have the potential to be used in the present methods directed to cancer therapy. In one embodiment, the invention provides for the use of the MN 88-36695 SVV-like picornavirus in any of the methods directed to cancer therapy, diagnosis, or screening.

[0307] Antisera to MN 88-36694 and SVV were tested in serum neutralization assays on each virus. Anti-SVV mouse serum was able to neutralize infection by both MN 88-36695 and SVV (neutralization titers on infection were 1:640 for MN 88-36695 and 1:1000 for SVV). Anti-MN 88-36695 gnotobiotic pig serum was able to neutralize infection by both MN 88-36695 and SVV (neutralization titers on infection were 1:5120 for MN 88-36695 and 1:100 for SVV).

[0308] These data indicate that SVV is genetically and serologically linked to the porcine USDA virus isolates.

Example 4

SDS-PAGE and N-Terminal Sequence Analysis of SVV Capsid Proteins

[0309] Purified SVV is subjected to electrophoresis using NuPAGE pre-cast Bis-Tris polyacrylamide mini-gel electrophoresis system (Novex, San Diego, Ca, USA). One half of the gel is visualized by silver stain while the other half is used to prepare samples for amino acid sequencing of the N-termini of the capsid proteins. Prior to transfer of proteins to membrane, the gel is soaked in 10 mM CAPS buffer, pH 11, for 1 hour, and a PVDF membrane (Amersham) is wetted in methanol. Proteins are transferred to the PVDF membrane. After transfer, proteins are visualized by staining with Amido black for approximately 1 minute, and bands of interest are excised with a scalpel and air dried. The proteins can be subjected to automated N-terminal sequence determination by Edman degradation using a pulsed phase sequencer.

[0310] Three major structural proteins of the purified SVV are shown in Figure 45 (approximately 36 kDa, 31 kDa, and 27 kDa).

Example 5

Assay for Neutralization Antibodies to SVV in Human Serum Samples

[0311] Preexisting antibodies to particular viral vectors may limit the use of such vectors for systemic delivery applications such as for treatment of metastatic cancer, because preexisting antibodies may bind to systemically delivered vectors and neutralize them before the vectors have a chance to transduce the targeted tissue or organ. Therefore, it is desirable to ensure that humans do not carry neutralization antibodies to viral vectors selected for systemic delivery. To determine whether human sera samples contain SVV-specific neutralizing antibodies, neutralization assays are carried out using randomly collected human sera samples.

[0312] Tissue culture infective dose 50: One day before the experiment, 180 μl of PER.C6 cell suspension containing 1×10^4 cells are plated in 96-well tissue culture dish. The crude virus lysate (CVL) of SVV is diluted in log steps from 10^0 to 10^{-11} in DMEM medium (Dulbecco's Modified Eagle's Medium) and 20 μl of each dilution is transferred to three wells of a Falcon 96-well tissue culture plate containing PER.C6 cells. The plates are incubated at 37°C in 5% CO_2 and read at 3 days for microscopic evidence of cytopathic effect (CPE), and the tissue culture infective dose 50 (TCID_{50}) is calculated.

[0313] Neutralization assay: First, 40 μl of medium is placed in all the wells and then 40 μl of heat-inactivated serum is added to the first well and mixed by pipeting, making a 1:4 dilution used for screening purposes. 40 μl is then transferred to the next well to perform a two-fold dilution of the serum samples. 40 μl of SVV virus, containing 100 TCID_{50} , is added to wells containing diluted serum samples. Plates are incubated at 37°C for 1 hour. 40 μl of the mix is taken and transferred to a plate containing PER.C6 cells (1×10^4 cells/160 μl /well). The plates are incubated at 37°C for 3 days. After this time, the cultures are read microscopically for CPE.

[0314] In a representative neutralization assay performed as described above, twenty-two human sera samples randomly collected from USA, Europe and Japan were examined for SVV specific neutralizing antibodies. The serum samples were serially diluted and mixed with a fixed amount of SVV containing 100 TCID_{50} . Serum-virus mixtures were then used to infect PER.C6 cells and incubated for 24 hours. Neutralizing antibody titer was determined as the reciprocal of the highest dilution of serum able to block CPE formation. In this

experiment, no dilution of serum blocked CPE formation indicating that the human serum samples did not contain SVV neutralizing antibodies.

[0315] Further SVV infection of PER.C6 was not inhibited by incubation with human blood (*see* Example 6), indicating that SVV infection was not inhibited by complement or by hemagglutination. As a result, SVV exhibits a longer circulation time *in vivo* than other oncolytic viruses, which is a significant problem with the use of oncolytic adenoviruses.

Example 6

Binding of SVV to Human Erythrocytes and Hemagglutination

[0316] Various viral serotypes have been shown to cause *in vitro* hemagglutination of erythrocytes isolated from blood of various animal species. Hemagglutination or binding to erythrocytes may cause toxicity *in vivo* and may also affect *in vivo* biodistribution and the efficacy of a viral vector. Therefore, it is desirable to analyze the erythrocyte agglutination properties of a viral vector selected for systemic administration to treat metastatic cancers.

[0317] Hemagglutination assay: To determine whether SVV causes agglutination of human erythrocytes, hemagglutination assays are carried out in U-bottom 96-well plates. Purified SVV is serially diluted in 25 μ l PBS (Phosphate Buffered Saline) in duplicates, and an equal volume of 1% erythrocyte suspension is added to each well. Blood samples used for isolation of erythrocytes are obtained from healthy individuals with heparin as an anticoagulant. Erythrocytes are prepared by washing the blood three times in cold PBS to remove the plasma and the white blood cells. After the last wash, erythrocytes are suspended in PBS to make a 1% (V/V) cell suspension. The virus and erythrocytes are gently mixed and the plates are incubated at room temperature for 1 hour and monitored for a hemagglutination pattern.

[0318] Whole blood inactivation assay: To rule out direct inactivation of SVV by blood components, aliquots of virus are incubated with heparinized human blood belonging to A, B, AB and O blood groups or PBS for 30 minutes or 1 hour at room temperature prior to separation of plasma, after which PER.C6 cells are infected and titers are calculated.

[0319] In representative assays performed as described above, no hemagglutination of human erythrocytes of different blood groups (A, B, AB and O) was seen at any tested dilutions of SVV. A slight increase in the virus titer is noticed when SVV is mixed with

blood human samples and incubated for 30 minutes and 1 hour, indicating that the virus is not inactivated by blood components but becomes more infectious under tested conditions.

Example 7

In vivo Clearance

[0320] Blood circulation time: To determine the blood circulation time and the amount of the virus in the tumor, H446 tumor bearing nude mice were treated with SVV at a dose of 1×10^{12} vp/kg by tail vein injection. The mice were bled at 0, 1, 3, 6, 24, 48, 72 hours and 7 days (189 hours) post-injection and the plasma was separated from the blood immediately after collection, diluted in infection medium, and used to infect PER.C6 cells. The injected mice were sacrificed at 6, 24, 48, 72 hours and 7 days post-injection and the tumors were collected. The tumors were cut into small sections and suspended in one ml of medium and subjected to three cycles of freeze and thaw to release the virus from the infected cells. Serial log dilutions of supernatants were made and assayed for titer on PER.C6 cells. SVV titers were expressed as pfu/ml. The tumor sections were also subjected to H&E staining and immunohistochemistry to detect the virus capsid proteins in the tumor.

[0321] The circulating levels of virus particles in the blood were determined based on the assumption that 7.3% of mouse body weight is blood. In representative assays performed as essentially as described above, within 6 hours of virus administration, the circulating levels of SVV reduced to zero particles and SVV was not detectable at later time points (Fig. 46A). In the tumor, SVV was detectable at 6 hours post-injection, after which the amount of the virus increased steadily by two logs (Fig. 46B). The virus was detectable in the tumor as late as 7 days postinjection (Fig. 46B). The tumor sections when subjected to immunohistochemistry, revealed SVV proteins in the tumor cells (Figure 47, top panels). When stained by H&E, the tumor sections revealed several rounded tumor cells (Figure 47, bottom panels).

[0322] SVV also exhibits a substantially longer resident time in the blood compared to similar doses of i.v. adenovirus. Following a single i.v. dose, SVV remains present in the blood for up to 6 hours (Figure 46C; Figure 46C is a duplication of Figure 46A for comparison purposes to Figure 46D), whereas adenovirus is cleared from the blood in about an hour (Figure 46D).

Example 8**Tumor Cell Selectivity**

[0323] *In vitro* cell killing activity of SVV: To determine the susceptibility of human, bovine, porcine, and mouse cells, normal and tumor cells were obtained from various sources and infected with SVV. All cell types were cultured in media and under the conditions recommended by the supplier. Primary human hepatocytes may be purchased from In Vitro Technologies (Baltimore, MD) and cultured in Hepatocyte Culture Media (HCMTM, BioWhittaker/Clonetics Inc., San Diego, CA).

[0324] *In vitro* cytopathic assay: To determine which types of cells are susceptible to SVV infection, monolayers of proliferating normal cells and tumor cells were infected with serial dilutions of purified SVV. The cells were monitored for CPE and compared with uninfected cells. Three days following infection, a MTS cytotoxic assay is performed and effective concentration 50 (EC₅₀) values in particles per cell are calculated. See Tables 5 and 6 below and Table 1A *supra*.

Table 5. Cell lines with EC₅₀ values less than 100

Cell lines with EC50 < 1	EC50 number
H446 (human sclc)	0.001197
PERC6	0.01996
H69AR (sclc-multidrug resisitant)	0.03477
293 (human kidney transformed with ad5E1)	0.03615
Y79 (human retinoblastoma)	0.0003505
IMR32 (human brain; neuroblastoma)	0.03509
D283med (human brain; cerebellum; medulloblastoma)	0.2503
SK-N-AS (human brain; neuroblastoma)	0.474
N1E-115 (mouse neuroblastoma)	0.002846
SK-NEP-1 (kidney, wilms' tumor, pleural effusion, human)	0.03434
BEKPCB3E1 (bovine embryonic kidney cells transformed with ad5E1)	0.99
Cell Lines with EC50 < 10 (1-10)	EC50 number
H1299 (human-non sclc)	7.656
ST (pig testes)	5.929
DMS 153 (human sclc)	9.233

Cell lines with EC50 < 1	EC50 number
Cell lines with EC50 < 100 (10-100)	EC50 number
BEK (bovine embryonic kidney)	17.55

Table 6. Cell lines with EC₅₀ values more than 1000

M059K (human brain; malignant glioblastoma)	HUVEC (human vein endothelial cells)	CMT-64 (mouse-sclc)
KK (human glioblastoma)	HAEC (human aortic endothelial cells)	LLC-1 (mouse-LCLC)
U-118MG (human glioblastoma)	WI38 (human lung fibroblast)	RM-1 (mouse-prostate)
DMS 79 (human sclc)	MRC-5 (human lung fibroblast)	RM-2 (mouse-prostate)
H69 (human sclc)	IMR90 (human lung fibroblast)	RM-9 (mouse-prostate)
DMS 114 (human sclc)	HMVEC (human microvascular endothelial cells-adult)	MLTC-1 (mouse-testes)
DMS 53 (human sclc)	HMVEC (human microvascular endothelial cells-neonatal)	KLN-205 (mouse-sqcc)
H460 (human-LCLC)	HCN-1A (human brain)	CMT-93 (mouse-rectal)
A375-S2 (human melanoma)	HRCE (human renal cortical epithelial cells)	B16F0 (mouse melanoma)
SK-MEL-28 (human melanoma)		Neuro-2A (mouse neuroblastoma)
PC3 (human prostate)		C8D30 (mouse brain)
PC3M2AC6 (human prostate)		PK15 (pig-kidney)
LNCaP (human prostate)		FBRC (fetal bovine retina)
DU145 (human prostate)		MDBK (bovine kidney)
Hep3B (human liver carcinoma)		CSL 503 (sheep lung cells transformed with ad5E1)
Hep2G (human liver carcinoma)		OFRC (ovine fetal retina cells)
SW620 (human-colon)		
SW839 (human kidney)		
5637 (human bladder)		
HeLa S3		
S8		

[0325] The MTS assay was performed according to the manufacturer's instructions (CellTiter 96® AQueous Assay by Promega, Madison, WI). The CellTiter 96® AQueous Assay preferably uses the tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) and an electron coupling reagent, phenazine methosulfate (PMS). Contact-inhibited normal human cells evaluated in the study include: HUVEC (human umbilical vein endothelial cells), HAEC (human aortic endothelial cells, Clonetics/BioWhittaker # CC-2535), Wi38 (normal human embryo lung fibroblasts, ATCC # CCL-75), IMR90 (human normal lung fibroblasts, ATCC

CCL-186), MRC-5 (human normal lung fibroblasts, ATCC, # CCL-171) and HRCE (human renal cortical epithelial cells, Clonetics/BioWhittaker # CC-2554).

[0326] SVV does not produce CPE in any of the above contact-inhibited normal cells. No virus-induced CPE was seen in the following human tumor cell lines: Hep3B (ATCC # HB-8064), HepG2 (human hepatocellular carcinoma, ATCC # HB-8065), LNCaP (human prostate carcinoma, ATCC # CRL-10995), PC3M-2AC6, SW620 (human colorectal adenocarcinoma, ATCC # CCL-227), SW 839 (human kidney adenocarcinoma, ATCC # HTB-49), 5637 (human urinary bladder carcinoma, ATCC # HTB-9), DMS-114 (small cell lung cancer, ATCC # CRL-2066), DMS 153 (human small cell lung cancer, ATCC # CRL-2064), A549 (human lung carcinoma, ATCC # CCL-185), HeLa S3 (human cervical adenocarcinoma, ATCC # CCL-2.2), NCI-H460 (human large cell lung cancer, ATCC # HTB-177), KK (glioblastoma), and U-118 MG (human glioblastoma, ATCC # HTB-15). Note - the cell lines in Table 6 with EC₅₀ values greater than 1000 are most likely not permissive for SVV replication and/or virion production; although the possibility remains that SVV can bind and enter into these cells but CPE is not observed because SVV replication cannot occur inside the cell or that replication does occur but CPE is not observed because there is some other post-entry block (*i.e.*, no packaging of replicated SVV genomes into virions). However, considering the absence of CPE in these cell lines, these cell-lines, and potentially tumor-types thereof, are good candidates to test which cell and tumor-types are permissive or non-permissive for SVV replication. Although wild-type SVV is tumor-specific, and has been shown to target neuroendocrine tumors, including small cell lung cancer and neuroblastomas, there may be individual patients that have types of etiologies such that SVV is not permissive in their form of neuroendocrine tumor. Therefore, the invention does contemplate the generation of SVV derivatives that can kill tumor cell-types isolated from individual patients where the tumors are non-permissive to the wild-type SVV, and the tumor-types isolated from these individuals can include, for example, glioblastoma, lymphoma, small cell lung cancer, large cell lung cancer, melanoma, prostate cancer, liver carcinoma, colon cancer, kidney cancer, colon cancer, bladder cancer, rectal cancer and squamous cell lung cancer.

[0327] SVV-mediated cytotoxicity on primary human hepatocytes (In Vitro Technologies) was determined by LDH release assay (CytoTox® 96 Non-Radioactive Cytotoxicity Assay, Promega, # G1780). Primary human hepatocytes plated in collagen

coated 12-well plates were infected with SVV at 1, 10 and 100 and 1000 particles per cell (ppc). After 3 hours of infection, the infection medium was replaced with 2 ml of growth medium and incubated for 3 days in a CO₂ incubator. The cell associated lactate dehydrogenase (LDH) and LDH in the culture supernatant was measured separately. Percent cytotoxicity is determined as a ratio of LDH units in supernatant over maximal cellular LDH plus supernatant LDH.

$$\text{Percent cytotoxicity} = \frac{\text{LDH units in culture supernatant} \times 100}{\text{Sum of LDH units in supernatant and cell lysate}}$$

The data shown in Figure 48 illustrates the absence of SVV mediated hepatotoxicity at all tested multiplicity of infections.

Example 10

Virus Production Assay

[0328] To assess the replicative abilities of SVV, several selected contact-inhibited normal cells and actively dividing tumor cells were infected with SVV at one virus particle per cell (ppc). After 72 hours, cells and the medium were subjected to three freeze-thaw cycles and centrifuged to collect the supernatant. Serial log dilutions of supernatants were made and assayed for titer on PER.C6 cells. For each cell line, the efficiency of SVV replication was expressed as pfu/ml (Figure 49).

Example 10

Toxicity

[0329] The maximum tolerated dose (MTD) is defined as the dosage immediately preceding the dose at which animals (*e.g.* mice) demonstrate a dose limiting toxicity (DLT) after the treatment with SVV. DLT is defined as the dose at which the animals exhibit a loss in body weight, symptoms, and mortality attributed to SVV administration during the entire duration of the study. Neutralizing antibodies to SVV were assessed at baseline, day 15, and day 21. Neutralization assays were carried as described earlier.

[0330] Escalating doses (1×10^8 - 1×10^{14} vp/kg) of SVV were administered intravenously into both immune deficient nude and caesarean derived-1 (CD-1) out-bred immune competent mice purchased from Harlan Sprague Dawley (Indianapolis, IN, USA) to determine the MTD with 10 mice per dose level. The virus was well-tolerated at all tested dose levels without exhibiting any clinical symptoms and without loss in body weight (Figure

50). Mice were bled at day 15 and 21 and the sera was monitored for the presence of SVV-specific neutralizing antibodies in neutralization assays. SVV injected CD1 mice develop neutralizing antibodies and the titers range from 1/1024 to greater than 1/4096.

[0331] Another toxicity study was conducted on the immunocompetent mouse strain (A/J). It has been demonstrated that SVV exhibits cell killing activity and replication in N1E-115 cells (see Table 1). The murine cell line N1E-115 (a neuroblastoma cell line, *i.e.*, neuroendocrine cancer) is derived from the A/J mouse strain. Thus, a syngeneic mouse model was established where N1E-115 cells were implanted subcutaneously in A/J mice to form tumors, and the mice were then treated with SVV to investigate its efficacy and toxicity.

[0332] In the A/J study, mice were i.v. injected with SVV to determine whether A/J mice can tolerate systemic administration of SVV. Blood hematology results were obtained to look for signs of toxicity, and serum chemistry results can also be obtained. The study design is shown in Table 7 below:

Table 7: A/J Study Design

Group #	Animals (Female)	Test Article	Dosage Level (particles/kg)	Dosage Volume (mL/kg)	Dosing regimen	Necropsy Day
1	5	Vehicle	0	10	IV on Day 1	Day 15
2	5	SVV	10^8	10	IV on Day 1	Day 15
3	5	SVV	10^{11}	10	IV on Day 1	Day 15
4	5	SVV	10^{14}	10	IV on Day 1	Day 15

[0333] The A/J mice were 8-10 week old females obtained from The Jackson Laboratory (Bar Harbor, Maine). SVV was prepared by storing isolated virions at -80°C until use. SVV was prepared fresh by thawing on ice and diluting with HBSS (Hank's balanced salt solution). SVV was diluted to concentrations of 10^7 particles/mL for group 2, 10^{10} particles/mL for group 3, and 10^{13} particle/mL for group 4. HBSS was used as the vehicle control for group 1. All dosing solutions were kept on wet ice until dosing.

[0334] SVV was administered to animals intravenous injection via the tail vein at a dose volume of 10 mL/kg body weight. Animals were weighed on the day of dosing and dose volumes were adjusted based on body weight (*i.e.*, a 0.0200 kg mouse gets 0.200 mL of

dosing solution). Mice were monitored twice daily for morbidity and mortality. Mice were weighed twice weekly. Information relating to moribund animals and animals exhibiting any unusual symptoms (physically or behaviorally) are recorded immediately.

[0335] Post-mortem observations and measurements entail the collection of blood from all surviving animals at terminal sacrifice for standard hematology and serum chemistry (AST, ALT, BUN, CK, LDH). The following organs are to be collected at sacrifice: brain, heart, lung, kidney, liver, and gonads. Half of each organ sample is snap frozen on dry ice and the other half will be placed in formalin.

[0336] Initial blood hematology results (CBC, differential) were obtained two weeks after SVV injection and the results are summarized below in Table 8 below. Five mice were tested from each test group (see Table 7):

Table 8: A/J Toxicity Results - Blood Hematology

	Test Group 1	Test Group 2	Test Group 3	Test Group 4
<u>Body Weight Result ± SD (g):</u>				
Day 0	21.48 ± 0.88	21.98 ± 1.93	22.58 ± 0.87	21.04 ± 1.67
Day 14	20.26 ± 0.93	20.92 ± 1.71	21.44 ± 0.84	21.26 ± 1.45
<u>CBC Wet (Result ± SD (ref range)):</u>				
White blood count (THSN/UL)	3.63 ± 1.57 (2.60-10.69)	4.5 ± 1.57 (2.60-10.69)	4.26 ± 0.94 (2.60-10.69)	4.72 ± 0.62 (2.60-10.69)
Red blood count (MILL/UL)	9.87 ± 0.03 (6.4-9.4)	9.49 ± 0.07 (6.4-9.4)	9.76 ± 0.37 (6.4-9.4)	9.71 ± 0.32 (6.4-9.4)
Hemoglobin (GM/DL)	15.37 ± 0.06 (11.5-16.1)	14.78 ± 0.29 (11.5-16.1)	15.12 ± 0.66 (11.5-16.1)	15.02 ± 0.63 (11.5-16.1)
Hematocrit (%)	46.03 ± 0.40 (36.1-49.5)	44.52 ± 0.49 (36.1-49.5)	45.7 ± 1.82 (36.1-49.5)	45.28 ± 1.69 (36.1-49.5)
MCV (FL)	46.67 ± 0.58 (45.4-60.3)	47.00 ± 0.0 (45.4-60.3)	47.0 ± 0.0 (45.4-60.3)	46.6 ± 0.55 (45.4-60.3)
MHC (PICO GM)	15.57 ± 0.06 (14.1-19.3)	15.70 ± 0.17 (14.1-19.3)	15.37 ± 0.06 (14.1-19.3)	15.43 ± 0.15 (14.1-19.3)
MCHC (%)	33.37 ± 0.12 (25.4-34.1)	33.14 ± 0.48 (25.4-34.1)	33.08 ± 0.22 (25.4-34.1)	33.14 ± 0.25 (25.4-34.1)
Platelet (THSN/UL)	885.33 ± 28.6 (592-2972)	758.2 ± 146.2 (592-2972)	874.8 ± 56.7 (592-2972)	897.2 ± 105.4 (592-2972)
<u>Differential (Result ± SD (ref range)):</u>				
Bands (THSN/UL)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.0 (0.0-0.1)
Seg. Neutrophils (THSN/UL)	0.92 ± 0.27 (0.13-2.57)	1.16 ± 0.37 (0.13-2.57)	1.09 ± 0.38 (0.13-2.57)	0.96 ± 0.20 (0.13-2.57)
Lymphocytes (THSN/UL)	2.64 ± 1.26 (1.43-9.94)	2.98 ± 1.41 (1.43-9.94)	3.10 ± 0.56 (1.43-9.94)	3.70 ± 0.41 (1.43-9.94)
Monocytes (THSN/UL)	0.06 ± 0.04 (0.0-0.39)	0.15 ± 0.05 (0.0-0.39)	0.06 ± 0.03 (0.0-0.39)	0.05 ± 0.02 (0.0-0.39)
Eosinophils (THSN/UL)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.003 ± 0.01

Basophils (THSN/UL)	(0.0-0.24) 0.0	(0.0-0.24) 0.004 ± 0.005	(0.0-0.24) 0.0	(0.0-0.24) 0.0
Atypical Lympho. (THSN/UL)	(0.0-0.0) 0.0	(0.0-0.0) 0.0	(0.0-0.0) 0.0	(0.0-0.0) 0.0
Metamyelocytes (THSN/UL)	(0.0-0.0) 0.0	(0.0-0.0) 0.0	(0.0-0.0) 0.0	(0.0-0.0) 0.0
Myelocytes (THSN/UL)	(0.0-0.0) 0.0	(0.0-0.0) 0.0	(0.0-0.0) 0.0	(0.0-0.0) 0.0
NRBC (/100WBC)	(0.0-0.0) 0.0 (0.0-0.0)	(0.0-0.0) 0.0 (0.0-0.0)	(0.0-0.0) 0.0 (0.0-0.0)	(0.0-0.0) 0.0 (0.0-0.0)
<u>Other (Result ± SD (ref range)):</u>				
AST (SGOT) (U/L)	1762.8 ± 1129.8 (72-288)	899.0 ± 234.6 (72-288)	779.8 ± 312.2 (72-288)	843.2 ± 653.4 (72-288)
ALT (SGPT) (U/L)	2171.8 ± 2792.9 (24-140)	535.2 ± 272.8 (24-140)	555 ± 350.8 (24-140)	380.2 ± 385.7 (24-140)
BUN (MG/DL)	27.2 ± 0.8 (9-28)			
Creatine phospho- kinase (U/L)	28312.8 ± 20534.4 (0-800)	24.8 ± 1.9 (9-28) 12194.4 ±	24.6 ± 5.5 (9-28) 10157 ± 5420.5	28.2 ± 12.8 (9-28) 11829 ± 10363.9
LDH (U/L)	6650.2 ± 4788.6 (260-680) 706.6 ± 423.4 (0-70)	4049.2 (0-800) 3661.6 ± 933.6 (260-680) 477.6 ± 195.7 (0-70)	(0-800) 3450.8 ± 972.6 (260-680) 589.6 ± 198.6 (0-70)	(0-800) 2808.4 ± 1709.1 (260-680) 496.4 ± 321.1 (0-70)
Hemolytic Index (MG/DL HGB)				

[0337] These results show that there are no abnormalities in blood hematology profiles obtained from mice treated with low, medium and high doses of SVV compared to blood hematology profiles obtained from untreated mice. From this study, it can be concluded that there are no measureable signs of toxicity following systemic administration of SVV, indicating that SVV is tolerated by A/J mice following i.v. injection.

Example 11

Efficacy

[0338] Athymic female nude mice (nu/nu) aged 6-7 weeks purchased from Harlan Sprague Dawley (Indianapolis, IN) were used in efficacy studies. Mice were injected subcutaneously with 5×10^6 H446 cells into the right flank using manual restraint. Tumor sizes were measured regularly, and the volumes were calculated using the formula $\pi/6 \times W \times L^2$, where L=length and W = width of the tumor. When the tumors reach approximately 100-150 mm³, mice (n=10) were randomly divided into groups. Mice were injected with escalating doses of SVV by tail vein injections at a dose volume of 10 ml/kg. A control group of mice was injected with an equivalent volume of HBSS. Dose escalation proceeds from 1×10^7 to 1×10^{13} particles per kilogram body weight. Antitumoral efficacy was

determined by measuring tumor volumes twice weekly following SVV administration. Complete response was defined as complete disappearance of xenograft; partial response as regression of the tumor volume by equal to or more than 50%; and no response as continuous growth of tumor as in the control group.

[0339] Tumors from mice treated with HBSS grew rapidly and the tumor volumes reached more than 2000 mm³ by study day 20 (Figure 51; *see* line with open diamond). In contrast, mice given one systemic injection of SVV at all tested doses (with the exception of the lowest dose) became tumor free by study day 20. In the lowest dose group, 8 mice became tumor free, one mouse had a very large tumor and the other had a small palpable tumor (25 mm³) by study day 31. To evaluate the antitumor activity of SVV on large sized tumors, five mice from HBSS group bearing tumors >2000 mm³ were systemically injected with a single dose of 1x10¹¹ vp/kg on study day 20. For the duration of the follow-up period (11 days of after SVV injection), a dramatic regression of the tumor volumes were noted (Figure 51).

[0340] Additional experiments to test the efficacy of a single intravenous dose of SVV was conducted in murine tumor models that express neuroendocrine markers. The tumor models tested included H446 (human SCLC) (*see* Figure 90A), Y79 (human retinoblastoma) (*see* Figure 90B), H69AR (human multi-drug resistant SCLC) (*see* Figure 90C), H1299 (human NSCLC) (*see* Figure 90D), and N1E-115 (murine neuroblastoma) (*see* Figure 90E).

[0341] The results show that a single intravenous dose of SVV has efficacy in all of the murine neuroendocrine tumor models. The results also show that SVV is efficacious in the N1E-115 immunocompetent murine neuroblastoma model.

[0342] Figure 52 shows a picture of mice that were “untreated” with SVV (*i.e.*, treated with HBSS) or “treated” with SVV. As can be seen, the untreated mice had very large tumors and the treated mice showed no visible signs of tumor. Further, for unsacrificed mice treated with SVV, no tumor regrowth was observed for the duration of the study, 200 days.

[0343] *In vitro* efficacy data for SVV for specific tumor cell lines is shown in Tables 1, 1A, and 5. The data shows that SVV specifically infects particular tumor cell types and does not infect normal adult cells (except for porcine normal cells), a significant advantage

over any other known oncolytic virus. SVV has been shown to have 1,000 times better cell killing specificity than chemotherapy treatments (cell killing specificity values for SVV have been shown to be greater than 10,000, whereas cell killing specificity values for chemotherapy are around 10).

[0344] Specific cytotoxic activity of SVV was demonstrated in H446 human SCLC cells. Following a two-day incubation with increasing concentrations of SVV, cell viability was determined. The results are shown in Figure 53. Figure 53 shows cell survival following incubation of SVV with either H446 SCLC tumor cells (top graph) or normal human H460 cells (bottom graph). SVV specifically killed the tumor cells with an EC_{50} of approximately 10^{-3} particles per cell. In contrast, normal human cells were not killed at any concentration of SVV. Further, as summarized in Tables 1, 1A-3, SVV was also cytotoxic toward a number of other tumor cell lines, including SCLC-multidrug resistant tumor cells, and some fetal cells and cell lines. The EC_{50} values for SVV cytotoxicity for the other tumor cell lines ranged from 10^{-3} to greater than 20,000 particles per cell. SVV was non-cytotoxic against a variety other non-neural tumors and normal human tissues. Additionally, SVV was not cytotoxic to primary human hepatocytes, as measured by LDH release at up to 1000 particles per cell (*see* Figure 48).

Example 12

Biodistribution and Pharmacokinetic Study in Rodents

[0345] Pharmacokinetic and biodistribution study of SVV is performed in normal mice and immunocompromised athymic nude mice bearing H446 SCLC tumors. This study evaluates the biodistribution, elimination and persistence of SVV following a single intravenous administration to both normal and immunocompromised tumor-bearing mice. Groups of mice each receive a single i.v. dose of control buffer or one of three doses of SVV (10^8 , 10^{10} , or 10^{12} vp/kg) and are monitored for clinical signs. Blood samples are obtained from groups of 5 mice at 1, 6, 24 and 48 hours post dose, and at 1, 2, 4, and 12 weeks post dose. Dose levels include a known low efficacious dose and two higher dose levels to determine linearity of virus elimination. Groups of mice are sacrificed at 24 hours, and 2, 4 and 12 weeks post dose. Selected tissues, including liver, heart, lung, spleen, kidney, lymph nodes, bone marrow, brain and spinal cord tissues are aseptically collected and tested for the presence of SVV RNA using a validated RT-PCR assay.

[0346] Samples of urine and feces are obtained at sacrifice, at 24 hours, and at 2, 4 and 12 weeks post dose and are examined for the presence of infectious virus. The design of the experiments in this Example are shown in Table 9 below:

Table 9: Biodistribution of SVV in CD-1 Mice and Athymic Nude Mice Bearing SCLC Tumors

Group	Treatment	Dose Level (vp/kg)	Route	# of Mice/Timepoint for Blood Sampling	# of Mice/Timepoint for PCR Tissue Distribution
Normal CD-1 Mice					
1	Saline	0	i.v.	5	5
2	SVV	10^8	i.v.	5	5
3	SVV	10^{10}	i.v.	5	5
4	SVV	10^{12}	i.v.	5	5
Athymic Tumor Bearing Mice					
5	Saline	0	i.v.	5	5
6	SVV	10^8	i.v.	5	5
7	SVV	10^{10}	i.v.	5	5
8	SVV	10^{12}	i.v.	5	5

[0347] Acute i.v. toxicology studies were also performed in both normal and immunocompromised athymic nude mice bearing H446 SCLC tumors. Preliminary i.v. studies in normal and SCLC tumor bearing mice indicate safety of SVV at doses up to 10^{14} vp/kg. No adverse clinical signs were observed and there was no loss of body weight up to 2 weeks following a single i.v. dose of 10^{14} vp/kg.

Example 13

Viral Transmission Study in Normal Adult and Pregnant Mice

[0348] The purpose of this Example is to determine if SVV is transmissible following cohabitation of noninfected normal mice with mice injected with a high concentration of SVV. Because SVV does not replicate in normal, non-tumor bearing mice, tumor bearing mice can also be injected with high concentrations of SVV and subsequently exposed to normal, healthy animals to better simulate the clinical scenario. A secondary purpose is to assess the potential transmissibility of SVV from an infected female to an uninfected pregnant DAM, and subsequently to the developing fetus.

[0349] Three groups of five naive male and female CD-1 mice are exposed to a single mouse of the same sex infected with either 10^8 , 10^{10} or 10^{12} vp/kg, and are monitored for the presence of SVV by blood sampling.

[0350] Similarly, an SVV exposed female is co-mingled with a number of timed pregnant females, and the ability of the virus to transmit from the infected female to an uninfected pregnant female, and subsequently to the developing fetus is determined.

Example 14

Non-Human Primate Studies

[0351] The safety, toxicity and toxicokinetics of SVV are also determined in non-human primates. In a dose range-finding phase, individual monkeys receive a single i.v. dose of SVV at 10^8 vp/kg and are closely monitored for clinical signs of infection or toxicity. If this dose is well tolerated, additional animals are treated with a higher i.v. dose until a dose of 10^{12} vp/kg is achieved. Subsequently, the main study consists of groups of three male and female monkeys, and each monkey is dosed once weekly for six weeks with either vehicle alone or one of three doses of SVV and monitored for signs of toxicity. An additional two monkeys per sex are dosed with the vehicle alone and with the high dose level of SVV for six weeks, and are allowed an additional four weeks recovery prior to sacrifice.

[0352] Blood samples are obtained following dosing during week 1 and week 6. Clinical pathological and hematology blood samples are obtained prior to the initial dose and prior to sacrifice. Additional blood samples are obtained following each dose for assessing the presence of neutralizing antibodies to SVV.

[0353] Surviving monkeys are euthanized and subjected to a full gross necropsy and a full tissue list is collected from the main study and recovery monkeys. Tissues from the control and high dose groups are evaluated histopathologically. Urine and fecal samples are collected following dosing on weeks 1 and 6 and are evaluated for presence of infectious SVV. The overall design of this Example is shown in Table 10 below.

Table10: Multiple Dose Toxicology Study of SVV in Primates

Dose Range-finding Phase							
Group	Treatment	Dose (vp/kg)	Route	Males		Females	
1	SVV	10^8	IV	1		1	
2	SVV	10^{10*}	IV	1		1	
3	SVV	10^{12*}	IV	1		1	
Main Phase				Main Phase		Recovery	
Group	Treatment	Dose (vp/kg)	Route	Male	Female	Male	Female
1	Control	-	IV	3	3	2	2
2	SVV	10^{8*}	IV	3	3	-	-
3	SVV	10^{10*}	IV	3	3	-	-
4	SVV	10^{12*}	IV	3	3	2	2

*Doses can vary based on results of Dose Range-finding phase

Example 15

Construction of an Infectious Full-Length and Functional Genomic SVV Plasmid

[0354] With SEQ ID NO:1, only about 1.5-2 Kb of the 5' genomic sequence of SVV remains to be sequenced, representing the nucleotide region covering the 5' UTR, 1A (VP4) and part of 1B (VP2). To clone the 5' end missing in SEQ ID NO:1, polymerases that function at high temperatures and reagents that can enable a polymerase to read through secondary structures were used. Additional SVV cDNAs were prepared from isolated SVV of ATCC deposit number PTA-5343. SVV particles were infected into a permissive cell line, such as PER.C6, and viruses are isolated. Viral RNA was then recovered from the virus particles such that cDNA copies are made therefrom. Individual cDNA clones were sequenced, such that selected cDNA clones are combined into one full-length clone in a plasmid having a T7 promoter upstream of the 5' end of the SVV sequence. The full-length genomic sequence of SVV is listed in Figures 83A-83H and SEQ ID NO:168. The full-length SVV from this plasmid is reverse-transcribed, by utilizing T7 polymerase and an *in vitro* transcription system, in order to generate full-length RNA (see Figure 55). The full-length RNA is then transfected into permissive cell lines to test the infectivity of the full-length clone (see Figure 55).

[0355] The methodology was as follows. *RNA Isolation*: SVV genomic RNA was extracted using guanidium thiocyanate and a phenol extraction method using Trizol (Invitrogen). Briefly, 250 μ l of the purified SVV ($\sim 3 \times 10^{12}$ virus particles) was mixed with 3

volumes of Trizol and 240 µl of chloroform. The aqueous phase containing RNA was precipitated with 600 µl isopropanol. The RNA pellet was washed twice with 70% ethanol, dried and dissolved in sterile DEPC-treated water. The quantity of RNA extracted can be estimated by optical density measurements at 260 nm. An aliquot of RNA can be resolved through a 1.25% denaturing agarose gel (Cambrex Bio Sciences Rockland Inc., Rockland, ME USA) and the band visualized by ethidium bromide staining and photographed.

[0356] *cDNA synthesis:* cDNA of the SVV genome was synthesized by RT-PCR. Synthesis of cDNA was performed under standard conditions using 1 µg of RNA, AMV reverse transcriptase, and oligo-dT primers. Random 14-mer oligonucleotide can also be used. Fragments of the cDNA were amplified and cloned into the plasmid pGEM-3Z (Promega) and the clones were sequenced. The sequence at the 5' end of the viral genome was cloned by RACE and the sequence determined. Sequence data was compiled to generate the complete genome sequence of SVV.

[0357] *Cloning of full length genome:* Three cDNA fragments representing the full-length SVV genome were amplified by three PCR reactions employing six sets of SVV-specific primers. Turbo pfu polymerase (Stratagene) was used in PCR reactions. First, a fragment representing the 5' end of SVV genome was amplified with primers 5'SVV-A (SEQ ID NO:219) and SVV1029RT-RI (SEQ ID NO:220) and the resulting fragment was cut with ApaI and EcoRI and gel purified. The gel purified fragment was ligated to Nde-ApaT7SVV (SEQ ID NO:221), an annealed oligo duplex containing engineered NdeI site at 5' end, T7 core promoter sequence in the middle and first 17 nucleotides of SVV with ready to use ApaI site at 3' end and cloned into Nde I and Eco RI sites of pGEM-3Z (Promega) by three-way ligation to generate pNTX-03. Second, a fragment representing 3' end of viral genome was amplified by PCR with primers SVV6056 (SEQ ID NO:222) and SVV7309NsiB (SEQ ID NO:223). The antisense primer, SVV7309NsiB was used to introduced poly(A) tail of 30 nucleotides in length and Nsi I recognition sequence at 3' end to clone into PstI site of pGEM-3Z plasmid. The resulting PCR product was digested with BamHI and gel purified. A fragment covering the internal part of the viral genome was amplified with primers SVV911L (SEQ ID NO:224) and SVV6157R (SEQ ID NO:225). The resulting PCR product was cut with EcoRI and BamHI and gel purified. The two gel purified fragments representing the middle and 3' end of SVV genome were cloned into EcoRI and SmaI sites of pGEM-4Z by three-way ligation to generate pNTX-02. To generate full-length SVV cDNA, pNTX-02 was

digested with EcoRI and NsiI and the resulting 6.3 kb fragment was gel purified cloned into EcoRI and PstI sites of pNTX-03. The resulting full-length plasmid was called pNTX-04.

[0358] The full-length plasmid pNTX-04 was further modified at both 5' and 3' ends to facilitate *in vitro* transcription and rescuing of the virus following RNA transfection into PER.C6 cells. First, a SmaI restriction enzyme site was inserted immediately downstream of the poly(A) tail to liberate the 3' end of SVV-cDNA from the plasmid backbone prior to *in vitro* transcription. A PCR approach was used to insert the site utilizing a primer pair of SVV6056 (SEQ ID NO:222) and SVVSmaRev (SEQ ID NO:226) and pNTX-04 as template. The antisense primer SVV3SmaRev contained 58 nucleotides representing the 3' end of the SVV sequence and recognition sequences for SmaI and SphI restriction enzyme sites. The resulting PCR fragment was digested with BamHI and SphI and used to replace the corresponding fragment from pNTX-04 to generate pNTX-06. Second, an extra four nucleotides present between the T7 promoter transcription start site and 5' end of SVV cDNA in pNTX-06 were removed using annealed oligo duplex approach. The duplex nucleotides were engineered to contain KpnI recognition site, T7 core promoter sequence and the first 17 nucleotides of SVV with a ready to use ApaI site at the 3' end (SEQ ID NO:227). The annealed oligos were used to replace the corresponding portion of pNTX-06 using KpnI and ApaI sites to generate pNTX-07. Finally, a two base pair deletion noticed in the polymerase encoding region of pNTX-07 was restored by replacing BamHI and SphI fragment with a corresponding fragment amplified from SVV cDNA by PCR to generate pNTX-09.

[0359] *In vitro transcription:* Infectivity of *in vitro* transcribed RNA was tested by first digesting pNTX-09 with SmaI to liberate 3' end of SVV sequence from plasmid backbone. The linearized plasmid was subjected to *in vitro* transcription using T7 polymerase (Promega).

[0360] *Transfection of in vitro transcribed RNA into PER.C6 cells:* One day prior to transfection, PER.C6 cells were plated in 6-well tissue-culture dishes. On the next day, Lipofetamine reagent (Invitrogen) was used to transfect *in vitro* transcribed RNA (1.5 µg) into the cells following the recommendations of the supplier. Cytopathic effect (CPE) due to virus production was noticed within 36 hour post-transfection. The transfected cells were subjected to three cycles of freeze-thaw and the viruses in lysate were further confirmed by infecting PER.C6 cells. Thus, the full-length SVV cDNA clone proved to be infectious.

[0361] As described above, the plasmid with the full-length genome of SVV can be reverse-transcribed following standard protocols. The viral RNA (100 ng) can be used to transfect any cell line known to be permissive for the native SVV, but the most efficient cell line for viral RNA transfection can be empirically determined among a variety of cell lines.

Example 16

Construction of an RGD-Displaying SVV Library

[0362] To find the optimal insertion position for the construction of SVV capsid mutants generated with random with oligonucleotides encoding random peptide sequences, a simple model system (RGD) is employed. RGD (arginine, glycine, aspartic acid) is a short peptide ligand that binds to integrins. A successful RGD-SVV derivative should contain the following characteristics: (1) the genetic insertion should not alter any of SVV's unique and desirable properties; and (2) a successful RGD derivative virus should have tropism toward $\alpha_v\beta_5$ integrin containing cells.

[0363] A SVV plasmid containing just the contiguous capsid region will be singly cleaved at random positions and a short model peptide sequence, referred to as RGD, will be inserted at each position. The virus SVV-RGD library will be constructed from this plasmid library utilizing the general technology described in Figures 56 and 57.

[0364] Random insertion of the cRGD oligonucleotide into the capsid region is conducted. In brief, a plasmid is constructed that just encodes the contiguous 2.1 Kb capsid region of SVV (*see* Figure 56, "pSVVcapsid"). A single random cleavage is made in pSVVcapsid by partially digesting the plasmid utilizing either CviJI or an endonuclease V method as described below (*see* Figure 57). After isolating the single cleaved plasmid the RGD oligonucleotide will be inserted to create a pSVVcapsid-RGD library.

[0365] The restriction enzyme CviJI has several advantages over other random cleavage methods such as sonication or shearing. First, as CviJI is a blunt ended cutter no repair is necessary. Second, CviJI has been demonstrated to cleave at random locations such that no hot spots will occur. The procedure is also simple and rapid. Briefly, the concentration of CviJI and/or time of digestion are increasingly lowered until the majority of cleaved DNA is a linearized plasmid, *i.e.* a single cleavage. This can be observed by standard agarose gel electrophoresis as depicted in Figure 57. The band is then isolated, purified and ligated with the RGD oligo.

[0366] Another method that may be utilized to randomly cleave DNA is the endonuclease V method (Kiyazaki, K., Nucleic Acids Res., 2002, 30(24): e139). Endonuclease V nicks uracil-containing DNA at the second or third phosphodiester bond 3' to uracil sites. This method is also expected to randomly cleave DNA, the frequency is simply determined by adjusting the concentration of dUTP in the polymerase chain reaction. Although the cleavage sites are always two or three bases downstream of a thymidine (substituted by uracil) site, this method is expected to produce much fewer hot and cold spots than other methodologies.

[0367] The randomly linearized plasmids are ligated with the cRGD oligonucleotides. The resultant pSVV capsid library is then amplified, such that a population of polynucleotides encoding the capsid region with randomly inserted cRGD regions can be purified (*see* Figures 57 and 58). The population of capsid polynucleotides is then subcloned into a vector containing the full-length SVV sequence minus the capsid region, such that a library of full-length SVV sequences are generated (where the library manifests sequence diversity in the capsid region as the cRGD sequence is randomly inserted). This library is then reverse transcribed into RNA, and the RNA is transfected into a permissive cell line to generate a population of SVV particles having different capsids (*see* Figure 59). Once this RGD-SVV population of virus particles is recovered ("RGD-SVV library"), a number of viruses (*i.e.*, 10 or more) will be randomly picked for sequencing to confirm the insertion of the RGD sequence and diversity of insertion site.

[0368] *In vitro* selection of the RGD-displaying SVV library. The SVV-RGD library is screened to determine which insertion site enabled an expanded tropism of SVV. The RGD-SVV library is allowed to infect $\alpha_v\beta_5$ integrin-expressing NSCLC lines (non-small cell lung cancer cell lines, *i.e.*, A549 expressing $\alpha_v\beta_5$). Only those SVV derivatives that contain a functional and properly displayed RGD motif can infect these cells and replicate.

[0369] *In vitro* screening is carried out by a high throughput automation system (TECAN) that is capable of liquid handling, concurrent incubation of 20 cell lines and measurement in 384-well plates (*see* Figure 62 and Figure 63). The cells are harvested 30 hr after infection when complete CPE is noticed and then cells are collected by centrifugation at 1500 rpm for 10 minutes at 4°C. The cell pellets are then resuspended in the cell culture supernatant and subjected to three cycles of freeze and thaw. The resulting suspension is clarified by centrifugation at 1500 rpm for 10 minutes at 4°C. Virus is purified by two rounds

of CsCl gradients: a one-step gradient (density of CsCl 1.24 g/ml and 1.4 g/ml) followed by one continuous gradient centrifugation (density of CsCl 1.33 g/ml). The purified virus concentration is determined spectrophotometrically, assuming $1A_{260} = 9.5 \times 10^{12}$ particles (Scraba, D.G. and Palmenberg, A.C., 1999). The process may be repeated multiple times until a sufficient amount of virus is recovered from $\alpha_V\beta_5$ cells.

[0370] *Analysis of recovered RGD-SVV derivatives.* A small pool of individual RGD-displaying SVV derivatives (about 10-50 different derivataives) are analyzed. The viral mixture is diluted and single viral particles are expanded for analysis. Each derivative is tested to determine whether they have gained the ability to infect $\alpha_V\beta_5$ -expressing cells efficiently and specifically. The capsid region of each derivative with this property is then be sequenced to determine the site of RGD insertion. The recovered cRGD-displaying SVV derivatives should possess the following properties: (1) the original properties of the virus are still intact; and (2) the derivatives have gained the ability to infect cells that express high levels of integrins that bind to RGD. This approach aims to identify one or more sites that enable an expanded tropism with RGD insertion, such that random oligonucleotides can be inserted into these sites to generate SVV derivatives with altered tropism.

[0371] The sequenced cRGD-SVV derivatives are numbered and ranked by their binding abilities to integrin. To test the binding activity, recombinant β_2 integrin is immobilized on a 96-well microtiter plate in PBS, washed twice with PBS, blocked with 3% BSA in PBS, and then incubated with a unique RGD-displaying virus. The native virus without peptide insertions is used as a negative control. After 1-5 hr of incubation, the wells are washed at least three times with PBS. Then, the viruses that are bound to the plate will be detected by anti-SVV antibodies. RGD peptide or antibodies against integrin should be able to compete with the binding of the RGD-SVV derivatives to the integrin-bound plate.

[0372] The cRGD-SVV derivatives (20) that have the strongest binding to integrin are analyzed to determine the 'successful' location(s) of cRGD oligonucleotide insertion. The insertion sites provide insights into the tropism of SVV. Based on the analysis of the insertion sites and other known structures, an ideal location to place a random peptide library can be determined (this method is an alternative method for generating SVV derivatives, where oligonucleotides (known sequence or random sequence) are inserted into random locations in the capsid). SVV derivatives generated with random sequence oligonucleotides are constructed in essentially the same manner as described above for the RGD-SVV library,

except for two additional and novel methodologies. To avoid unwanted stop codons and deleterious amino acid insertions (e.g. cysteines or prolines) within a desired coding region, TRIM (trinucleotide-mutagenesis) technology developed by Morphosys (Munich, Germany) can be used to generate random oligonucleotides for capsid insertion. TRIM utilizes trinucleotides which only code for amino acids at the desired position (Virnekas, B. *et al.*, Nucleic Acids Res, 1994, 22(25): 5600-5607). The random-peptide displaying SVV with a diversity of 10^8 is believed to be sufficient and expected to yield peptides that specifically direct the virus to targeted tumor tissues. Random-peptide displaying SVV is tested *in vitro* as described above, or *in vivo* using tumor-bearing mice.

Example 17: Serum Studies

[0373] Pigs are a permissive host for the USDA virus isolates identified above. The isolate MN 88-36695 was inoculated into a gnotobiotic pig and antisera generated (GP102). The antisera binds to all of the other USDA isolates listed above and to SVV. The antisera does not react with 24 common porcine virus pathogens indicating its specificity. Porcine sera was also tested for neutralizing antibodies to 1278 (Plum Island virus). Sera were collected in the US and 8/29 sera were positive with titers ranging from 1:57 to 1:36,500.

[0374] To test whether the pig is the natural source for SVV, serum samples from various animals were obtained and tested for their ability to act as neutralizing antibodies against SVV infection of permissive cells. The Serum Neutralization Assay is conducted as follows: (1) Dilute various serums 1:2 and 1:4 and serially in increasing dilutions if necessary; (2) Mix with 100 TCID₅₀ of virus (SVV; but any virus can be tested to determine whether a serum can neutralize its infection); (3) Incubate at 37°C for 1 hour; (4) Add the mixture to 1×10^4 PER.C6[®] cells (or other permissive cell type); (5) Incubate at 37°C for 3 days; and (6) Measure CPE using a tetrazolium based dye cytotoxicity (such as MTS) assay. The neutralization titer is defined as the highest dilution of sera that neutralizes SVV (or other virus in question) at 100%.

[0375] The serum neutralization results showed that there is a minimal or no presence of neutralizing antibodies in human and primate populations. In one experiment, 0/22 human sera contained neutralizing antibodies to SVV. In another experiment, only 1/28 human sera contained neutralizing antibodies. In a third experiment, 0/50 human sera from Amish farmers were neutralizing. In another experiment, 0/52 primate sera from four species were neutralizing.

[0376] The serum neutralization results showed that there is a prevalence of SVV neutralizing antibodies in farm animal populations. In one experiment, 27/71 porcine sera from farms were neutralizing. In another experiment, 4/30 porcine sera from a disease-free farm were neutralizing. In another experiment, 10/50 bovine sera were neutralizing. In yet another experiment, 5/35 wild mouse sera were neutralizing.

[0377] Antisera to MN 88-36694 were tested in serum neutralization assays on SVV (see Example 2). Anti-MN 88-36695 gnotobiotic pig serum was able to neutralize infection by SVV (neutralization titer on infection was 1:100 for SVV). As stated above, the antisera binds to all of the other USDA isolates and to SVV, indicating that the herein disclosed USDA isolates are SVV-like picornaviruses due to their serological cross-reactivity with the gnotobiotic pig serum as measured in an indirect immunofluorescence assay.

[0378] These data indicate that SVV is genetically and serologically linked to the porcine USDA virus isolates.

Example 18: SVV and SVV-like picornaviruses

[0379] The grouping of the following isolates: MN 88-36695, NC 88-23626, IA 89-47552, NJ 90-10324, IL 92-48963, CA 131395; LA 1278; IL 66289; IL 94-9356; MN/GA 99-29256; MN 99197; and SC 363649, was deduced in part from indirect immunofluorescence experiments. Antisera GP102 was raised against isolate MN 88-36695 by inoculation of the virus into a gnotobiotic pig. The antisera binds to all twelve isolates demonstrating that they are serologically related to one another.

[0380] The GP102 antisera was tested in a neutralization assay with SVV. In this assay, serial dilutions of antisera are mixed with a known quantity of SVV (100 TCID₅₀). The mixtures are placed at 37°C for 1 hour. An aliquot of the mixture is then added to 1x10⁴ PER.C6[®] cells, or another cell line that is also permissive for SVV, and the mixtures are placed at 37°C for 3 days. The wells are then checked for a cytopathic effect of the virus (CPE). If the serum contains neutralizing antibodies, it would neutralize the virus and inhibit the infection of the PER.C6[®] cells by the virus. CPE is measured quantitatively by using a tetrazolium based dye reagent that changes absorbance based on the number of live cells present. The results are expressed as the percent of viable cells of an uninfected control vs. the log dilution of serum, and are shown in Figure 93. This data indicates that SVV is serologically linked to the porcine USDA virus isolates.

[0381] Additionally, the viral lysate of MN 88-36695 was tested in cytotoxicity assays with four different cell lines and the results are shown in Table 4. The permissivity profile is identical to that of SVV in that NCI-H446 and HEK293 are permissive for SVV, and NCI-H460 and S8 are not. Additionally, MN 88-36695, like SVV, was cytotoxic to PER.C6[®] cells. Further, polyclonal antisera to SVV raised in mice was used in a neutralization assay along with MN 88-36695 virus. The results are shown in Figure 94. The anti-SVV antisera neutralized MN 88-36695, further linking SVV to the USDA viruses serologically.

Table 11: MN 88-36695 Cytotoxicity Results

Cell Line	TCID ₅₀ (pfu/ml)	Result
NCI-H446	1.6 x 10 ⁻⁶	Permissive
HEK293	1.3 x 10 ⁻²	Permissive
NCI-H460	0	Nonpermissive
S8	0	Nonpermissive

[0382] Partial genomic sequence analysis of several of the USDA isolates revealed that they are all very closely related to SVV (see Figures 87-89 for sequence alignments). Table 12 shows the percent sequence identity between SVV and six of the isolates. It was found that about 95-98% identity exists at the nucleotide (nt) level over 460 nt of the 3' end of the genome encoding 3D^{pol} and the 3'UTR (Fig. 89). Each of the USDA viruses is unique and is about 95-98% identical to SVV at the nucleotide level.

Table 12: Percent Sequence Identity Between SVV and Six USDA Isolates

1	2	3	4	5	6	7		Virus Name
	96.5	99.1	97.2	97.0	97.4	97.0	1	NJ 90-10324
		97.0	95.7	94.8	95.0	98.3*	2	CA 13195
			97.6	97.2	97.6	97.2	3	IA 89-47752
				95.4	96.1	96.3	4	IL 92-48963
					98.9	95.2	5	MN 88-36695
						95.4	6	NC 88-23626
							7	SVV-001 (SVV)

[0383] Further sequencing of parts of the P1 (Fig. 87) and 2C (Fig. 88) genes of two of the isolates has confirmed this close relationship with SVV. The USDA isolates are more highly related to SVV than any other known viruses, including members of the genus

Cardiovirus. Sequences from several regions of seven of the USDA viruses were compared with SVV and neighbor-joining trees were constructed (Figure 95A and 95B). These trees further confirm the high degree of relation between the viruses, and identifying CA 131395 as SVV's current closest relative.

DEMANDES OU BREVETS VOLUMINEUX

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JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
THAN ONE VOLUME.**

THIS IS VOLUME __1__ OF __2__

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WHAT IS CLAIMED:

1. An isolated nucleic acid comprising a nucleic acid sequence having at least 75% sequence identity to: (i) SEQ ID NO: 168, or (ii) a contiguous portion of SEQ ID NO: 168 that is at least 20 nucleotides in length.
2. The isolated nucleic acid of claim 1, wherein the nucleic acid is RNA or DNA.
3. An isolated polypeptide comprising an amino acid sequence having at least 75% sequence identity to: (i) SEQ ID NO: 169, or (ii) a contiguous portion of SEQ ID NO: 169 that is at least 10 amino acids in length.
4. An isolated Seneca Valley virus or derivative or relative thereof, having a genome comprising a sequence that is at least 95%, 90%, 85%, 80%, 75%, 70%, or 65% identical to SEQ ID NO:168.
5. The virus of claim 4 comprising the following characteristics: replication competence in tumor cells, tumor-cell tropism, and lack of cytolysis in normal cells.
6. The virus of claim 5, wherein said virus is replication competent in tumor cell types having neuroendocrine properties.
7. A pharmaceutical composition comprising an effective amount of the virus of any one of claims 4-6 and a pharmaceutically acceptable carrier.
8. An isolated antibody that specifically binds to the polypeptide of claim 3 or to any epitope of the isolated virus of claim 4 or 5.
9. The use of a virus with a genome that comprises a sequence that is at least 75% identical to a contiguous sequence of SEQ ID NO:168 that is at least 100 nucleotides for the treatment of cancer.
10. The method of claim 9, wherein the virus is a picornavirus.
11. The method of claim 10, wherein the picornavirus is a cardiovirus.
12. The method of claim 11, wherein the cardiovirus is selected from the group consisting of: vilyuisk human encephalomyelitis virus, Theiler's murine encephalomyelitis virus, and encephalomyocarditis virus.

13. The method of claim 12, wherein the picornavirus is a member of a genus to which Seneca Valley virus belongs.
14. The method of claim 10, wherein the picornavirus is Seneca Valley virus.
15. The method of claim 10, wherein the picornavirus is a Seneca Valley virus-like picornavirus.
16. The method of claim 15, wherein the Seneca Valley virus-like picornavirus is selected from the group of isolates consisting of: MN 88-36695, NC 88-23626, IA 89-47552, NJ 90-10324, IL 92-48963, CA 131395, LA 1278, IL 66289, IL 94-9356, MN/GA 99-29256, MN 99197, and SC 363649.
17. A method of killing an abnormally proliferative cell comprising contacting the cell with the virus of any one of claims 4-6.
18. A method for making an oncolytic virus, the method comprising:
 - (a) comparing a Seneca Valley virus genomic sequence with a test virus genomic sequence, wherein the Seneca Valley virus genome comprises a sequence that is at least 95% identical to SEQ ID NO:168;
 - (b) identifying at least a first amino acid difference between a polypeptide encoded by the Seneca Valley virus genomic sequence and a polypeptide encoded by the test virus genomic sequence;
 - (c) mutating the test virus genomic sequence such that the polypeptide encoded by the test virus genomic sequence has at least one less amino acid difference to the polypeptide encoded by the Seneca Valley virus genomic sequence;
 - (d) transfecting the mutated test virus genomic sequence into a tumor cell; and
 - (e) determining whether the tumor cell is lytically infected by the mutated test virus genomic sequence.
19. The method of claim 18, wherein the test virus is a picornavirus.
20. The method of claim 19, wherein the test virus is a Seneca Valley virus-like picornavirus.
21. The method of claim 18, wherein the amino acid differences are between a Seneca Valley virus capsid protein and a test virus capsid protein.

22. The method of claim 18, wherein mutating the test virus genomic sequence comprises mutating a cDNA having the test virus genomic sequence.
23. The method of claim 18, wherein transfecting the mutated test virus genomic sequence comprises transfecting RNA, wherein the RNA is generated from the cDNA having the mutated test virus genomic sequence.
24. A method for making a mutant virus having an altered cell-type tropism, the method comprising:
- (a) creating a library of viral mutants comprising a plurality of nucleic acid sequences, wherein the library of viral mutants is created from a parental sequence comprising a nucleic acid sequence that is at least 75% identical to a contiguous portion of SEQ ID NO:168 that is at least 100 nucleotides in length;
 - (b) transfecting the library of viral mutants into a permissive cell, such that a plurality mutant viruses are produced;
 - (c) isolating the plurality of mutant viruses;
 - (d) incubating a non-permissive cell with the isolated plurality of mutant viruses; and
 - (e) recovering a mutant virus that was produced in the non-permissive cell, thereby making a mutant virus having an altered tropism.
25. The method of claim 24, further comprising:
- (f) incubating the recovered mutant virus in the non-permissive cell; and
 - (g) recovering a mutant virus that that was produced in the non-permissive cell.
26. The method of claim 25, further comprising iteratively repeating steps (f) and (g).
27. The method of claim 25, wherein the incubating is conducted in a multi-well high-throughput platform wherein the platform comprises a different non-permissive cell-type in each well.
28. The method of claim 27, further comprising screening the platform to identify which wells contain a mutant virus that kills the cells.
29. The method of claim 28, wherein the screening is conducted by analyzing light absorbance in each well.
30. The method of claim 24, wherein the non-permissive cell is a tumor cell.

31. The method of claim 24, wherein creating the library of viral mutants comprises:
- (i) providing a polynucleotide having a sequence identical to a portion of the parental sequence;
 - (ii) mutating the polynucleotide in order to generate a plurality of different mutant polynucleotide sequences; and
 - (iii) ligating the plurality of mutated polynucleotides into a vector having the genomic sequence of the virus except for the portion of the genomic sequence of the virus that the polynucleotide in step (i) contains, thereby creating the library of viral mutants.
32. The method of claim 31, wherein in step (i), the genomic sequence of a virus is from a picornavirus.
33. The method of claim 32, wherein the picornavirus is a Seneca Valley virus-like picornavirus.
34. The method of claim 31, wherein the mutating of step (ii) is conducted by random insertion of nucleotides into the polynucleotide.
35. The method of claim 34, wherein the mutating of step (ii) is conducted in a capsid encoding region of the polynucleotide.
36. The method of claim 34, wherein the random insertion of nucleotides is conducted by trinucleotide-mutagenesis (TRIM).
37. The method of claim 36, wherein at least a portion of the nucleotides inserted into the polynucleotide encodes an epitope tag.

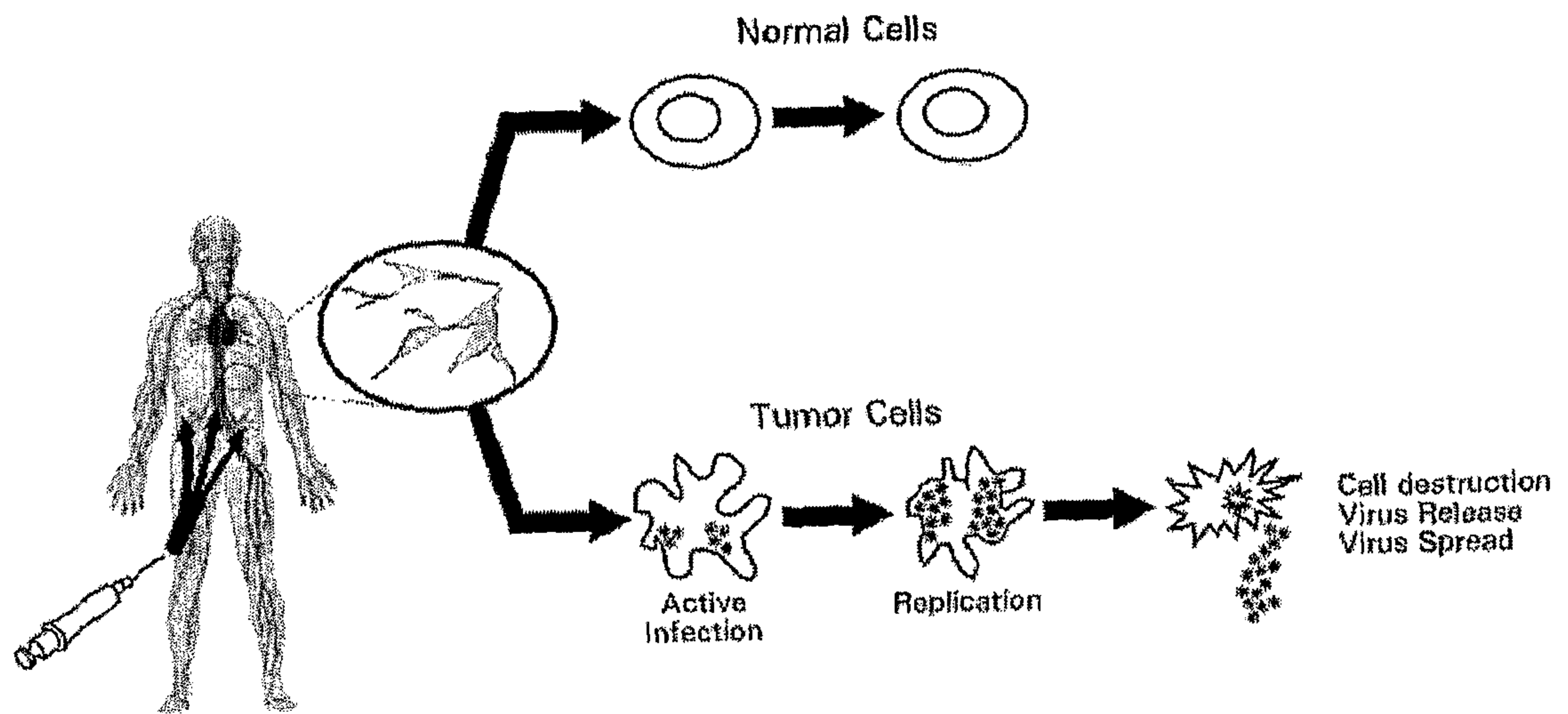
**FIG. 1**



FIG. 2

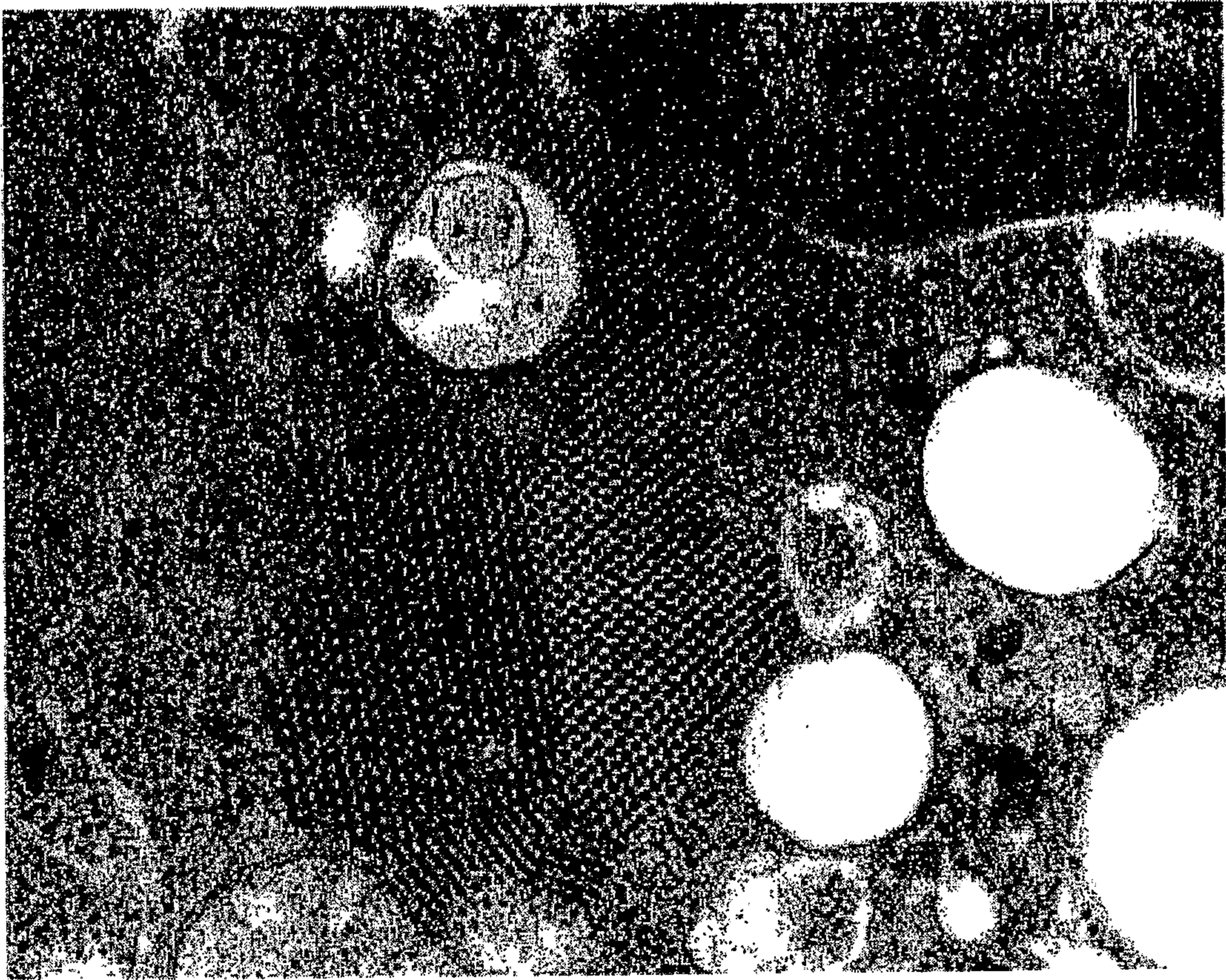


FIG. 3

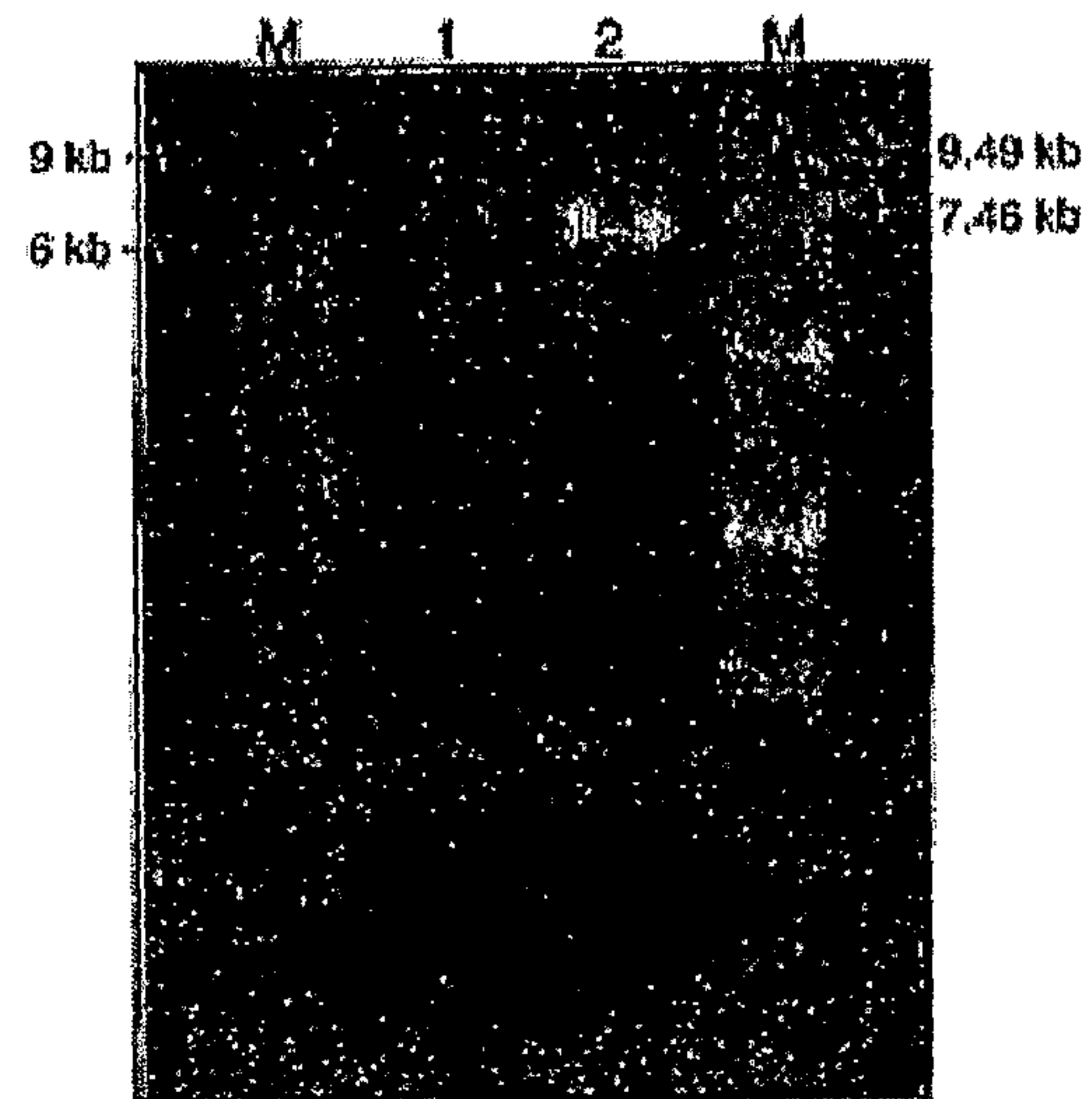


FIG. 4A

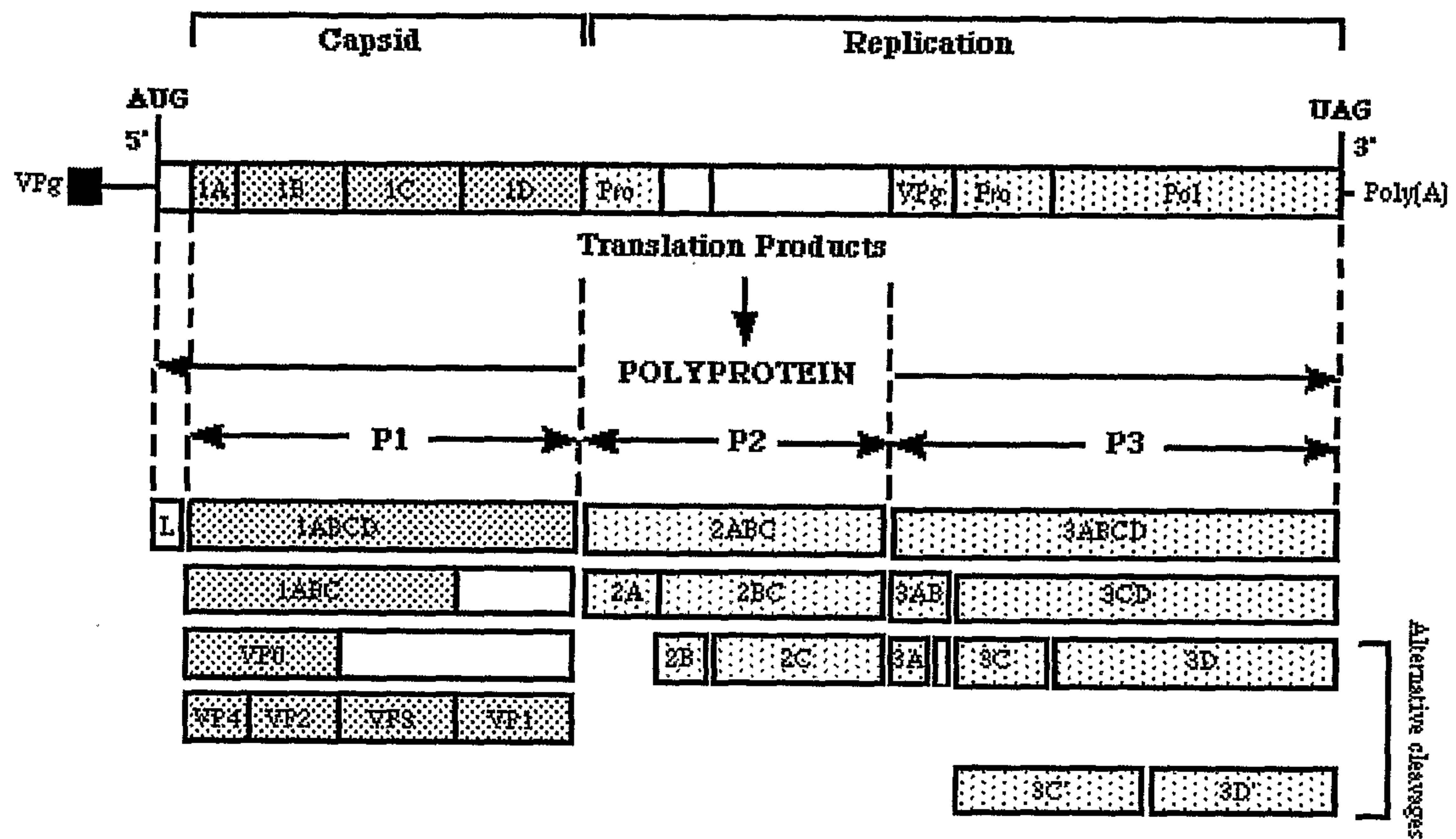


FIG. 4B

10 20 30 40 50 60 70 80 90
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
NNTCTAGCCCACCATGGCAACAAGAAGAGCTTACAGGAGCTGAATGAAGAACAGTGGGTGGAAATGTCTGACGATTACCGGACCGGGAAA
X L A H H G N K K S L Q E L N E E Q W V E M S D D Y R T G K

100 110 120 130 140 150 160 170 180
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AACATGCCTTTTCAGTCTCTTGGCACATACTATCGGCCCCCTAACTGGACTTGGGGTCCCAATTTTCATCAACCCCTATCAAGTAACGGTT
N M P F Q S L G T Y Y R P P N W T W G P N F I N P Y Q V T V

190 200 210 220 230 240 250 260 270
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTCCACACCAAATTCCTGAACGCGAGAACCTCTACCTCGGTAGACATAAACGTCCCATACATCGGGGAGACCCCCACGCAATCCTCAGAG
F P H Q I L N A R T S T S V D I N V P Y I G E T P T Q S S E

280 290 300 310 320 330 340 350 360
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ACACAGAACTCCTGGACCCTCCTCGTTATGGTGCTCGTTCCCCCTAGACTATAAGGAAGGAGCCACAACCTGACCCAGAAATTACATTTTCT
T Q N S W T L L V M V L V P L D Y K E G A T T D P E I T F S

370 380 390 400 410 420 430 440 450
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GTAAGGCCTACAAGTCCCTACTTCAATGGGCTTCGCAACCGCTACACGGCCGGGACGGACGAAGAACAGGGGGCCCATTCCTACGGCACCC
V R P T S P Y F N G L R N R Y T A G T D E E Q G P I P T A P

460 470 480 490 500 510 520 530 540
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AGAGAAAATTCGCTTATGTTTCTCTCAACCCCTCCCTGACGACACTGTCCCTGCTTACGGGAATGTGCGTACCCCTCCTGTCAATTACCTC
R E N S L M F L S T L P D D T V P A Y G N V R T P P V N Y L

550 560 570 580 590 600 610 620 630
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CCTGGTGAAATAACCGACCTTTTGCAACTGGCCCGCATACCCACTCTCATGGCATTGAGCGGGTGCCTGAACCCGTGCCTGCCTCAGAC
P G E I T D L L Q L A R I P T L M A F E R V P E P V P A S D

640 650 660 670 680 690 700 710 720
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ACATATGTGCCCTACGTTGCCGTTCCCACCCAGTTCGATGACAGGCCTCTCATCTCCTTCCCGATCACCCCTTTCAGATCCCGTCTATCAG
T Y V P Y V A V P T Q F D D R P L I S F P I T L S D P V Y Q

730 740 750 760 770 780 790 800 810
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AACACCCTGGTTGGCGCCATCAGTTCAAATTCGCCAATTACCGTGGGTGTATCCAAATCACTCTGACATTTTGTGGACCCATGATGGCG
N T L V G A I S S N F A N Y R G C I Q I T L T F C G P M M A

820 830 840 850 860 870 880 890 900
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AGAGGGAAATTCCTGCTCTCGTATTCTCCCCAAATGGAACGCAACCACAGACTCTTCCGAAGCTATGCAGTGCACATACTCTATTTGG
R G K F L L S Y S P P N G T Q P Q T L S E A M Q C T Y S I W

910 920 930 940 950 960 970 980 990
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GACATAGGCTTGAACCTAGTTGGACCTTCGTCGTCCCCTACATCTCGCCAGTGACTACCGTGAAACTCGAGCCATTACCAACTCGGTT
D I G L N S S W T F V V P Y I S P S D Y R E T R A I T N S V

FIG. 5A

1000 1010 1020 1030 1040 1050 1060 1070 1080
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TACTCCGCTGATGGTTGGTTAGCCTGCACAAGTTGACCAAAATTACTCTACCACCTGACTGTCCGCAAAGTCCCTGCATTCTCTTTTC
Y S A D G W F S L H K L T K I T L P P D C P Q S P C I L F F

1090 1100 1110 1120 1130 1140 1150 1160 1170
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GCTTCTGCTGGTGAGGATTACACTCTCCGTCTCCCGTTGATTGTAATCCTTCCTATGTGTTCCACTCCACCGACAACGCCGAGACCGGG
A S A G E D Y T L R L P V D C N P S Y V F H S T D N A E T G

1180 1190 1200 1210 1220 1230 1240 1250 1260
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GTTATTGAGGCGGGTAACACTGACACCGATTCTCTGGTGAAGTGGCGGCTCCTGGCCCTAACACACTAATGTCAAGTTCCTGTTTGT
V I E A G N T D T D F S G E L A A P G P N H T N V K F L F D

1270 1280 1290 1300 1310 1320 1330 1340 1350
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CGATCTCGATTATTGAATGTAATCAAGGTACTGGAGAAGGACGCCGTTTCCCCCGCCCTTCCCTACACAAGAAGGTGCGCAGCAGGAT
R S R L L N V I K V L E K D A V F P R P F P T Q E G A Q Q D

1360 1370 1380 1390 1400 1410 1420 1430 1440
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GATGGTTACTTTTGTCTCTGACCCCCGCCCAACAGTCGCTTCCCGACCCGCCACTCGTTTCGGCCTGTACGCCAATCCGTCCGGCAGT
D G Y F C L L T P R P T V A S R P A T R F G L Y A N P S G S

1450 1460 1470 1480 1490 1500 1510 1520 1530
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GGTGTCTTGCTAACACTTCACTGGACTTCAATTTTATAGCTTGGCCTGTTTCACTTACTTTAGATCGGACCTTGAGGTTACGGTGGTC
G V L A N T S L D F N F Y S L A C F T Y F R S D L E V T V V

1540 1550 1560 1570 1580 1590 1600 1610 1620
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TCACTAGAGCCGATCTGGAATTTGCTGTAGGGTGCTTTCCTTCTGGCAGTGAATACCAGGCTTCCAGCTTGTCTACGACCAGCTGCAT
S L E P D L E F A V G W F P S G S E Y Q A S S F V Y D Q L H

1630 1640 1650 1660 1670 1680 1690 1700 1710
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GTGCCCTTCCACTTTACTGGGCGCACTCCCCGCGCTTTCGCTAGCAAGGGTGGGAAGGTATCTTTTCGTGCTCCCTTGGAAGTCTGTCTCG
V P F H F T G R T P R A F A S K G G K V S F V L P W N S V S

1720 1730 1740 1750 1760 1770 1780 1790 1800
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TCTGTGCTCCCGTGCCTGGGGGGGGGCTTCCAAGCTCTCTTCTGCTACGCGGGGTCTACCGGCGCATGCTGATTGGGGGACTATTTAC
S V L P V R W G G A S K L S S A T R G L P A H A D W G T I Y

1810 1820 1830 1840 1850 1860 1870 1880 1890
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GCCTTTGTCCCCGTCTAATGAGAAGAAAAGCACCGCTGTAACACAGTGGCCGTGTACATTCGGTACAAGAACGCACGTGCCTGGTGC
A F V P R P N E K K S T A V K H V A V Y I R Y K N A R A W C

1900 1910 1920 1930 1940 1950 1960 1970 1980
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CCCAGCATGCTTCCCTTTTCGACGCTACAAGCAGAAGATGCTGATGCAATCTGGCGATATCGAGACCAATCCTGGTCCTGCTTCTGACAAC
P S M L P F R S Y K Q K M L M Q S G D I E T N P G P A S D N

1990 2000 2010 2020 2030 2040 2050 2060 2070
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CCAATTTTGGAGTTTCTTGAAGCAGAAAATGATCTAGTCACTCTGGCCTCTCTCTGGAAGATGGTGCACCTCTGTTCAACAGACCTGGAGA
P I L E F L E A E N D L V T L A S L W K M V H S V Q Q T W R

2080 2090 2100 2110 2120 2130 2140 2150 2160
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AAGTATGTGAAGAACGATGATTTTGGCCCAATTTACTCAGCGAGCTAGTGGGGGAAGGCTCTGTGCGCTTGGCCGCCACGCTATCCAAC
K Y V K N D D F W P N L L S E L V G E G S V A L A A T L S N

FIG. 5B

FIG. 5C

3340 3350 3360 3370 3380 3390 3400 3410 3420
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GATGACACCCCGTCGACGAGGCGTTGGGTAGAGTTCTCTCCCCGCTGCGGTCGATGAGGCGCTTGTCTGACCTCACTCCAGAGGCCGAC
D D T P V D E A L G R V L S P A A V D E A L V D L T P E A D

3430 3440 3450 3460 3470 3480 3490 3500 3510
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CCGGTTGGCCGTTTGGCTATTCTTGCCAAGCTAGGTCTTGCCCTAGCTGCGGTCACCCCTGGTCTGATAATCTTGGCAGTGGGACTCTAC
P V G R L A I L A K L G L A L A A V T P G L I I L A V G L Y

3520 3530 3540 3550 3560 3570 3580 3590 3600
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AGGTACTTCTCTGGCTCTGATGCAGACCAAGAAGAAACAGAAAGTGAGGGATCTGTCAAGGCACCCAGGAGCGAAAATGCTTATGACGGC
R Y F S G S D A D Q E E T E S E G S V K A P R S E N A Y D G

3610 3620 3630 3640 3650 3660 3670 3680 3690
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CCGAAGAAAACTCTAAGCCCCCTGGAGCACTCTCTCTCATGGAATGCAACAGCCCCAACGTGGACATGGGCTTTGAGGCTGCGGTCGCT
P K K N S K P P G A L S L M E M Q Q P N V D M G F E A A V A

3700 3710 3720 3730 3740 3750 3760 3770 3780
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAGAAAGTGGTCGTCCCATTTACCTTCATGGTTCCCAACAGACCTTCTGGGCTTACACAGTCCGCTCTTCTGGTGACCGGCCGGACCTTC
K K V V V P I T F M V P N R P S G L T Q S A L L V T G R T F

3790 3800 3810 3820 3830 3840 3850 3860 3870
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CTAATCAATGAACATACATGGTCCAATCCCTCCTGGACCAGCTTCACAATCCGCGGTGAGGTACACACTCGTGATGAGCCCTTCCAAACG
L I N E H T W S N P S W T S F T I R G E V H T R D E P F Q T

3880 3890 3900 3910 3920 3930 3940 3950 3960
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GTTCAATTTCACTCACCACGGTATTTCCACAGATCTGATGATGGTACGTCTCGGACCGGGCAATTCTTTCCCTAACAATCTAGACAAGTTT
V H F T H H G I P T D L M M V R L G P G N S F P N N L D K F

3970 3980 3990 4000 4010 4020 4030 4040 4050
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GGACTTGACCAGATGCCGGCAGCAACTCCCGTGTGGTTGGCGTTTCGTCCAGTTACGGAAACTTCTTCTCTCTGGAATTTCTCCTCGGA
G L D Q M P A R N S R V V G V S S S Y G N F F F S G N F L G

4060 4070 4080 4090 4100 4110 4120 4130 4140
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTTGTTGATTCCGTCACCTCTGAACAAGGAAGTACGCAAGACTCTTTAGGTACAGGGTGACGACCTACAAAGGATGGTGCGGCTCGGCC
F V D S V T S E Q G T Y A R L F R Y R V T T Y K G W C G S A

4150 4160 4170 4180 4190 4200 4210 4220 4230
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CTGGTCTGTGAGGCCGGTGGCGTCCGACGCATCATTTGGCCTGCATTCTGCTGGCGCCGCGGTATCGGCGCCGGGACCTATATCTCAAAA
L V C E A G G V R R I I G L H S A G A A G I G A G T Y I S K

4240 4250 4260 4270 4280 4290 4300 4310 4320
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTAGGACTAATCAAAGCCCTGAAACACCTCGGTGAACCTTTGGCCACAATGCAAGGACTGATGACTGAATTAGAGCCTGGAATCACCGTA
L G L I K A L K H L G E P L A T M Q G L M T E L E P G I T V

4330 4340 4350 4360 4370 4380 4390 4400 4410
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CATGTACCCCGGAAATCCAAATTGAGAAAGACGACCGCACACGCGGTGTACAAACCGGAGTTTGAGCCTGCTGTGTTGTCAAAATTTGAT
H V P R K S K L R K T T A H A V Y K P E F E P A V L S K F D

4420 4430 4440 4450 4460 4470 4480 4490 4500
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CCCAGACTGAACAAGGATGTTGACTTGGATGAAGTAATTTGGTCTAAACACACTGCCAATGTCCCTTACCAACCTCCTTTGTTCTACACA
P R L N K D V D L D E V I W S K H T A N V P Y Q P P L F Y T

FIG. 5D

4510 4520 4530 4540 4550 4560 4570 4580 4590
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TACATGTCAGAGTACGCTCATCGAGTCTTCTCCTTCTTGGGGAAGACAATGACATTCTGACCGTCAAAGAAGCAATTCTGGGCATCCCC
Y M S E Y A H R V F S F L G K D N D I L T V K E A I L G I P

4600 4610 4620 4630 4640 4650 4660 4670 4680
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GGACTAGACCCCATGGATCCCCACACAGCTCCGGGTCTGCCTTACGCCATCAACGGCCTTCGACGTACTGATCTCGTCGATTTTGTGAAC
G L D P M D P H T A P G L P Y A I N G L R R T D L V D F V N

4690 4700 4710 4720 4730 4740 4750 4760 4770
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GGTACAGTAGATGCGGCGCTGGCTGTACAAATCCAGAAATTCTTAGACGGTGACTACTCTGACCATGTCTTCCAAACTTTTCTGAAAGAT
G T V D A A L A V Q I Q K F L D G D Y S D H V F Q T F L K D

4780 4790 4800 4810 4820 4830 4840 4850 4860
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GAGATCAGACCCTCAGAGAAAGTCCGAGCGGGAAAAACCCGCATTGTTGATGTGCCCTCCCTGGCGCATTCGATTGTGGGCAGAATGTTG
E I R P S E K V R A G K T R I V D V P S L A H C I V G R M L

4870 4880 4890 4900 4910 4920 4930 4940 4950
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CTTGGGCGCTTTTGCTGCCAAGTTTCAATCCCATCCTGGCTTTCTCCTCGGCTCTGCTATCGGGTCTGACCCTGATGTTTTCTGGACCGTC
L G R F A A K F Q S H P G F L L G S A I G S D P D V F W T V

4960 4970 4980 4990 5000 5010 5020 5030 5040
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ATAGGGGCTCAACTCGAGGGGAGAAAGAACACGTATGACGTGGACTACAGTGCCTTTGACTCTTCACACGGCACTGGCTCCTTCGAGGCT
I G A Q L E G R K N T Y D V D Y S A F D S S H G T G S F E A

5050 5060 5070 5080 5090 5100 5110 5120 5130
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CTCATCTCTCACTTTTTACCGTGGACAATGGTTTTAGCCCTGCGCTGGGACCGTATCTCAGATCCCTGGCTGTCTCGGTGCACGCTTAC
L I S H F F T V D N G F S P A L G P Y L R S L A V S V H A Y

5140 5150 5160 5170 5180 5190 5200 5210 5220
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GGCGAGCGTCGCATCAAGATTACCGGTGGCCTCCCCTCCGGTTGTGCCGCGACCAGCCTGCTGAACACAGTGCTCAACAATGTGATCATC
G E R R I K I T G G L P S G C A A T S L L N T V L N N V I I

5230 5240 5250 5260 5270 5280 5290 5300 5310
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AGGACTGCTCTGGCATTGACTTACAAGGAATTTGAGTATGACACGGTTGATATCATCGCCTACGGTGACGACCTTCTGGTTGGCACGGAT
R T A L A L T Y K E F E Y D T V D I I A Y G D D L L V G T D

5320 5330 5340 5350 5360 5370 5380 5390 5400
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TACGATCTGGACTTCAATGAGGTGGCACGACGCGCTGCCAAGTTGGGGTATAAGATGACTCCTGCCAACAAGGGTTCTGTCTTCCCTCCG
Y D L D F N E V A R R A A K L G Y K M T P A N K G S V F P P

5410 5420 5430 5440 5450 5460 5470 5480 5490
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ACTTCCTCTCTTTCCGATGCTGTTTTTCTAAAGCGCAAATTCGTCCAAAACAACGACGGCTTATACAAACCAGTTATGGATTAAAGAAT
T S S L S D A V F L K R K F V Q N N D G L Y K P V M D L K N

5500 5510 5520 5530 5540 5550 5560 5570 5580
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TTGGAAGCCATGCTCTCCTACTTCAAACCAGGAACACTACTCGAGAAGCTGCAATCTGTTTCTATGTTGGCTCAACATTCTGGAAAAGAA
L E A M L S Y F K P G T L L E K L Q S V S M L A Q H S G K E

5590 5600 5610 5620 5630 5640 5650 5660 5670
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GAATATGATAGATTGATGCACCCCTTCGCTGACTACGGTGCCGTACCGAGTCACGAGTACCTGCAGGCAAGATGGAGGGCCTTGTTTCGAC
E Y D R L M H P F A D Y G A V P S H E Y L Q A R W R A L F D

5680 5690 5700 5710 5720 5730 5740 5750
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TGACCCAGATAGCCCAAGGCGCTTCGGTGCTGCCGGCGATTCTGGGAGAACTCAGTCGGAACAGAAAAAAAAAAAAAAAAAAAAA

FIG. 5E

NNTCTAGCCC ACCATGGCAA CAAGAAGAGC TTACAGGAGC TGAATGAAGA ACAGTGGGTG 60
GAAATGTCTG ACGATTACCG GACCGGGAAA AACATGCCTT TTCAGTCTCT TGGCACATAC 120
TATCGGCCCC CTAAGTGGAC TTGGGGTCCC AATTTTCATCA ACCCCTATCA AGTAACGGTT 180
TTCCCACACC AAATTCTGAA CGCGAGAACC TCTACCTCGG TAGACATAAA CGTCCCATAC 240
ATCGGGGAGA CCCCCACGCA ATCCTCAGAG ACACAGAACT CCTGGACCCT CCTCGTTATG 300
GTGCTCGTTC CCCTAGACTA TAAGGAAGGA GCCACAACCTG ACCCAGAAAT TACATTTTCT 360
GTAAGGCCTA CAAGTCCCTA CTTCAATGGG CTTGCAACC GCTACACGGC CGGGACGGAC 420
GAAGAACAGG GGCCCATTC TACGGCACCC AGAGAAAATT CGCTTATGTT TCTCTCAACC 480
CTCCCTGACG ACACTGTCCC TGCTTACGGG AATGTGCGTA CCCCTCCTGT CAATTACCTC 540
CCTGGTGAAA TAACCGACCT TTTGCAACTG GCCCGCATAC CCACTCTCAT GGCATTTGAG 600
CGGGTGCCTG AACCCGTGCC TGCCTCAGAC ACATATGTGC CCTACGTTGC CGTTCCCACC 660
CAGTTCGATG ACAGGCCTCT CATCTCCTTC CCGATCACCC TTTCAGATCC CGTCTATCAG 720
AACACCCTGG TTGGCGCCAT CAGTTCAAAT TTCGCCAATT ACCGTGGGTG TATCCAAATC 780
ACTCTGACAT TTTGTGGACC CATGATGGCG AGAGGGAAAT TCCTGCTCTC GTATTCTCCC 840
CCAAATGGAA CGCAACCACA GACTCTTTCC GAAGCTATGC AGTGACACATA CTCTATTTGG 900
GACATAGGCT TGAAGTCTAG TTGGACCTTC GTCGTCCCCT ACATCTCGCC CAGTGACTION 960
CGTGAAACTC GAGCCATTAC CAACTCGGTT TACTCCGCTG ATGGTTGGTT TAGCCTGCAC 1020
AAGTTGACCA AAATTACTCT ACCACCTGAC TGTCCGCAA GTCCCTGCAT TCTCTTTTTC 1080
GCTTCTGCTG GTGAGGATTA CACTCTCCGT CTCCCCGTTG ATTGTAATCC TTCCTATGTG 1140
TTCCACTCCA CCGACAACGC CGAGACCGGG GTTATTGAGG CGGGTAACAC TGACACCGAT 1200
TTCTCTGGTG AACTGGCGGC TCCTGGCCCT AACCACACTA ATGTCAAGTT CCTGTTTGAT 1260
CGATCTCGAT TATTGAATGT AATCAAGGTA CTGGAGAAGG ACGCCGTTTT CCCCCGCCCT 1320
TTCCCTACAC AAGAAGGTGC GCAGCAGGAT GATGGTTACT TTTGTCTTCT GACCCCCCGC 1380
CCAACAGTCG CTTCCCGACC CGCCACTCGT TTCGGCCTGT ACGCCAATCC GTCCGGCAGT 1440
GGTGTCTTGT CTAACACTTC ACTGGACTTC AATTTTATA GCTTGGCCTG TTCACTTAC 1500

FIG. 6A

TTTAGATCGG ACCTTGAGGT TACGGTGGTC TCACTAGAGC CGGATCTGGA ATTTGCTGTA 1560
GGGTGGTTTC CTTCTGGCAG TGAATACCAG GCTTCCAGCT TTGTCTACGA CCAGCTGCAT 1620
GTGCCCTTCC ACTTTACTGG GCGCACTCCC CGCGCTTTCG CTAGCAAGGG TGGGAAGGTA 1680
TCTTTCGTGC TCCCTTGGA CTCTGTCTCG TCTGTGCTCC CCGTGCGCTG GGGGGGGGCT 1740
TCCAAGCTCT CTTCTGCTAC GCGGGGTCTA CCGGCGCATG CTGATTGGGG GACTATTTAC 1800
GCCTTTGTCC CCCGTCCTAA TGAGAAGAAA AGCACCGCTG TAAACACGT GGCCGTGTAC 1860
ATTCGGTACA AGAACGCACG TGCCTGGTGC CCCAGCATGC TTCCCTTTCG CAGCTACAAG 1920
CAGAAGATGC TGATGCAATC TGGCGATATC GAGACCAATC CTGGTCCTGC TTCTGACAAC 1980
CCAATTTTGG AGTTTCTTGA AGCAGAAAAT GATCTAGTCA CTCTGGCCTC TCTCTGGAAG 2040
ATGGTGCACT CTGTTCAACA GACCTGGAGA AAGTATGTGA AGAACGATGA TTTTGGCCC 2100
AATTTACTCA GCGAGCTAGT GGGGGAAGGC TCTGTCGCCT TGGCCGCCAC GCTATCCAAC 2160
CAAGCTTCAG TAAAGGCTCT TTTGGGCCTG CACTTCTCT CTCGGGGGCT CAATTACACT 2220
GACTTTTACT CTTTACTGAT AGAGAAATGC TCTAGTTTCT TTACCGTAGA ACCACCTCCT 2280
CCACCAGCTG AAAACCTGAT GACCAAGCCC TCAGTGAAGT CGAAATTCCG AAAACTGTTT 2340
AAGATGCAAG GACCCATGGA CAAAGTCAAA GACTGGAACC AAATAGCTGC CGGCTTGAAG 2400
AATTTTCAAT TTGTTTCGTGA CCTAGTCAAA GAGGTGGTCG ATTGGCTGCA GGCCTGGATC 2460
AACAAAGAGA AAGCCAGCCC TGTCTCCAG TACCAGTTGG AGATGAAGAA GCTCGGGCCT 2520
GTGGCCTTGG CTCATGACGC TTTCATGGCT GGTTCGGGC CCCCTCTTAG CGACGACCAG 2580
ATTGAATACC TCCAGAACCT CAAATCTCTT GCCCTAACAC TGGGGAAGAC TAATTTGGCC 2640
CAAAGTCTCA CCACTATGAT CAATGCCAAA CAAAGTTCAG CCCAACGAGT TGAACCCGTT 2700
GTGGTGGTCC TTAGAGGCAA GCCGGGATGC GGCAAGGGCT TGGCCTCTAC GTTGATTGCC 2760
CAGGCTGTGT CCAAGCGCCT CTATGGCTCC CAAAGTGTAT ATTCTCTTCC CCCAGATCCA 2820
GATTTCTTCG ATGGATACAA AGGACAGTTC GTGACCTTGA TGGATGATTT GGGACAAAAC 2880
CCGGATGGAC AAGATTTCCC CACCTTTTGT CAGATGGTGT CGACCGCCCA ATTTCTCCCC 2940
AACATGGCGG ACCTTGCAGA GAAAGGGCGT CCCTTTACCT CCAATCTCAT CATTGCAACT 3000

FIG. 6B

ACAAATCTCC CCCACTTCAG TCCTGTCACC ATTGCTGATC CTTCTGCAGT CTCTCGCCGT 3060
ATCAACTACG ATCTGACTCT AGAAGTATCT GAGGCCTACA AGAAACACAC ACGGCTGAAT 3120
TTTGACTTGG CTTTCAGGCG CACAGACGCC CCCCCATTT ATCCTTTTGC TGCCCATGTG 3180
CCCTTTGTGG ACGTAGCTGT GCGCTTCAAA AATGGTCACC AGAATTTTAA TCTCCTAGAG 3240
TTGGTCGATT CCATTTGTAC AGACATTCGA GCCAAGCAAC AAGGTGCCCCG AAACATGCAG 3300
ACTCTGGTTC TACAGAGCCC CAACGAGAAT GATGACACCC CCGTCGACGA GCGGTTGGGT 3360
AGAGTTCTCT CCCCCGCTGC GGTCGATGAG GCGCTTGTCG ACCTCACTCC AGAGGCCGAC 3420
CCGGTTGGCC GTTTGGCTAT TCTTGCCAAG CTAGGTCTTG CCCTAGCTGC GGTCACCCCT 3480
GGTCTGATAA TCTTGGCAGT GGGACTCTAC AGGTACTTCT CTGGCTCTGA TGCAGACCAA 3540
GAAGAAACAG AAAGTGAGGG ATCTGTCAAG GCACCCAGGA GCGAAAATGC TTATGACGGC 3600
CCGAAGAAAA ACTCTAAGCC CCCTGGAGCA CTCTCTCTCA TGGAAATGCA ACAGCCCAAC 3660
GTGGACATGG GCTTTGAGGC TGCGGTCGCT AAGAAAGTGG TCGTCCCCAT TACCTTCATG 3720
GTTCCCAACA GACCTTCTGG GCTTACACAG TCCGCTCTTC TGGTGACCGG CCGGACCTTC 3780
CTAATCAATG AACATACATG GTCCAATCCC TCCTGGACCA GCTTCACAAT CCGCGGTGAG 3840
GTACACACTC GTGATGAGCC CTTCCAAACG GTTCATTTC A CTCACCACGG TATTCCCACA 3900
GATCTGATGA TGGTACGTCT CGGACCGGGC AATTCTTTCC CTAACAATCT AGACAAGTTT 3960
GGACTTGACC AGATGCCGGC ACGCAACTCC CGTGTGGTTG GCGTTTCGTC CAGTTACGGA 4020
AACTTCTTCT TCTCTGGAAA TTTCTCGGA TTTGTTGATT CCGTCACCTC TGAACAAGGA 4080
ACTTACGCAA GACTCTTTAG GTACAGGGTG ACGACCTACA AAGGATGGTG CGGCTCGGCC 4140
CTGGTCTGTG AGGCCGGTGG CGTCCGACGC ATCATTGGCC TGCATTCTGC TGGCGCCGCC 4200
GGTATCGGCG CCGGGACCTA TATCTCAAAA TTAGGACTAA TCAAAGCCCT GAAACACCTC 4260
GGTGAACCTT TGGCCACAAT GCAAGGACTG ATGACTGAAT TAGAGCCTGG AATCACCGTA 4320
CATGTACCCC GGAAATCCAA ATTGAGAAAG ACGACCGCAC ACGCGGTGTA CAAACCGGAG 4380
TTTGAGCCTG CTGTGTTGTC AAAATTTGAT CCCAGACTGA ACAAGGATGT TGAATTGGAT 4440
GAAGTAATTT GGTCTAAACA CACTGCCAAT GTCCCTTACC AACCTCCTTT GTTCTACACA 4500

FIG. 6C

TACATGTCAG AGTACGCTCA TCGAGTCTTC TCCTTCTTGG GGAAAGACAA TGACATTCTG 4560
ACCGTCAAAG AAGCAATTCT GGGCATCCCC GGACTAGACC CCATGGATCC CCACACAGCT 4620
CCGGGTCTGC CTTACGCCAT CAACGGCCTT CGACGTACTG ATCTCGTCGA TTTTGTGAAC 4680
GGTACAGTAG ATGCGGCGCT GGCTGTACAA ATCCAGAAAT TCTTAGACGG TGACTACTCT 4740
GACCATGTCT TCCAAACTTT TCTGAAAGAT GAGATCAGAC CCTCAGAGAA AGTCCGAGCG 4800
GGAAAAACCC GCATTGTTGA TGTGCCCTCC CTGGCGCATT GCATTGTGGG CAGAATGTTG 4860
CTTGGGCGCT TTGCTGCCAA GTTTC AATCC CATCCTGGCT TTCTCCTCGG CTCTGCTATC 4920
GGGTCTGACC CTGATGTTTT CTGGACCGTC ATAGGGGCTC AACTCGAGGG GAGAAAGAAC 4980
ACGTATGACG TGGACTACAG TGCCTTTGAC TCTTCACACG GCACTGGCTC CTTGAGGGCT 5040
CTCATCTCTC ACTTTTTTAC CGTGGACAAT GGTTTTAGCC CTGCGCTGGG ACCGTATCTC 5100
AGATCCCTGG CTGTCTCGGT GCACGCTTAC GGCGAGCGTC GCATCAAGAT TACCGGTGGC 5160
CTCCCCTCCG GTTGTGCCGC GACCAGCCTG CTGAACACAG TGCTCAACAA TGTGATCATC 5220
AGGACTGCTC TGGCATTGAC TTACAAGGAA TTTGAGTATG ACACGGTTGA TATCATCGCC 5280
TACGGTGACG ACCTTCTGGT TGGCACGGAT TACGATCTGG ACTTCAATGA GGTGGCACGA 5340
CGCGCTGCCA AGTTGGGGTA TAAGATGACT CCTGCCAACA AGGGTTCTGT CTTCCCTCCG 5400
ACTTCCTCTC TTTCCGATGC TGTTTTTCTA AAGCGCAAAT TCGTCCAAAA CAACGACGGC 5460
TTATACAAAC CAGTTATGGA TTAAAGAAT TTGGAAGCCA TGCTCTCCTA CTTCAAACCA 5520
GGAACACTAC TCGAGAAGCT GCAATCTGTT TCTATGTTGG CTCAACATTC TGGAAAAGAA 5580
GAATATGATA GATTGATGCA CCCCTTCGCT GACTACGGTG CCGTACCGAG TCACGAGTAC 5640
CTGCAGGCAA GATGGAGGGC CTTGTTTCGAC TGACCCAGAT AGCCCAAGGC GCTTCGGTGC 5700
TGCCGGCGAT TCTGGGAGAA CTCAGTCGGA ACAGAAAAAA AAAAAAAAAA AA 5752

(SEQ ID NO:1)

FIG. 6D

XLAHHGNKKS LQELNEEQWV EMSDDYRTGK NMPFQSLGTY YRPPNWTWGP NFINPYQVTV 60
FPHQILNART STSVDINVPY IGETPTQSSE TQNSWTLLVM VLVPLDYKEG ATTDPEITFS 120
VRPTSPYFNG LRNRYTAGTD EEQGPIPTAP RENSLMFLST LPDDTVPAYG NVRTPPVNYL 180
PGEITDLLQL ARIPTLMAFE RVPEPVPASD TYVPYVAVPT QFDDRPLISF PITLSDPVYQ 240
NTLVGAISSN FANYRGCIQI TLTFCGPMMA RGKFLLSYSP PNGTQPQTLS EAMQCTYSIW 300
DIGLNSSWTF VVPYISPSDY RETRAITNSV YSADGWFS LH KLTKITLPPD CPQSPCILFF 360
ASAGEDYTLR LPVDCNPSYV FHSTDNAETG VIEAGNTDTD FSGELAAPGP NHTNVKFLFD 420
RSRLNVIKV LEKDAVFPRP FPTQEGAQQD DGYFCLLTPR PTVASRPATR FGLYANPSGS 480
GVLANTSLDF NFYSLACFTY FRSDLEVTVV SLEPDLEFAV GWFPSPGSEYQ ASSEFVYDQLH 540
VPFHFTGRTP RAFASKGGKV SFVLPWNSVS SVLPVRWGGA SKLSSATRGL PAHADWGTYI 600
AFVPRPNEKK STAVKHAVY IRYKNARAWC PSMLPFRSYK QKMLMQSGDI ETNPGPASDN 660
PILEFLEAEN DLVTIASLWK MVHSVQQTWR KYVKNDDFWP NLLSELVGEG SVALAATLSN 720
QASVKALLGL HFSLRGLNYT DFYSLLEK C SSFFTVEPPP PPAENLMTKP SVKSKFRKLF 780
KMQGPMDKVK DWNQIAAGLK NFQFVRDLVK EVVDWLQAWI NKEKASPVLO YQLEMKKLGP 840
VALAHDAFMA GSGPPLSDDQ IEYLQNLKSL ALTIGKTNLA QSLTTMINAK QSSAQRVEPV 900
VVVLRGKPGC GKGLASTLIA QAVSKRLYGS QSVYSLPPDP DFFDGYKGQF VTLMDDLGQN 960
PDGQDEFSTFC QMVSTAQFLP NMADLAEKGR PFTSNLIIAT TNLPHFSPVT IADPSAVSRR 1020
INYDLTLEVS EAYKKHTRLN FDLAFRRRTDA PPIYPFAAHV PFVDVAVREFK NGHQNFNLLE 1080
LVDSICTDIR AKQOGARNMQ TLVLQSPNEN DDTFVDEALG RVLSPA AVDE ALVDLTPEAD 1140
PVGR LAILAK LGLALAAVTP GLIILAVGLY RYFSGSDADQ EETSEGSVK APRSENAYDG 1200

FIG. 7A

PKKNSKPPGA LSLMEMQQPN VDMGFEEA VA KKVVPITFM VPNRPSGLTQ SALLVTGRTE 1260
LINEHTWSNP SWTSFTIRGE VHTRDEPFQT VHFTHHGIPT DLMMVRLGPG NSFPNNLDKF 1320
GLDQMPARNS RVVGVSSSYG NFFFSGNFLG FVDSVTSEQG TYARLFYRV TTYKGWCGSA 1380
LVCEAGGVRR IIGLHSAGAA GIGAGTYISK LGLIKALKHL GEPLATMQGL MTELEPGITV 1440
HVPRKSKLRK TTAHAVYKPE FEPAVLSKFD PRLNKDVLDD EVIWSKHTAN VPYQPPLFYT 1500
YMSEYAHRVF SFLGKDNDIL TVKEAILGIP GLDPMDPHTA PGLPYAINGL RRTDLVDFVN 1560
GTVDAALAVQ IQKFLDGDYS DHVFQTFELKD EIRPSEKVRA GKTRIVDVPS LAHCIVGRML 1620
LGRFAAKFQS HPGFLLGSAI GSDPDVFWTV IGAQLEGRKN TYDVDYSAFD SSHGTGSFEA 1680
LISHFFTVDN GFSPALGPYL RSLAVSVHAY GERRIKITGG LPSGCAATSL LNTVLNNVII 1740
RTALALTYKE FEYDTVDIIA YGDDLLVGTD YDLDFNEVAR RAAKLGKMT PANKGSVFPP 1800
TSSLSDAVFL KRKFVQNNNG LYKPVMDLKN LEAMLSYFKP GTLLEKLQSV SMLAQHSGKE 1860
EYDRLMHPFA DYGAVPSHEY LQARWRALFD * (SEQ ID NO:2) 1890

FIG. 7B

CTAGCCCACC ATGGCAACAA GAAGAGCTTA CAGGAGCTGA ATGAAGAACA GTGGGTGGAA 60
ATGTCTGACG ATTACCGGAC CGGGAAAAAC ATGCCTTTTC AGTCTCTTGG CACATACTAT 120
CGGCCCCCTA ACTGGACTTG GGGTCCCAAT TTCATCAACC CCTATCAAGT AACGGTTTTTC 180
CCACACCAAA TTCTGAACGC GAGAACCTCT ACCTCGGTAG ACATAAACGT CCCATACATC 240
GGGGAGACCC CCACGCAATC CTCAGAGACA CAGAACTCCT GGACCCTCCT CGTTATGGTG 300
CTCGTTCCCC TAGACTATAA GGAAGGAGCC ACAACTGACC CAGAAATTAC ATTTTCTGTA 360
AGGCCTACAA GTCCCTACTT CAATGGGCTT CGCAACCGCT ACACGGCCGG GACGGACGAA 420
GAACAG (SEQ ID NO:3) 426

FIG. 8

LAHHGNKKS L QELNEEQWVE MSDDYRTGKN MPFQSLGTYR RPPNWTWGP N FINPYQVTVF 60
PHQILNARTS TSVDINVPYI GETPTQSSET QNSWTLLVMV LVPLDYKEGA TTDPEITFSV 120
RPTSPYFNGL RNRYTAGTDE EQ (SEQ ID NO:4) 142

FIG. 9

GGGCCCATTCTACGGCACC CAGAGAAAAT TCGCTTATGT TTCTCTCAAC CCTCCCTGAC 60
GACACTGTCC CTGCTTACGG GAATGTGCGT ACCCCTCCTG TCAATTACCT CCCTGGTGAA 120
ATAACCGACC TTTTGCAACT GGCCCGCATA CCCACTCTCA TGGCATTGGA GCGGGTGCCT 180
GAACCCGTGC CTGCCTCAGA CACATATGTG CCCTACGTTG CCGTTCCCAC CCAGTTCGAT 240
GACAGGCCTC TCATCTCCTT CCCGATCACC CTTTCAGATC CCGTCTATCA GAACACCCTG 300
GTTGGCGCCA TCAGTTCAAA TTTCGCCAAT TACCGTGGGT GTATCCAAAT CACTCTGACA 360
TTTTGTGGAC CCATGATGGC GAGAGGGAAA TTCCTGCTCT CGTATTCTCC CCCAAATGGA 420
ACGCAACCAC AGACTCTTTC CGAAGCTATG CAGTGCACAT ACTCTATTTG GGACATAGGC 480
TTGAACTCTA GTTGGACCTT CGTCGTCCCC TACATCTCGC CCAGTGACTA CCGTGAAACT 540
CGAGCCATTA CCAACTCGGT TTA CTCCGCT GATGGTTGGT TTAGCCTGCA CAAGTTGACC 600
AAAATTACTC TACCACCTGA CTGTCCGCAA AGTCCCTGCA TTCTCTTTTT CGCTTCTGCT 660
GGTGAGGATT ACACTCTCCG TCTCCCCGTT GATTGTAATC CTTCTATGT GTTCCAC 717
(SEQ ID NO:5)

FIG. 10

GPIPTAPREN SLMFLSTLPD DTVPAYGNVR TPPVNYLPGE ITDLLQLARI PTLMAFERVP 60
EPVPASDTYV PYVAVPTQFD DRPLISFPIT LSDPVYQNTL VGAISSNFAN YRGCIQITLT 120
FCGPMMARGK FLLSYSPPNG TQPQTLSEAM QCTYSIWDIG LNSSWTFVVP YISPSDYRET 180
RAITNSVYSA DGWESLHKLT KITLPPDCPQ SPCILFFASA GEDYTLRLPV DCNPSYVFH 239
(SEQ ID NO:6)

FIG. 11

TCCACCGACA ACGCCGAGAC CGGGGTTATT GAGGCGGGTA AACTGACAC CGATTTCTCT 60
GGTGAAC TGG CGGCTCCTGG CCCTAACCAC ACTAATGTCA AGTTCCTGTT TGATCGATCT 120
CGATTATTGA ATGTAATCAA GGTACTGGAG AAGGACGCCG TTTTCCCCCG CCCTTCCCT 180
ACACAAGAAG GTGCGCAGCA GGATGATGGT TACTTTTGTC TTCTGACCCC CCGCCAACA 240
GTCGCTTCCC GACCCGCCAC TCGTTTCGGC CTGTACGCCA ATCCGTCCGG CAGTGGTGTT 300
CTTGCTAACA CTTCACTGGA CTTCAATTTT TATAGCTTGG CCTGTTTCAC TTACTTTAGA 360
TCGGACCTTG AGGTTACGGT GGTCTCACTA GAGCCGGATC TGGAATTTGC TGTAGGGTGG 420
TTTCCTTCTG GCAGTGAATA CCAGGCTTCC AGCTTTGTCT ACGACCAGCT GCATGTGCCC 480
TTCCACTTTA CTGGGCGCAC TCCCCGCGCT TTCGCTAGCA AGGGTGGGAA GGTATCTTTC 540
GTGCTCCCTT GGAAC TCTGT CTCGTCTGTG CTCCCCGTGC GCTGGGGGGG GGCTTCCAAG 600
CTCTCTTCTG CTACGCGGGG TCTACCGGCG CATGCTGATT GGGGACTAT TTACGCCTTT 660
GTCCCCCGTC CTAATGAGAA GAAAAGCACC GCTGTAAAC ACGTGGCCGT GTACATTCGG 720
TACAAGAACG CACGTGCCTG GTGCCCCAGC ATGCTTCCCT TTCGCAGCTA CAAGCAG 777
(SEQ ID NO:7)

FIG. 12

STDNAETGVI EAGNTDTDFS GELAAPGPNH TNVKFLFDRS RLLNVIKVLE KDAVFPRPFP 60
TQEGAQQDDG YFCLLTPRPT VASRPATREG LYANPSGSGV LANTSLDFNF YSLACFTYFR 120
SDLEVTVVSL EPDLEFAVGW FPSGSEYQAS SFVYDQLHVP FHFTGRTPRA FASKGGKVSF 180
VLPWNSVSSV LPVRWGGASK LSSATRGLPA HADWG TIYAF VPRPNEKKST AVKHAVYIR 240
YKNARAWCPS MLPFRSYKQ (SEQ ID NO:8) 259

FIG. 13

AAGATGCTGA TGCAATCTGG CGATATCGAG ACCAATCCTG GT (SEQ ID NO:9) 42

FIG. 14

KMLMQSGDIE TNPG (SEQ ID NO:10) 14

FIG. 15

CCTGCTTCTG ACAACCCAAT TTTGGAGTTT CTTGAAGCAG AAAATGATCT AGTCACTCTG 60
GCCTCTCTCT GGAAGATGGT GCACTCTGTT CAACAGACCT GGAGAAAGTA TGTGAAGAAC 120
GATGATTTTT GGCCCAATTT ACTCAGCGAG CTAGTGGGGG AAGGCTCTGT CGCCTTGGCC 180
GCCACGCTAT CCAACCAAGC TTCAGTAAAG GCTCTTTTGG GCCTGCACTT TCTCTCTCGG 240
GGGCTCAATT ACACTGACTT TTACTCTTTA CTGATAGAGA AATGCTCTAG TTTCTTTACC 300
GTAGAACCAC CTCCTCCACC AGCTGAAAAC CTGATGACCA AGCCCTCAGT GAAGTCGAAA 360
TTCCGAAAAC TGTTTAAGAT GCAA (SEQ ID NO:11) 384

FIG. 16

PASDNPILEF LEAENDLVTL ASLWKMVHSV QQWRKYVKN DDFWPPLLSE LVGEGSVALA 60
ATLSNQASVK ALLGLHFSLR GLNYTDFYSL LIEKCSSEFT VEPPPPPAEN LMTKPSVKSK 120
FRKLFKMQ (SEQ ID NO:12) 128

FIG. 17

GGACCCATGG ACAAAGTCAA AGACTGGAAC CAAATAGCTG CCGGCTTGAA GAATTTTCAA 60
TTTGTTCTGT ACCTAGTCAA AGAGGTGGTC GATTGGCTGC AGGCCTGGAT CAACAAAGAG 120
AAAGCCAGCC CTGTCCTCCA GTACCAGTTG GAGATGAAGA AGCTCGGGCC TGTGGCCTTG 180
GCTCATGACG CTTTCATGGC TGGTTCCGGG CCCCCTCTTA GCGACGACCA GATTGAATAC 240
CTCCAGAACC TCAAATCTCT TGCCTAACA CTGGGGAAGA CTAATTTGGC CCAAAGTCTC 300
ACCACTATGA TCAATGCCAA ACAAAGTTCA GCCCAACGAG TTGAACCCGT TGTGGTGGTC 360
CTTAGAGGCA AGCCGGGATG CGGCAAGGGC TTGGCCTCTA CGTTGATTGC CCAGGCTGTG 420
TCCAAGCGCC TCTATGGCTC CCAAAGTGTA TATTCTCTTC CCCAGATCC AGATTTCTTC 480
GATGGATACA AAGGACAGTT CGTGACCTTG ATGGATGATT TGGGACAAAA CCCGGATGGA 540
CAAGATTTCC CCACCTTTTG TCAGATGGTG TCGACCGCCC AATTTCTCCC CAACATGGCG 600
GACCTTGCAG AGAAAGGGCG TCCCTTTACC TCCAATCTCA TCATTGCAAC TACAAATCTC 660
CCCCACTTCA GTCCTGTCAC CATTGCTGAT CCTTCTGCAG TCTCTCGCCG TATCAACTAC 720
GATCTGACTC TAGAAGTATC TGAGGCCTAC AAGAAACACA CACGGCTGAA TTTTGACTTG 780
GCTTTCAGGC GCACAGACGC CCCCCCATT TATCCTTTTG CTGCCCATGT GCCCTTTGTG 840
GACGTAGCTG TGCGCTTCAA AAATGGTCAC CAGAATTTTA ATCTCCTAGA GTTGGTCGAT 900
TCCATTTGTA CAGACATTCG AGCCAAGCAA CAAGGTGCCC GAAACATGCA GACTCTGGTT 960
CTACAG (SEQ ID NO:13) 966

FIG. 18

GPMDKVKDWN QIAAGLKNFQ FVRDLVKEVV DWLQAWINKE KASPVLQYQL EMKKLGPVAL 60
AHDAFMAGSG PPLSDDQIEY LQNLKSLALT LGKTNLAQSL TTMINAKQSS AQRVEPVVVV 120
LRGKPGCGKG LASTLIAQAV SKRLYGSQSV YSLPPDPDFD DGYKGQFVTL MDDLQONPDG 180
QDFSTFCQMV STAQFLPNMA DLAEKGRPFT SNLIIATTNL PHFSPVTIAD PSAVSRRINY 240
DLTLEVSEAY KKHTRLNFDL AFRRTDAPPI YPFAAHVPFV DVAVRFKNGH QNFNLLELVD 300
SICTDIRAKQ QGARNMQTLV LQ (SEQ ID NO:14) 322

FIG. 19

AGCCCCAACG AGAATGATGA CACCCCCGTC GACGAGGCGT TGGGTAGAGT TCTCTCCCCC 60
GCTGCGGTCG ATGAGGCGCT TGTCGACCTC ACTCCAGAGG CCGACCCGGT TGGCCGTTTG 120
GCTATTCTTG CCAAGCTAGG TCTTGCCCTA GCTGCGGTCA CCCCTGGTCT GATAATCTTG 180
GCAGTGGGAC TCTACAGGTA CTTCTCTGGC TCTGATGCAG ACCAAGAAGA AACAGAAAGT 240
GAGGGATCTG TCAAGGCACC CAGGAGCGAA (SEQ ID NO:15) 270

FIG. 20

SPNENDDTPV DEALGRVLSP AAVDEALVDL TPEADPVGRL AILAKLGLAL AAVTPGLIIL 60
AVGLYRYFSG SDADQEETES EGSVKAPRSE (SEQ ID NO:16) 90

FIG. 21

AATGCTTATG ACGGCCCGAA GAAAACTCT AAGCCCCCTG GAGCACTCTC TTCATGGAA 60
ATGCAA (SEQ ID NO:17) 66

FIG. 22

NAYDGPCKNS KPPGALSLME MQ (SEQ ID NO:18) 22

FIG. 23

CAGCCCAACG TGGACATGGG CTTTGAGGCT GCGGTCGCTA AGAAAGTGGT CGTCCCCATT 60
ACCTTCATGG TTCCCAACAG ACCTTCTGGG CTTACACAGT CCGCTCTTCT GGTGACCGGC 120
CGGACCTTCC TAATCAATGA ACATACATGG TCCAATCCCT CCTGGACCAG CTTCACAATC 180
CGCGGTGAGG TACACACTCG TGATGAGCCC TTCCAAACGG TTCATTTTAC TCACCACGGT 240
ATTCCACAG ATCTGATGAT GGTACGTCTC GGACCGGGCA ATTCTTTCCC TAACAATCTA 300
GACAAGTTTG GACTTGACCA GATGCCGGCA CGCAACTCCC GTGTGGTTGG CGTTTCGTCC 360
AGTTACGGAA ACTTCTTCTT CTCTGGAAAT TTCCTCGGAT TTGTTGATTC CGTCACCTCT 420
GAACAAGGAA CTTACGCAAG ACTCTTTAGG TACAGGGTGA CGACCTACAA AGGATGGTGC 480
GGCTCGGCCC TGGTCTGTGA GGCCGGTGGC GTCCGACGCA TCATTGGCCT GCATTCTGCT 540
GGCGCCGCCG GTATCGGCGC CGGGACCTAT ATCTCAAAT TAGGACTAAT CAAAGCCCTG 600
AAACACCTCG GTGAACCTTT GGCCACAATG CAA (SEQ ID NO:19) 633

FIG. 24

QPNVDMGFEA AVAKKVVVPI TFMVPNRPSG LTQSALLVTG RTFLINEHTW SNPSWTSFTI 60
RGEVHTRDEP FQTVHFTHHG IPTDLMMVRL GPGNSFPNNL DKFGLDQMPA RNSRVVGVSS 120
SYGNFFFSGN FLGFVDSVTS EQGTYARLFR YRVTTYKGWC GSALVCEAGG VVRIIGLHSA 180
GAAGIGAGTY ISKLGLIKAL KHLGEPLATM Q (SEQ ID NO:20) 211

FIG. 25

GGACTGATGA CTGAATTAGA GCCTGGAATC ACCGTACATG TACCCCGGAA ATCCAAATTG 60
AGAAAGACGA CCGCACACGC GGTGTACAAA CCGGAGTTTG AGCCTGCTGT GTTGTCAAAA 120
TTTGATCCCA GACTGAACAA GGATGTTGAC TTGGATGAAG TAATTTGGTC TAAACACACT 180
GCCAATGTCC CTTACCAACC TCCTTTGTTC TACACATACA TGTCAGAGTA CGCTCATCGA 240
GTCTTCTCCT TCTTGGGGAA AGACAATGAC ATTCTGACCG TCAAAGAAGC AATTCTGGGC 300
ATCCCCGGAC TAGACCCCAT GGATCCCCAC ACAGCTCCGG GTCTGCCTTA CGCCATCAAC 360
GGCCTTCGAC GTACTGATCT CGTCGATTTT GTGAACGGTA CAGTAGATGC GGCCTGGCT 420
GTACAAATCC AGAAATTCTT AGACGGTGAC TACTCTGACC ATGTCTTCCA AACTTTTCTG 480
AAAGATGAGA TCAGACCCTC AGAGAAAGTC CGAGCGGGAA AAACCCGCAT TGTGATGTG 540
CCCTCCCTGG CGCATTGCAT TGTGGGCAGA ATGTTGCTTG GGCCTTTGC TGCCAAGTTT 600
CAATCCCATC CTGGCTTTCT CCTCGGCTCT GCTATCGGGT CTGACCCTGA TGTTTTCTGG 660
ACCGTCATAG GGGCTCAACT CGAGGGGAGA AAGAACACGT ATGACGTGGA CTACAGTGCC 720
TTTGACTCTT CACACGGCAC TGGCTCCTTC GAGGCTCTCA TCTCTCACTT TTTCACCGTG 780
GACAATGGTT TTAGCCCTGC GCTGGGACCG TATCTCAGAT CCCTGGCTGT CTCGGTGAC 840
GCTTACGGCG AGCGTCGCAT CAAGATTACC GGTGGCCTCC CCTCCGGTTG TGCCGCGACC 900
AGCCTGCTGA ACACAGTGCT CAACAATGTG ATCATCAGGA CTGCTCTGGC ATTGACTTAC 960
AAGGAATTTG AGTATGACAC GGTTGATATC ATCGCCTACG GTGACGACCT TCTGGTTGGC 1020
ACGGATTACG ATCTGGACTT CAATGAGGTG GCACGACGCG CTGCCAAGTT GGGGTATAAG 1080
ATGACTCCTG CCAACAAGGG TTCTGTCTTC CCTCCGACTT CCTCTCTTTC CGATGCTGTT 1140
TTTCTAAAGC GCAAATTCGT CCAAACAAC GACGGCTTAT ACAAACCAGT TATGGATTTA 1200
AAGAATTTGG AAGCCATGCT CTCCTACTTC AAACCAGGAA CACTACTCGA GAAGCTGCAA 1260
TCTGTTTCTA TGTTGGCTCA ACATTCTGGA AAAGAAGAAT ATGATAGATT GATGCACCCC 1320
TTCGCTGACT ACGGTGCCGT ACCGAGTCAC GAGTACCTGC AGGCAAGATG GAGGGCCTTG 1380
TTCGACTGA (SEQ ID NO:21) 1389

FIG. 26

GLMTELEPGI TVHVPRKSKL RKTTHAVYK PEFEPAVLSK FDPRLNKDVD LDEVIWSKHT 60
ANVPYQPPLF YTYMSEYahr VFSFLGKDND ILTVKEAILG IPGLDPMDPH TAPGLPYAIN 120
GLRRTDLVDF VNGTVDAALA VQIQKFLDGD YSDHVFQTFI KDEIRPSEKV RAGKTRIVDV 180
PSLAHCIVGR MLLGRFAAKE QSHPGFLLGS AIGSDPDVFW TVIGAQLEGR KNTYDVDYSA 240
FDSSHGTGSF EALISHFFTV DNGFSPALGP YLRSLAVSVH AYGERRIKIT GGLPSGCAAT 300
SLLNTVLNNV IIRTALALTY KEFEYDTVDI IAYGDDLLVG TDYDLDFNEV ARRAAKLGYK 360
MTPANKGSVF PPTSSLSDAV FLKRKFVQNN DGLYKPVMDL KNLEAMLSYF KPGTLLEKLQ 420
SVSMLAQHSG KEEYDRLMHP FADYGAVPSH EYLQARWRAL FD * (SEQ ID NO:22) 462

FIG. 27

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10      20      30      40      50      60      70      80      90      100
SVV-----
EMCV-R  NATTMEQETCAHSLTFECCKCSALQYRNG-FYLLKYDEEATPEELLT-DGEDDVDFD-----ELDMEVVVELQ/GHNTSSDKNNSSSG
EMCV-FV21 NATTMEQETCAHSLTFECCKCSALQYRNG-FYLLKYDEEATPEELLT-DGEDDVDFD-----ELDMEVVVELQ/GHNTSSDKNNSSSG
EMCV-B  NATTMEQETCAHSLTFECCKCSALQYRNG-FYLLKYDEEATPEELLT-DGEDDVDFD-----ELDMEVVVELQ/GHNTSSDKNNSSSG
EMCV-Da NATTMEQETCAHSLTFECCKCSALQYRNG-FYLLKYDEEATPEELLT-DGEDDVDFD-----ELDMEVVVELQ/GHNTSSDKNNSSSG
EMCV-Db NATTMEQETCAHSLTFECCKCSALQYRNG-FYLLKYDEEATPEELLT-DGEDDVDFD-----ELDMEVVVELQ/GHNTSSDKNNSSSG
EMCV-FV2 NATTMEQETCAHSLTFECCKCSALQYRNG-FYLLKYDEEATPEELLT-DGEDDVDFD-----ELDMEVVVELQ/GHNTSSDKNNSSSG
EMCV-Mengo NATTMEQETCAHSLTFECCKCSALQYRNG-FYLLKYDEEATPEELLT-DGEDDVDFD-----ELDMEVVVELQ/GHNTSSDKNNSSSG
TMHV/DA [M20301] HA-----CKHGYF-DVCPICIAVDVTFGEFYLLADGEXFTDILCVLDDDDVDFNPDSNQCQTHETDILPLVVDIVMHPQ/GHNTSSDKNNSSSG
TMHV/GDVII [M20562] HA-----CKHGYF-DVCPICIAVDATTFGEFYLLADGEXFTDILCVLDDDDVDFNPDSNQCQTHETDILPLVVDIVMHPQ/GHNTSSDKNNSSSG
TMHV/BeAnS86 [M16020] HA-----CKHGYF-DVCPICIAVDATTFGEFYLLADGEXFTDILCVLDDDDVDFNPDSNQCQTHETDILPLVVDIVMHPQ/GHNTSSDKNNSSSG
TLV-N3S910 HA-----CKHGYF-SVCPICIAVDKSDGFYLLADGEXFTDILCVLDDDDVDFNPDSNQCQTHETDILPLVVDIVMHPQ/GHNTSSDKNNSSSG
VHEV/Siberia-55 [M1668] HA-----CKHGYF-DVCPICIAVDVTFGEFYLLADGEXFTDILCVLDDDDVDFNPDSNQCQTHETDILPLVVDIVMHPQ/GHNTSSDKNNSSSG

110      120      130      140      150      160      170      180      190      200
SVV-----
EMCV-R  NEGVIINHYFNQYQNSIDLSAN-AAGSDPRTYQCFENLLSGAVHAFBNMIPLLA/DQNTHEHNLSDRVSQDTAGNTVTNTQSTVGRLVGYGTHDGE
EMCV-FV21 NEGVIINHYFNQYQNSIDLSAN-AAGSDPRTYQCFENLLSGAVHAFBNMIPLLA/DQNTHEHNLSDRVSQDTAGNTVTNTQSTVGRLVGYGTHDGE
EMCV-B  NEGVIINHYFNQYQNSIDLSAN-ATGSDPRTYQCFENLLSGAVHAFBNMIPLLA/DQNTHEHNLSDRVSQDTAGNTVTNTQSTVGRLVGYGTHDGE
EMCV-Da NEGVIINHYFNQYQNSIDLSAN-ATGSDPRTYQCFENLLSGAVHAFBNMIPLLA/DQNTHEHNLSDRVSQDTAGNTVTNTQSTVGRLVGYGTHDGE
EMCV-Db NEGVIINHYFNQYQNSIDLSAN-ATGSDPRTYQCFENLLSGAVHAFBNMIPLLA/DQNTHEHNLSDRVSQDTAGNTVTNTQSTVGRLVGYGTHDGE
EMCV-FV2 NEGVIINHYFNQYQNSIDLSAN-ATGSDPRTYQCFENLLSGAVHAFBNMIPLLA/DQNTHEHNLSDRVSQDTAGNTVTNTQSTVGRLVGYGTHDGE
EMCV-Mengo NEGVIINHYFNQYQNSIDLSAN-ATGSDPRTYQCFENLLSGAVHAFBNMIPLLA/DQNTHEHNLSDRVSQDTAGNTVTNTQSTVGRLVGYGTHDGE
TMHV/DA [M20301] NEGVIINHYFNQYQNSIDLSAGGNAAGAPQNNQCLLENILGGAANAFATMAPIIL/DQNTHEHNLSDRVASDKAGNSATNTQSTVGRLOCYGHAHGE
TMHV/GDVII [M20562] NEGVIINHYFNQYQNSIDLSAGGNAAGAPQNNQCLLENILGGAANAFATMAPIIL/DQNTHEHNLSDRVASDKAGNSATNTQSTVGRLOCYGHAHGE
TMHV/BeAnS86 [M16020] NEGVIINHYFNQYQNSIDLSAGGNAAGAPQNNQCLLENILGGAANAFATMAPIIL/DQNTHEHNLSDRVASDKAGNSATNTQSTVGRLOCYGHAHGE
TLV-N3S910 NEGVIINHYFNQYQNSIDLSAGGNAAGAPQNNQCLLENILGGAANAFATMAPIIL/DQNTHEHNLSDRVASDKAGNSATNTQSTVGRLOCYGHAHGE
VHEV/Siberia-55 [M1668] NEGVIINHYFNQYQNSIDLSAGGNAAGAPQNNQCLLENILGGAANAFATMAPIIL/DQNTHEHNLSDRVASDKAGNSATNTQSTVGRLOCYGHAHGE

210      220      230      240      250      260      270      280      290      300
SVV-----LAHHEKKEKLLQELNBEQ
EMCV-R  EPASCADTASEKILAVERYTTFKVDWTSTQKPFYIRIPLFHVLSGEDGQGVFGAALRRHYLVKTCGRVQVQCNAQPHAGSLVFMASBYTT-----
EMCV-FV21 EPASCADTASEKILAVERYTTFKVDWTSTQKPFYIRIPLFHVLSGEDGQGVFGAALRRHYLVKTCGRVQVQCNAQPHAGSLVFMASBYTT-----
EMCV-B  EPASCADTASEKILAVERYTTFKVDWTSTQKPFYIRIPLFHVLSGEDGQGVFGAALRRHYLVKTCGRVQVQCNAQPHAGSLVFMASBYTT-----
EMCV-Da EPASCADTASEKILAVERYTTFKVDWTSTQKPFYIRIPLFHVLSGEDGQGVFGAALRRHYLVKTCGRVQVQCNAQPHAGSLVFMASBYTT-----
EMCV-Db EPASCADTASEKILAVERYTTFKVDWTSTQKPFYIRIPLFHVLSGEDGQGVFGAALRRHYLVKTCGRVQVQCNAQPHAGSLVFMASBYTT-----
EMCV-FV2 EPASCADTASEKILAVERYTTFKVDWTSTQKPFYIRIPLFHVLSGEDGQGVFGAALRRHYLVKTCGRVQVQCNAQPHAGSLVFMASBYTT-----
EMCV-Mengo EPASCADTASEKILAVERYTTFKVDWTSTQKPFYIRIPLFHVLSGEDGQGVFGAALRRHYLVKTCGRVQVQCNAQPHAGSLVFMASBYTT-----
TMHV/DA [M20301] EPASCADTATKVLAAERYTTIDLSGTTTQAPSHIRIPLFHVLSGEDGQGVFGATLRHYLVKTCGRVQVQCNAQPHAGSLVFMASBYTTCKGTETG
TMHV/GDVII [M20562] EPASCADTATKVLAAERYTTIDLSGTTTQAPSHIRIPLFHVLSGEDGQGVFGATLRHYLVKTCGRVQVQCNAQPHAGSLVFMASBYTTCKGTETG
TMHV/BeAnS86 [M16020] EPASCADTATKVLAAERYTTIDLSGTTTQAPSHIRIPLFHVLSGEDGQGVFGATLRHYLVKTCGRVQVQCNAQPHAGSLVFMASBYTTCKGTETG
TLV-N3S910 EPASCADTATKVLAAERYTTIDLSGTTTQAPSHIRIPLFHVLSGEDGQGVFGATLRHYLVKTCGRVQVQCNAQPHAGSLVFMASBYTTCKGTETG
VHEV/Siberia-55 [M1668] EPASCADTATKVLAAERYTTIDLSGTTTQAPSHIRIPLFHVLSGEDGQGVFGATLRHYLVKTCGRVQVQCNAQPHAGSLVFMASBYTTCKGTETG

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Fig. 28A

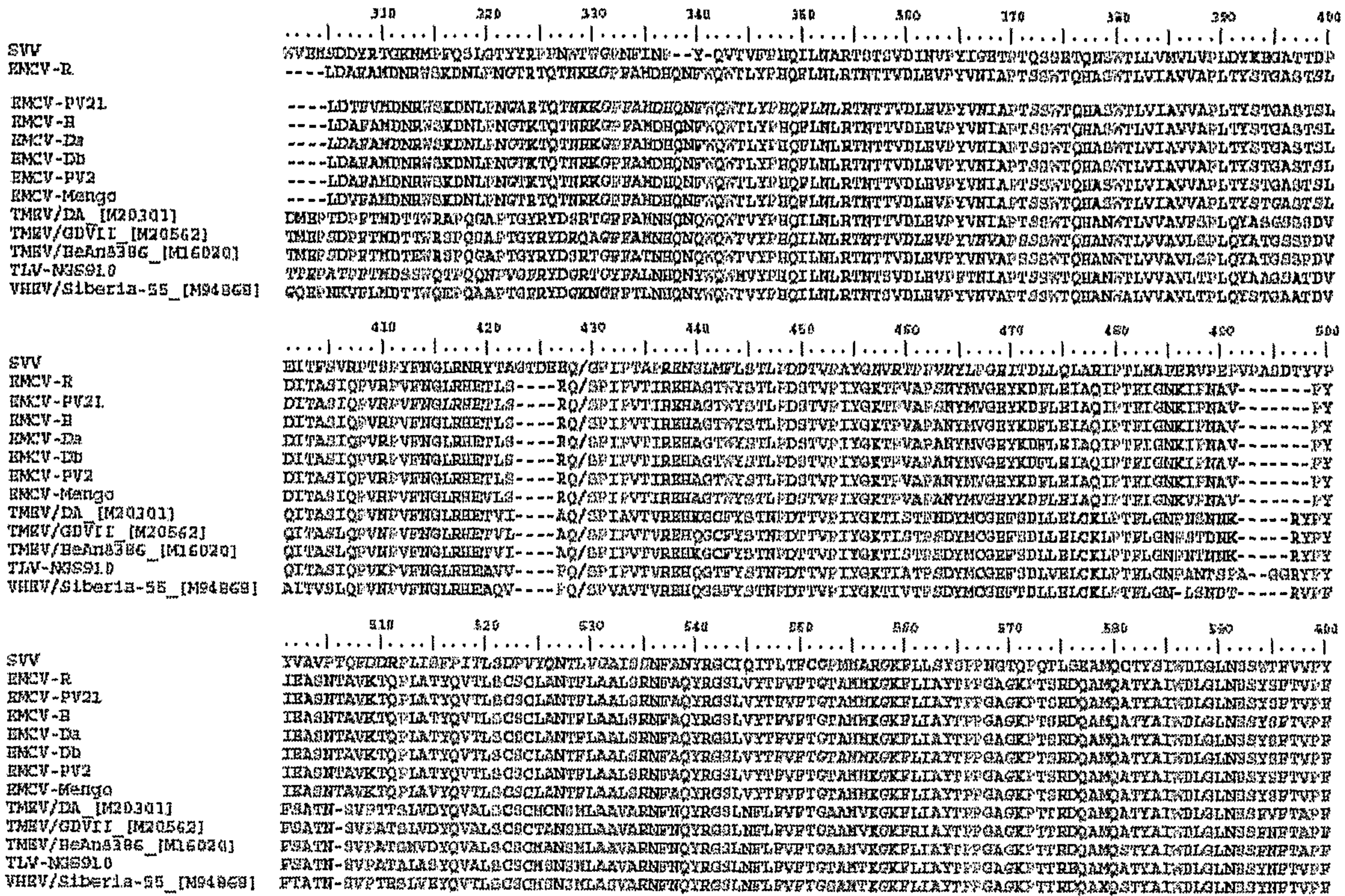


Fig. 28B

Fig. 28C

Fig. 28D

	1314	1320	1330	1340	1350	1360	1370	1380	1390	1400
SVV	DLVKEVVDWFLQAWINKKKAHFWLQYQLEHKKLSPVALAHDAFHAG-DCGFLSDDDQIBFLQHLKOLALITLQKTNLAQHLFEMINAKQSSAQVVEFVVVVLR									
EMCV-R	KTVEKVVDFHGTAVVQRE--KEQTLDQLLQRFPEHAKRISDLRNGHSAAYVECKESFDFPEELYNQAVKKEKRTGLAAYCKEPQKHDHATAACHPVVIVLR									
EMCV-FV21	KTVEKVVDFHGTAVVQRE--KEQTLDQLLQRFPEHAKRISDLRNGHSAAYVECKESFDFPEELYNQAVKKEKRTGLAAYCKEPQKHDHATAACHPVVIVLR									
EMCV-H	KTVEKVVDFHGTAVVQRE--KEQTLDQLLQRFPEHAKRISDLRNGHSAAYVECKESFDFPEELYNQAVKKEKRTGLAAYCKEPQKHDHATAACHPVVIVLR									
EMCV-Da	KTVEKVVDFHGTAVVQRE--KEQTLDQLLQRFPEHAKRISDLRNGHSAAYVECKESFDFPEELYNQAVKKEKRTGLAAYCKEPQKHDHATAACHPVVIVLR									
EMCV-Db	KTVEKVVDFHGTAVVQRE--KEQTLDQLLQRFPEHAKRISDLRNGHSAAYVECKESFDFPEELYNQAVKKEKRTGLAAYCKEPQKHDHATAACHPVVIVLR									
EMCV-FV2	KTVEKVVDFHGTAVVQRE--KEQTLDQLLQRFPEHAKRISDLRNGHSAAYVECKESFDFPEELYNQAVKKEKRTGLAAYCKEPQKHDHATAACHPVVIVLR									
EMCV-Mango	KTVEKVVDFHGTAVVQRE--KEQTLDQLLQRFPEHAKRISDLRNGHSAAYVECKESFDFPEELYNQAVKKEKRTGLAAYCKEPQKHDHATAACHPVVIVLR									
TNEV/DA [M20301]	ETIQSIVNHLTSKHKQHDHPQSKLKLKLMERFDHCRNIMDMRHRKAYCROTASVKKYFDELYNLAFTCKRIPLASLOCKKRNHDSVTREHPVVVLR									
TNEV/GDVII [M20562]	ETIQSIVNHLTSKHKQHDHPQSKLKLKLMERFDHCRNIMDMRHRKAYCROTASVKKYFDELYNLAFTCKRIPLASLOCKKRNHDSVTREHPVVVLR									
TNEV/HeAnS3B6 [M16020]	ETIQSIVNHLTSKHKQHDHPQSKLKLKLMERFDHCRNIMDMRHRKAYCROTASVKKYFDELYNLAFTCKRIPLASLOCKKRNHDSVTREHPVVVLR									
TLV-N3S910	ETVQKIVDHLMSKFKQRAHFQKLIKHLADFFERQSSILAMRHRKAYTDCAGAKYFDELYNLAFTCKRIPLASLOCKKRNHDSVTREHPVVVLR									
VHEV/Siberia-55 [M94869]	ETVQKIVDHLMSKFKQRAHFQKLIKHLADFFERQSSILAMRHRKAYTDCAGAKYFDELYNLAFTCKRIPLASLOCKKRNHDSVTREHPVVVLR									
	1410	1420	1430	1440	1450	1460	1470	1480	1490	1500
SVV	GKAGQCKSLSSQVIAQAVSKTIPGRQSVYSLPFDHDFDGYENQFAAITHDILGQHPDGSDFTFQCQHVSTTNFLNMAHLEBKGTFTSQIIVVATTNLEP									
EMCV-R	GKAGQCKSLSSQVIAQAVSKTIPGRQSVYSLPFDHDFDGYENQFAAITHDILGQHPDGSDFTFQCQHVSTTNFLNMAHLEBKGTFTSQIIVVATTNLEP									
EMCV-FV21	GKAGQCKSLSSQVIAQAVSKTIPGRQSVYSLPFDHDFDGYENQFAAITHDILGQHPDGSDFTFQCQHVSTTNFLNMAHLEBKGTFTSQIIVVATTNLEP									
EMCV-H	GKAGQCKSLSSQVIAQAVSKTIPGRQSVYSLPFDHDFDGYENQFAAITHDILGQHPDGSDFTFQCQHVSTTNFLNMAHLEBKGTFTSQIIVVATTNLEP									
EMCV-Da	GKAGQCKSLSSQVIAQAVSKTIPGRQSVYSLPFDHDFDGYENQFAAITHDILGQHPDGSDFTFQCQHVSTTNFLNMAHLEBKGTFTSQIIVVATTNLEP									
EMCV-Db	GKAGQCKSLSSQVIAQAVSKTIPGRQSVYSLPFDHDFDGYENQFAAITHDILGQHPDGSDFTFQCQHVSTTNFLNMAHLEBKGTFTSQIIVVATTNLEP									
EMCV-FV2	GKAGQCKSLSSQVIAQAVSKTIPGRQSVYSLPFDHDFDGYENQFAAITHDILGQHPDGSDFTFQCQHVSTTNFLNMAHLEBKGTFTSQIIVVATTNLEP									
EMCV-Mango	GKAGQCKSLSSQVIAQAVSKTIPGRQSVYSLPFDHDFDGYENQFAAITHDILGQHPDGSDFTFQCQHVSTTNFLNMAHLEBKGTFTSQIIVVATTNLEP									
TNEV/DA [M20301]	GKAGQCKSVTSQIIAQSVKMAFGRQSVYSLPFDHDFDGYENQFAAITHDILGQHPDGSDFTFQCQHVSTTNFLNMAHLEBKGTFTSQIIVVATTNLEP									
TNEV/GDVII [M20562]	GKAGQCKSVTSQIIAQSVKMAFGRQSVYSLPFDHDFDGYENQFAAITHDILGQHPDGSDFTFQCQHVSTTNFLNMAHLEBKGTFTSQIIVVATTNLEP									
TNEV/HeAnS3B6 [M16020]	GKAGQCKSVTSQIIAQSVKMAFGRQSVYSLPFDHDFDGYENQFAAITHDILGQHPDGSDFTFQCQHVSTTNFLNMAHLEBKGTFTSQIIVVATTNLEP									
TLV-N3S910	GKAGQCKSVTSQIIAQSVKMAFGRQSVYSLPFDHDFDGYENQFAAITHDILGQHPDGSDFTFQCQHVSTTNFLNMAHLEBKGTFTSQIIVVATTNLEP									
VHEV/Siberia-55 [M94869]	GKAGQCKSVTSQIIAQSVKMAFGRQSVYSLPFDHDFDGYENQFAAITHDILGQHPDGSDFTFQCQHVSTTNFLNMAHLEBKGTFTSQIIVVATTNLEP									
	1510	1520	1530	1540	1550	1560	1570	1580	1590	1600
SVV	FRPVTIAHYFAVERRTTEDYSVSAGFVCKTRAGYKVLQVERAER--PTGDAPLPCFQHNCLFLEKAGLQFEDNRTEKILSLVDVIERAVARIERKKVIL									
EMCV-R	FRPVTIAHYFAVERRTTEDYSVSAGFVCKTRAGYKVLQVERAER--PTGDAPLPCFQHNCLFLEKAGLQFEDNRTEKILSLVDVIERAVARIERKKVIL									
EMCV-FV21	FRPVTIAHYFAVERRTTEDYSVSAGFVCKTRAGYKVLQVERAER--PTGDAPLPCFQHNCLFLEKAGLQFEDNRTEKILSLVDVIERAVARIERKKVIL									
EMCV-H	FRPVTIAHYFAVERRTTEDYSVSAGFVCKTRAGYKVLQVERAER--PTGDAPLPCFQHNCLFLEKAGLQFEDNRTEKILSLVDVIERAVARIERKKVIL									
EMCV-Da	FRPVTIAHYFAVERRTTEDYSVSAGFVCKTRAGYKVLQVERAER--PTGDAPLPCFQHNCLFLEKAGLQFEDNRTEKILSLVDVIERAVARIERKKVIL									
EMCV-Db	FRPVTIAHYFAVERRTTEDYSVSAGFVCKTRAGYKVLQVERAER--PTGDAPLPCFQHNCLFLEKAGLQFEDNRTEKILSLVDVIERAVARIERKKVIL									
EMCV-FV2	FRPVTIAHYFAVERRTTEDYSVSAGFVCKTRAGYKVLQVERAER--PTGDAPLPCFQHNCLFLEKAGLQFEDNRTEKILSLVDVIERAVARIERKKVIL									
EMCV-Mango	FRPVTIAHYFAVERRTTEDYSVSAGFVCKTRAGYKVLQVERAER--PTGDAPLPCFQHNCLFLEKAGLQFEDNRTEKILSLVDVIERAVARIERKKVIL									
TNEV/DA [M20301]	FRPVTIAHYFAVERRTTEDYSVSAGFVCKTRAGYKVLQVERAER--PTGDAPLPCFQHNCLFLEKAGLQFEDNRTEKILSLVDVIERAVARIERKKVIL									
TNEV/GDVII [M20562]	FRPVTIAHYFAVERRTTEDYSVSAGFVCKTRAGYKVLQVERAER--PTGDAPLPCFQHNCLFLEKAGLQFEDNRTEKILSLVDVIERAVARIERKKVIL									
TNEV/HeAnS3B6 [M16020]	FRPVTIAHYFAVERRTTEDYSVSAGFVCKTRAGYKVLQVERAER--PTGDAPLPCFQHNCLFLEKAGLQFEDNRTEKILSLVDVIERAVARIERKKVIL									
TLV-N3S910	FRPVTIAHYFAVERRTTEDYSVSAGFVCKTRAGYKVLQVERAER--PTGDAPLPCFQHNCLFLEKAGLQFEDNRTEKILSLVDVIERAVARIERKKVIL									
VHEV/Siberia-55 [M94869]	FRPVTIAHYFAVERRTTEDYSVSAGFVCKTRAGYKVLQVERAER--PTGDAPLPCFQHNCLFLEKAGLQFEDNRTEKILSLVDVIERAVARIERKKVIL									

Fig. 28E

Fig. 28F

	1810	1820	1830	1840	1850	1860	1870	1880	1890	1900
SVV	CHAGVVRRI	DLHHAAGAGI	BAETYSKLG	ILIZALKHL	GEPLATMQ	/GLMTELEP	QITVHVPR	REKXLRKTT	ANAVYKPE	REPAVLGKED
EMCV-R	ADLGGGKKIL	STHSAGSNGI	AAASIVS	QKHIRAVVN	----	AFEPQ/GAL	ERLFDGFR	IHVPRKTA	LRPTVARQ	VQPAYAPAVL
EMCV-FV21	ADLGGGKKIL	STHSAGSNGI	AAASIVS	QKHIRAVVN	----	AFEPQ/GAL	ERLFDGFR	IHVPRKTA	LRPTVARQ	VQPAYAPAVL
EMCV-B	ADLGGGKKIL	STHSAGSNGI	AAASIVS	QKHIRAVVN	----	AFEPQ/GAL	ERLFDGFR	IHVPRKTA	LRPTVARQ	VQPAYAPAVL
EMCV-Da	ADLGGGKKIL	STHSAGSNGI	AAASIVS	QKHIRAVVN	----	AFEPQ/GAL	ERLFDGFR	IHVPRKTA	LRPTVARQ	VQPAYAPAVL
EMCV-Dh	ADLGGGKKIL	STHSAGSNGI	AAASIVS	QKHIRAVVN	----	AFEPQ/GAL	ERLFDGFR	IHVPRKTA	LRPTVARQ	VQPAYAPAVL
EMCV-FV2	ADLGGGKKIL	STHSAGSNGI	AAASIVS	QKHIRAVVN	----	AFEPQ/GAL	ERLFDGFR	IHVPRKTA	LRPTVARQ	VQPAYAPAVL
EMCV-Mango	ADLGGGKKIL	STHSAGSNGI	AAASIVS	QKHIRAVVN	----	AFEPQ/GAL	ERLFDGFR	IHVPRKTA	LRPTVARQ	VQPAYAPAVL
TNEV/DA [M20301]	CHVNGKKA	VYGMHSAGG	GGLAAAT	ITTEHL	IEAARKM	----	LALFPQ/GA	IVDI	STG	SVVHVPRK
TNEV/GDVII [M20562]	CHVNGKKA	VYGMHSAGG	GGLAAAT	ITTEHL	IEAARKM	----	LALFPQ/GA	IVDI	STG	SVVHVPRK
TNEV/BeAn5386 [M16020]	CHVNGKKA	VYGMHSAGG	GGLAAAT	ITTEHL	IEAARKM	----	LALFPQ/GA	IVDI	STG	SVVHVPRK
TLV-M3591b	CHVNGKKA	VYGMHSAGG	GGLAAAT	ITTEHL	IEAARKM	----	LALFPQ/GA	IVDI	STG	SVVHVPRK
VHEV/Siberia-55 [M94869]	CHVNGKKA	VYGMHSAGG	GGLAAAT	ITTEHL	IEAARKM	----	LALFPQ/GA	IVDI	STG	SVVHVPRK
SVV	VIWSKHTAN	VPYQPF	LYTMS	HYAEW	ESFLG	KNNDIL	TVREAIL	LRPGL	DPHDEHTA	FGLPYA
EMCV-R	VAFSEKHTSN	QESLP	FVPRH	VAKHYAN	RVTLL	GKDN	GRLLTV	QALD	GLESGM	DFMDKNT
EMCV-FV21	VAFSEKHTSN	QESLP	FVPRH	VAKHYAN	RVTLL	GKDN	GRLLTV	QALD	GLESGM	DFMDKNT
EMCV-B	VAFSEKHTSN	QESLP	FVPRH	VAKHYAN	RVTLL	GKDN	GRLLTV	QALD	GLESGM	DFMDKNT
EMCV-Da	VAFSEKHTSN	QESLP	FVPRH	VAKHYAN	RVTLL	GKDN	GRLLTV	QALD	GLESGM	DFMDKNT
EMCV-Dh	VAFSEKHTSN	QESLP	FVPRH	VAKHYAN	RVTLL	GKDN	GRLLTV	QALD	GLESGM	DFMDKNT
EMCV-FV2	VAFSEKHTSN	QESLP	FVPRH	VAKHYAN	RVTLL	GKDN	GRLLTV	QALD	GLESGM	DFMDKNT
EMCV-Mango	VAFSEKHTSN	QESLP	FVPRH	VAKHYAN	RVTLL	GKDN	GRLLTV	QALD	GLESGM	DFMDKNT
TNEV/DA [M20301]	VAFSEKHTSN	QESLP	FVPRH	VAKHYAN	RVTLL	GKDN	GRLLTV	QALD	GLESGM	DFMDKNT
TNEV/GDVII [M20562]	VAFSEKHTSN	QESLP	FVPRH	VAKHYAN	RVTLL	GKDN	GRLLTV	QALD	GLESGM	DFMDKNT
TNEV/BeAn5386 [M16020]	VAFSEKHTSN	QESLP	FVPRH	VAKHYAN	RVTLL	GKDN	GRLLTV	QALD	GLESGM	DFMDKNT
TLV-M3591b	VAFSEKHTSN	QESLP	FVPRH	VAKHYAN	RVTLL	GKDN	GRLLTV	QALD	GLESGM	DFMDKNT
VHEV/Siberia-55 [M94869]	VAFSEKHTSN	QESLP	FVPRH	VAKHYAN	RVTLL	GKDN	GRLLTV	QALD	GLESGM	DFMDKNT
SVV	RVFPQFLK	DEIR	RPSEK	VRAK	TRIV	DPV	SLAEC	IVG	HNLL	SPFAK
EMCV-R	VVYQFLKDE	IRL	RPSEK	VRAK	TRIV	DPV	SLAEC	IVG	HNLL	SPFAK
EMCV-FV21	VVYQFLKDE	IRL	RPSEK	VRAK	TRIV	DPV	SLAEC	IVG	HNLL	SPFAK
EMCV-B	VVYQFLKDE	IRL	RPSEK	VRAK	TRIV	DPV	SLAEC	IVG	HNLL	SPFAK
EMCV-Da	VVYQFLKDE	IRL	RPSEK	VRAK	TRIV	DPV	SLAEC	IVG	HNLL	SPFAK
EMCV-Dh	VVYQFLKDE	IRL	RPSEK	VRAK	TRIV	DPV	SLAEC	IVG	HNLL	SPFAK
EMCV-FV2	VVYQFLKDE	IRL	RPSEK	VRAK	TRIV	DPV	SLAEC	IVG	HNLL	SPFAK
EMCV-Mango	VVYQFLKDE	IRL	RPSEK	VRAK	TRIV	DPV	SLAEC	IVG	HNLL	SPFAK
TNEV/DA [M20301]	VVYQFLKDE	IRL	RPSEK	VRAK	TRIV	DPV	SLAEC	IVG	HNLL	SPFAK
TNEV/GDVII [M20562]	VVYQFLKDE	IRL	RPSEK	VRAK	TRIV	DPV	SLAEC	IVG	HNLL	SPFAK
TNEV/BeAn5386 [M16020]	VVYQFLKDE	IRL	RPSEK	VRAK	TRIV	DPV	SLAEC	IVG	HNLL	SPFAK
TLV-M3591b	VVYQFLKDE	IRL	RPSEK	VRAK	TRIV	DPV	SLAEC	IVG	HNLL	SPFAK
VHEV/Siberia-55 [M94869]	VVYQFLKDE	IRL	RPSEK	VRAK	TRIV	DPV	SLAEC	IVG	HNLL	SPFAK

Fig. 28G

	2210	2220	2230	2240	2250	2260	2270	2280	2290	2300
SVV	ISHEFTVDNGEPALQFFYLRSLAVGWHAYGEREIKITGGLPSGCAATSHLNTIMNNTIIRAGLYLTYYKHFFEDDVKVLSTYGDLLVATNYQLNEDKVRAS									
EMCV-R	AREHFTTPENGDFPLTRYLRSLATSTHAFBEKRFLLITGGLPSGCAATSHLNTIMNNTIIRAGLYLTYYKHFFEDDVKVLSTYGDLLVATNYQLNEDKVRAS									
EMCV-PV21	AREHFTTPENGDFPLTRYLRSLATSTHAFBEKRFLLITGGLPSGCAATSHLNTIMNNTIIRAGLYLTYYKHFFEDDVKVLSTYGDLLVATNYQLNEDKVRAS									
EMCV-B	AREHFTTPENGDFPLTRYLRSLATSTHAFBEKRFLLITGGLPSGCAATSHLNTIMNNTIIRAGLYLTYYKHFFEDDVKVLSTYGDLLVATNYQLNEDKVRAS									
EMCV-Da	AREHFTTPENGDFPLTRYLRSLATSTHAFBEKRFLLITGGLPSGCAATSHLNTIMNNTIIRAGLYLTYYKHFFEDDVKVLSTYGDLLVATNYQLNEDKVRAS									
EMCV-Db	AREHFTTPENGDFPLTRYLRSLATSTHAFBEKRFLLITGGLPSGCAATSHLNTIMNNTIIRAGLYLTYYKHFFEDDVKVLSTYGDLLVATNYQLNEDKVRAS									
EMCV-EV2	AREHFTTPENGDFPLTRYLRSLATSTHAFBEKRFLLITGGLPSGCAATSHLNTIMNNTIIRAGLYLTYYKHFFEDDVKVLSTYGDLLVATNYQLNEDKVRAS									
EMCV-Mango	AREHFTTPENGDFPLTRYLRSLATSTHAFBEKRFLLITGGLPSGCAATSHLNTIMNNTIIRAGLYLTYYKHFFEDDVKVLSTYGDLLVATNYQLNEDKVRAS									
TMRV/DA [M20301]	IKNFTTEQNGFDRRIARYLRSLAVGRHAYEDRRVLIIRGGLPSGCAATSHLNTIMNNTIIRAGLYLTYYKHFFEDDVKVLSTYGDLLVATNYQLNEDKVRAS									
TMRV/GDVII [M20562]	IKNFTTEQNGFDRRIARYLRSLAVGRHAYEDRRVLIIRGGLPSGCAATSHLNTIMNNTIIRAGLYLTYYKHFFEDDVKVLSTYGDLLVATNYQLNEDKVRAS									
TMRV/HeAnS385 [M16020]	IKNFTTEQNGFDRRIARYLRSLAVGRHAYEDRRVLIIRGGLPSGCAATSHLNTIMNNTIIRAGLYLTYYKHFFEDDVKVLSTYGDLLVATNYQLNEDKVRAS									
TIV-NGS910	IKNFTTEQNGFDRRIARYLRSLAVGRHAYEDRRVLIIRGGLPSGCAATSHLNTIMNNTIIRAGLYLTYYKHFFEDDVKVLSTYGDLLVATNYQLNEDKVRAS									
VHRV/Siberia-55 [M94869]	IKNFTTEQNGFDRRIARYLRSLAVGRHAYEDRRVLIIRGGLPSGCAATSHLNTIMNNTIIRAGLYLTYYKHFFEDDVKVLSTYGDLLVATNYQLNEDKVRAS									
SVV	AAKLGYYKITEANKTSSTFFLDSLTEDVFLERKFKKE-GPLYRFVMMHREALRANLSYYRFGTLSEKLTSTTHLAVHSGKQBYDRLRAPPREVGIVVPTYES									
EMCV-R	AAKLGYYKITEANKTSSTFFLDSLTEDVFLERKFKKE-GPLYRFVMMHREALRANLSYYRFGTLSEKLTSTTHLAVHSGKQBYDRLRAPPREVGIVVPTYES									
EMCV-PV21	AAKLGYYKITEANKTSSTFFLDSLTEDVFLERKFKKE-GPLYRFVMMHREALRANLSYYRFGTLSEKLTSTTHLAVHSGKQBYDRLRAPPREVGIVVPTYES									
EMCV-B	AAKLGYYKITEANKTSSTFFLDSLTEDVFLERKFKKE-GPLYRFVMMHREALRANLSYYRFGTLSEKLTSTTHLAVHSGKQBYDRLRAPPREVGIVVPTYES									
EMCV-Da	AAKLGYYKITEANKTSSTFFLDSLTEDVFLERKFKKE-GPLYRFVMMHREALRANLSYYRFGTLSEKLTSTTHLAVHSGKQBYDRLRAPPREVGIVVPTYES									
EMCV-Db	AAKLGYYKITEANKTSSTFFLDSLTEDVFLERKFKKE-GPLYRFVMMHREALRANLSYYRFGTLSEKLTSTTHLAVHSGKQBYDRLRAPPREVGIVVPTYES									
EMCV-EV2	AAKLGYYKITEANKTSSTFFLDSLTEDVFLERKFKKE-GPLYRFVMMHREALRANLSYYRFGTLSEKLTSTTHLAVHSGKQBYDRLRAPPREVGIVVPTYES									
EMCV-Mango	AAKLGYYKITEANKTSSTFFLDSLTEDVFLERKFKKE-GPLYRFVMMHREALRANLSYYRFGTLSEKLTSTTHLAVHSGKQBYDRLRAPPREVGIVVPTYES									
TMRV/DA [M20301]	LAFFGYKITEANKTSSTFFLDSLTEDVFLERKFKKE-GPLYRFVMMHREALRANLSYYRFGTLSEKLTSTTHLAVHSGKQBYDRLRAPPREVGIVVPTYES									
TMRV/GDVII [M20562]	LAFFGYKITEANKTSSTFFLDSLTEDVFLERKFKKE-GPLYRFVMMHREALRANLSYYRFGTLSEKLTSTTHLAVHSGKQBYDRLRAPPREVGIVVPTYES									
TMRV/HeAnS385 [M16020]	LAFFGYKITEANKTSSTFFLDSLTEDVFLERKFKKE-GPLYRFVMMHREALRANLSYYRFGTLSEKLTSTTHLAVHSGKQBYDRLRAPPREVGIVVPTYES									
TIV-NGS910	LAFFGYKITEANKTSSTFFLDSLTEDVFLERKFKKE-GPLYRFVMMHREALRANLSYYRFGTLSEKLTSTTHLAVHSGKQBYDRLRAPPREVGIVVPTYES									
VHRV/Siberia-55 [M94869]	LAFFGYKITEANKTSSTFFLDSLTEDVFLERKFKKE-GPLYRFVMMHREALRANLSYYRFGTLSEKLTSTTHLAVHSGKQBYDRLRAPPREVGIVVPTYES									
SVV	IQARWRSLER									
EMCV-R	VEYWRSLER									
EMCV-PV21	VEYWRSLER									
EMCV-B	VEYWRSLER									
EMCV-Da	VEYWRSLER									
EMCV-Db	VEYWRSLER									
EMCV-EV2	VEYWRSLER									
EMCV-Mango	VEYWRSLER									
TMRV/DA [M20301]	HLVWRSLER									
TMRV/GDVII [M20562]	HLVWRSLER									
TMRV/HeAnS385 [M16020]	HLVWRSLER									
TIV-NGS910	HLVWRSLER									
VHRV/Siberia-55 [M94869]	-----									

Fig. 28H

Polypeptide	aa	Predicted sequence
L Leader	?	No data
VP4 (1A)	?	No data
VP2 (1B)	>142	LAHHGNKKSLOELNEEQWVMSDDYRTGKNMPFQSLGTYRPPNWTWGP FINPYQVTVFPHQILNARTSTSVDINVPYIGETPTQSSETQNSWTLLVMV LVPLDYKEGATTDPEITFSVRPTSPYFNGLRNRYTAGTDEEQ
VP3 (1C)	239	GPIPTAPRENSLMFLSTLPDDTVPAYGNVRTPPVNYLPGEITDLLQLARI PTLMAFERVPEPVASDTYVPYVAVPTQFDDRPLISFPITLSDPVYQNTL VGAISSNFANYRGCITLTFCGPMARGKFLLSYSPNGTQPQTLSEAM QCTYSIWDIGLNSSWTFVVPYISPSDYRETRAITNSVYSADGWFSLHKLT KITLPPDCPQSPCILFFASAGEDYTLRLPVDCNPSYVFH
VP1 (1D)	259	STDNAETGVIEAGNTDTEFSGELAAPGPNHTNVKFLFDRSRLNVIKVLE KDAVFPRPFPTQEGAQQDDGYFCLLTPRPTVASRPATRFGLYANPSGSGV LANTSLDNFYSLACFTYFRSDLEVTVVSLEPDLEFAVGWFPSGSEYQAS SFVYDQLHVPFHFTGRTPRAFASKGGKVSFVLPWNSVSSVLPVRWGGASK LSSATRGLPAHADWGTIYAFVPRPNEKKSTAVKHVAVYIRYKNARAWCPS MLPFRSYKQ
2A	14	KMLMQSGDIET <u>NPG</u>
2B	128	<u>P</u> ASDNPILEFLEAENDLVTLASLWKMVHSVQQTWRKYVKNDDEFWPNLLSE LVGEGSVALAATLSNQASVKALLGLHFLSRGLNYTDFYSLLEKCSSFFT VEPPPPPAENLMTKPSVKSKFRKLFKMQ
2C	322	GPMDKVKDWNQIAAGLKNFQFVRDLVKEVVDWLQAWINKEKASPVLYQYL EMKKLGPVALAHDAFMAGSGPPLSDDQIEYLQNLKSLALTGLKTNLAQSL TTMINAKQSSAQRVEPVVVVLR <u>GKPGCGKGL</u> ASTLIAQAVSKRLYGSQSV YSLPPDPDFFDGYKGQF <u>VTLMDDL</u> GQNPDGQDFSTFCQMVSTAQFLPNMA DLAEKGRPFTSNLIIATTNLPHFSPVTIADPSAVSRRINYDLTLEVSEAY KKHTRLNFDLAFRRTDAPPIYPFAAHVPFVDVAVRFKNGHQNFNLLELVD SICTDIRAKQQGARNMQTLVLQ
3A	90	SPNENDDTPVDEALGRVLSAAVDEALVDLTPEADPVGRLAILAKLGLAL AAVTPGLIILAVGLYRYFSGSDADQEETESSEGSVKAPRSE
3B (VPg)	22	NA <u>Y</u> DGPKKNSKPPGALSIMEMQ
3C (pro)	211	QPNVDMGFEEAAVAKKVVPITFMVFNRPSTGLTQSALLVTGRFTLINE <u>H</u> TW SNPSWTSFTIRGEVHTRDEPFQTVHFTHHGIPTDLMMVRLGPGNSFPNNL DKFGLDQMPARNSRVVGVSSSYGNFFSGNFLGFVDSVTSEQTYARLER YRVTTYKGW <u>C</u> GSALVCEAGGVRRIIGL <u>H</u> SAGAAGIGAGTYISKLGLIKAL KHLGEPLATMQ
3D (pol)	462	GLMTELEPGITVHVPRKSKLRKTTAHAVYKPEFEPVLSKFDPRLNKDVD LDEVIWSKHTANVPYQPPLFYTYMSEYAHRVFSFLGKDNDILTVKEAILG IPGLDPMDPHTAPGLPYAINGLRRTDLVDFVNGTVDAALAVQIQKFLDGD YSDHVFQTFLL <u>KDEIR</u> PSEKVRAGKTRIVDVPSLAHCIVGRMLLGRFAAKF QSHPGFLLGSAIGSDPDVFWTVIGAQLEGRKNTYDVDYSAFDSSHGTGSF EALISHFFTVDNGFSPALGPYLRSLAVSVHAYGERRIKITGGL <u>PSG</u> CAAT SLLNTVLNNVIIRTALALTYKEFEYDVTVDIIA <u>YGDD</u> LLVGTDYDLDFNEV ARRAAKLGKMTTPANKGSVFPPPTSSLSDAV <u>FLKR</u> KFVQNNNGLYKPVMDL KNLEAMLSYFKPGTLLLEKLQSVSMLAQHSGKEEYDRLMHPFADYGAVPSH EYLQARWRALFD

Fig. 29

<u>Genus</u>	<u>Species</u>	<u>Abbrev.</u>	<u>Serotype</u>
Enterovirus	Poliovirus	PV-1	= Poliovirus 1
	<i>Human enterovirus A</i>	CV-A16	= Cocksackievirus A16
	<i>Human enterovirus B</i>	CV-B5	= Cocksackievirus B5
	<i>Human enterovirus C</i>	CV-A21	= Cocksackievirus A21
	<i>Human enterovirus D</i>	EV-70	= Enterovirus 70
	<i>Simian enterovirus A</i>	SEV-A	= Simian enterovirus A1
	<i>Bovine enterovirus</i>	BEV-1	= Bovine enterovirus 1
	<i>Porcine enterovirus B</i>	PEV-9	= Porcine enterovirus 9
Rhinovirus	<i>Human rhinovirus A</i>	HRV-2	= Human rhinovirus 2
	<i>Human rhinovirus B</i>	HRV-14	= Human rhinovirus 14
New genus?	Not yet designated	SV2	= Simian virus 2
	<i>Porcine enterovirus A</i>	PEV-8	= Porcine enterovirus 8
Cardiovirus	<i>Encephalomyocarditis virus</i>	EMCV	= Encephalomyocarditis virus
	<i>Theilovirus</i>	TMEV	= Theiler's murine encephalomyelitis virus
	?	virus	
Aphthovirus	<i>Foot-and-mouth disease virus</i>	SVV	= Seneca Valley virus
	<i>Equine rhinitis A virus</i>	FMDV-O	= Foot-and-mouth disease virus O
Hepatovirus	<i>Hepatitis A virus</i>	ERAV	= Equine rhinitis A virus
	<i>Avian encephalomyelitis-like viruses</i>	HAV	= Hepatitis A virus
	<i>Human parechovirus</i>	AEV	= Avian encephalomyelitis virus
Parechovirus	<i>Ljungan virus</i>	HPeV-1	= Human parechovirus 1
	<i>Aichi virus</i>	LV	= Ljungan virus
Kobuvirus	<i>Bovine kobuvirus</i>	AIv	= Aichi virus
	<i>Equine rhinitis B virus</i>	BKV	= Bovine kobuvirus
Erbovirus	<i>Porcine teschovirus</i>	ERBV-1	= Equine rhinitis B virus 1
Teschovirus		PTV-1	= Porcine teschovirus 1

Fig. 30

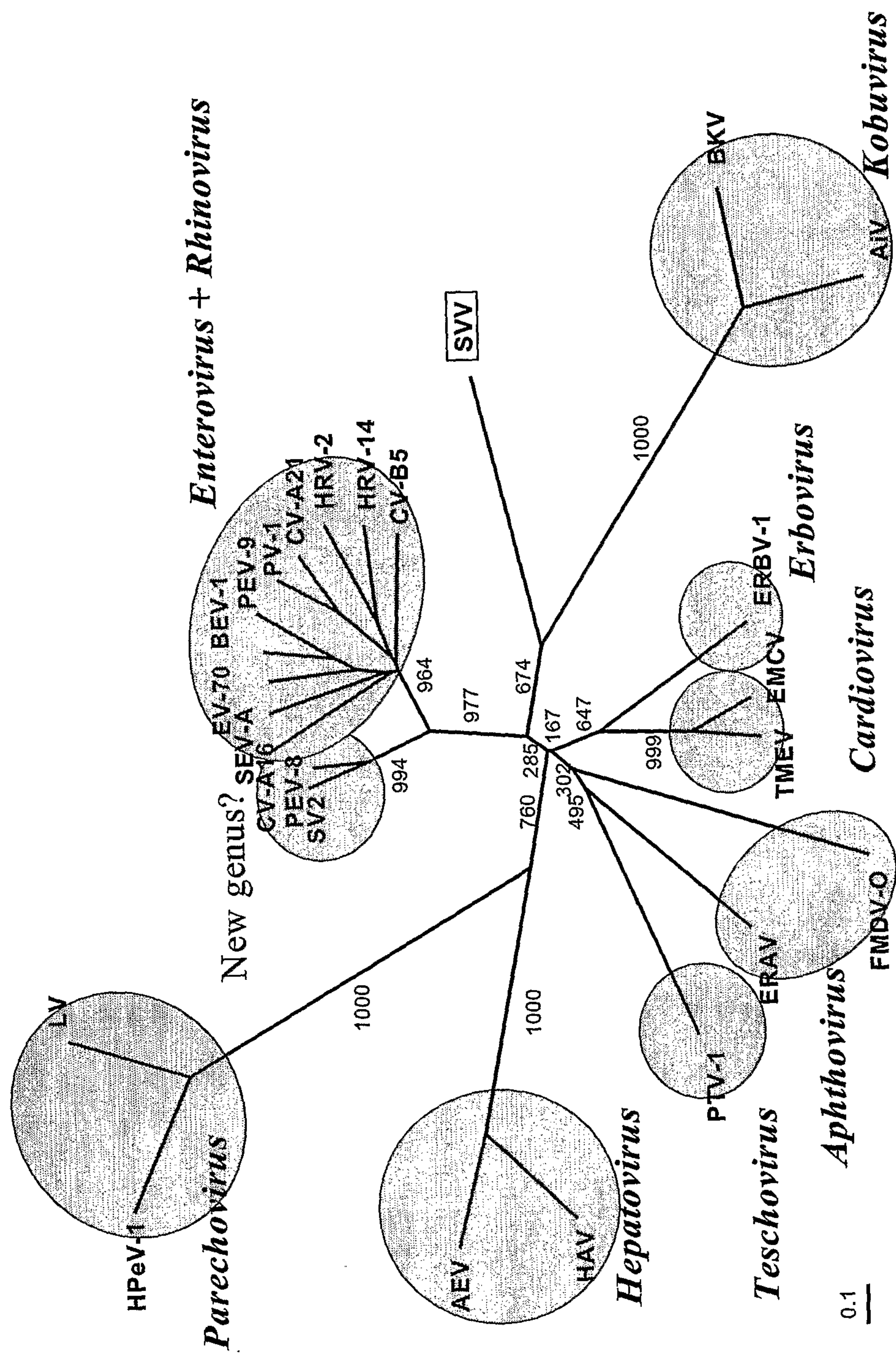


Fig. 31

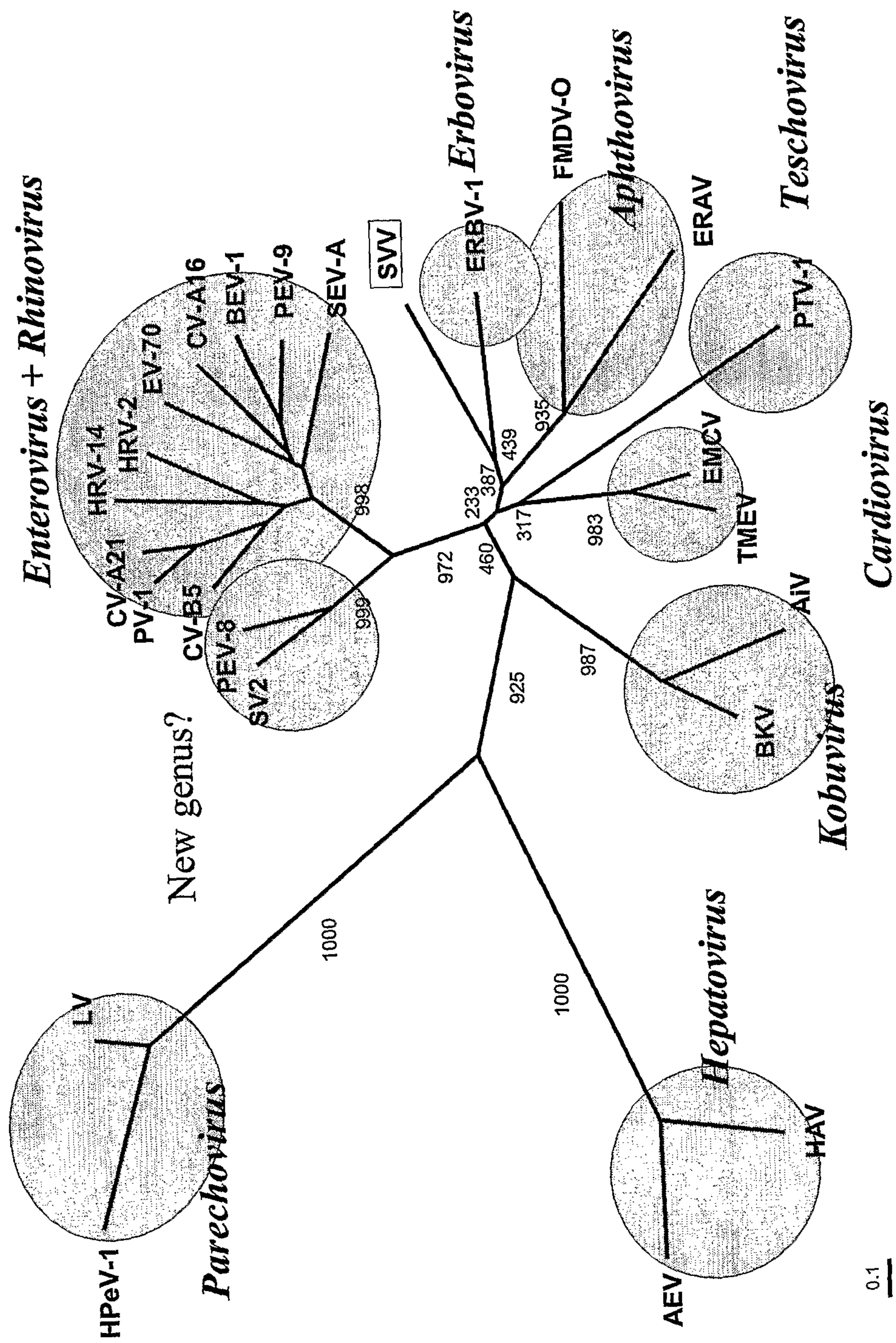


Fig. 32

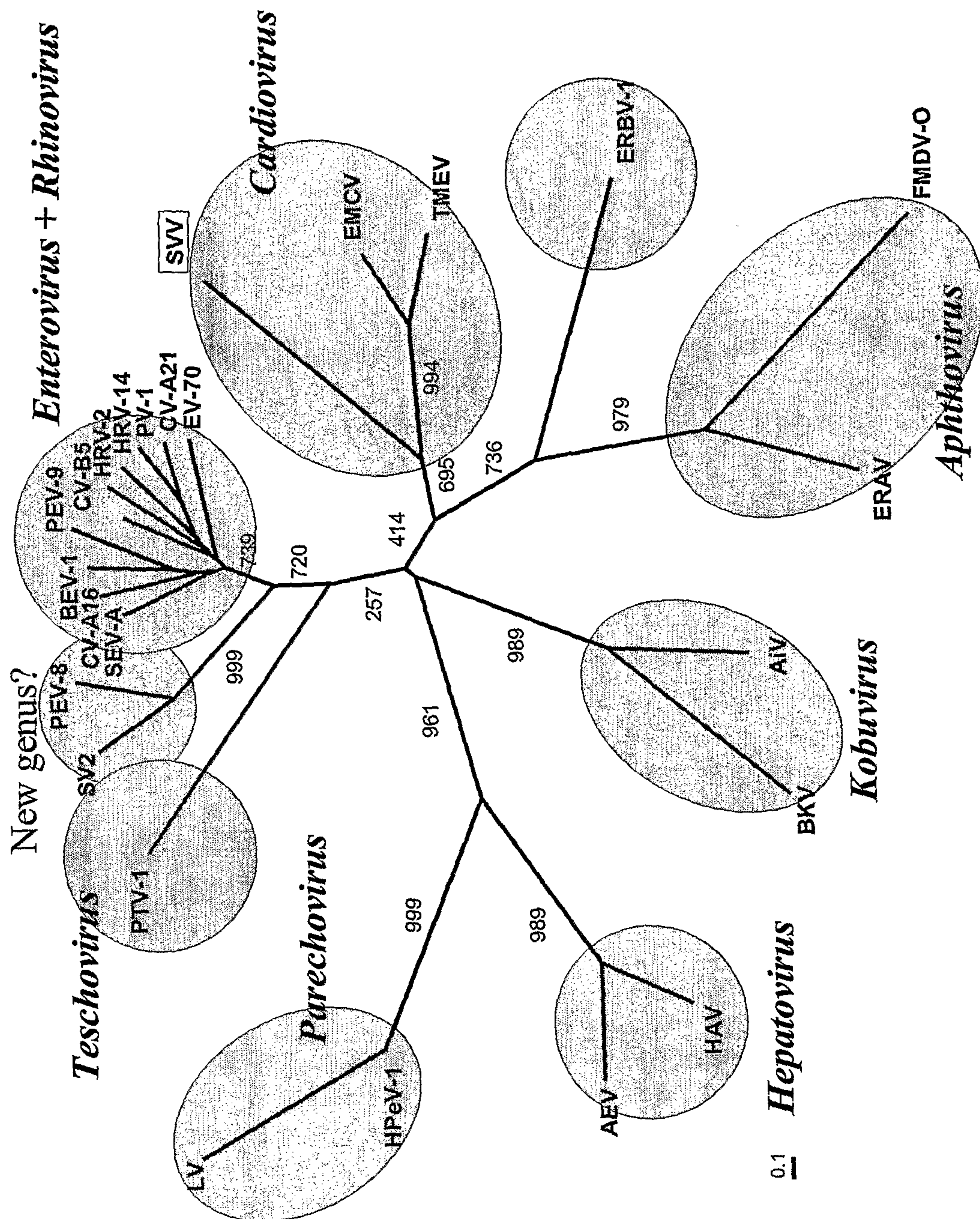


Fig. 33

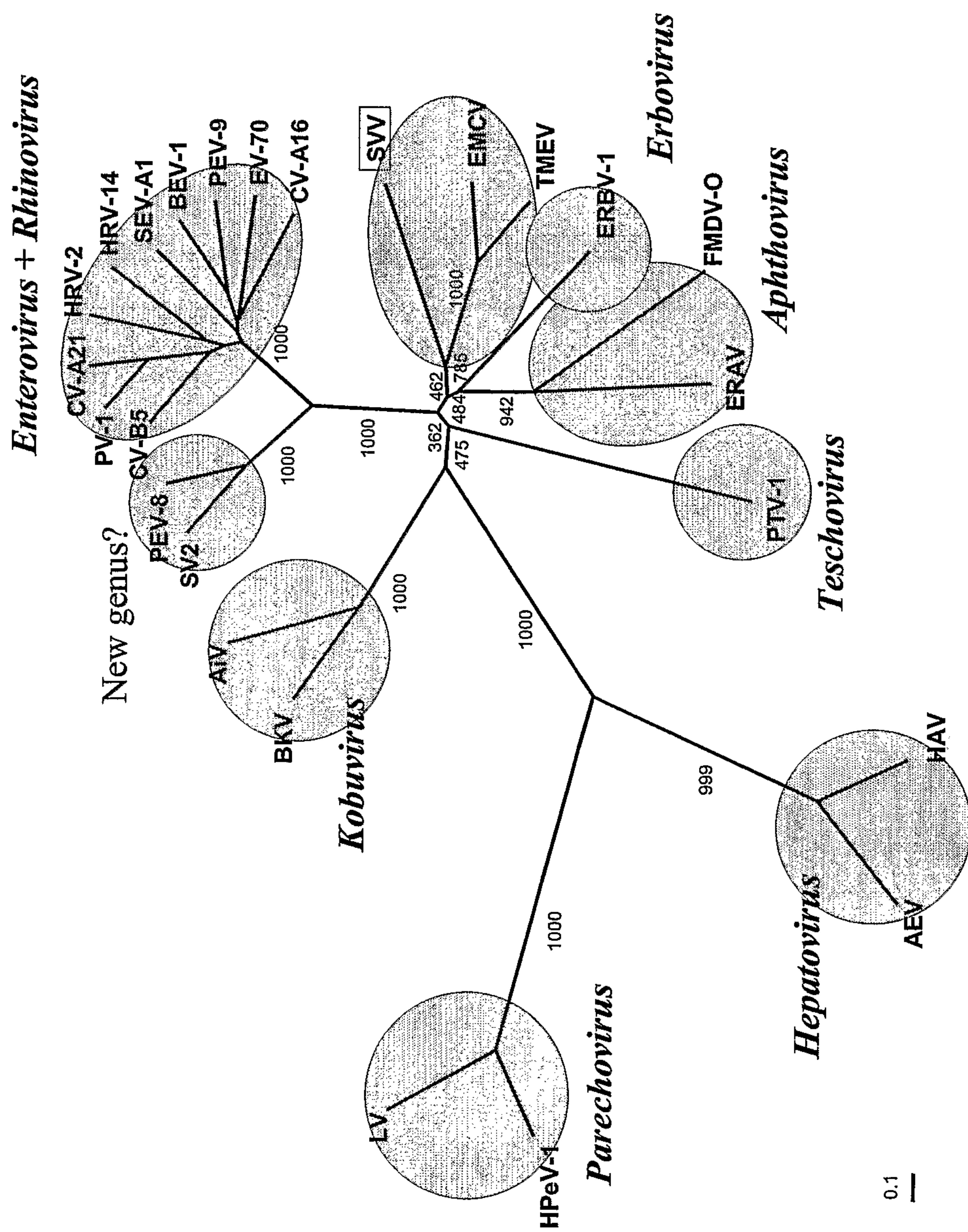


Fig. 34

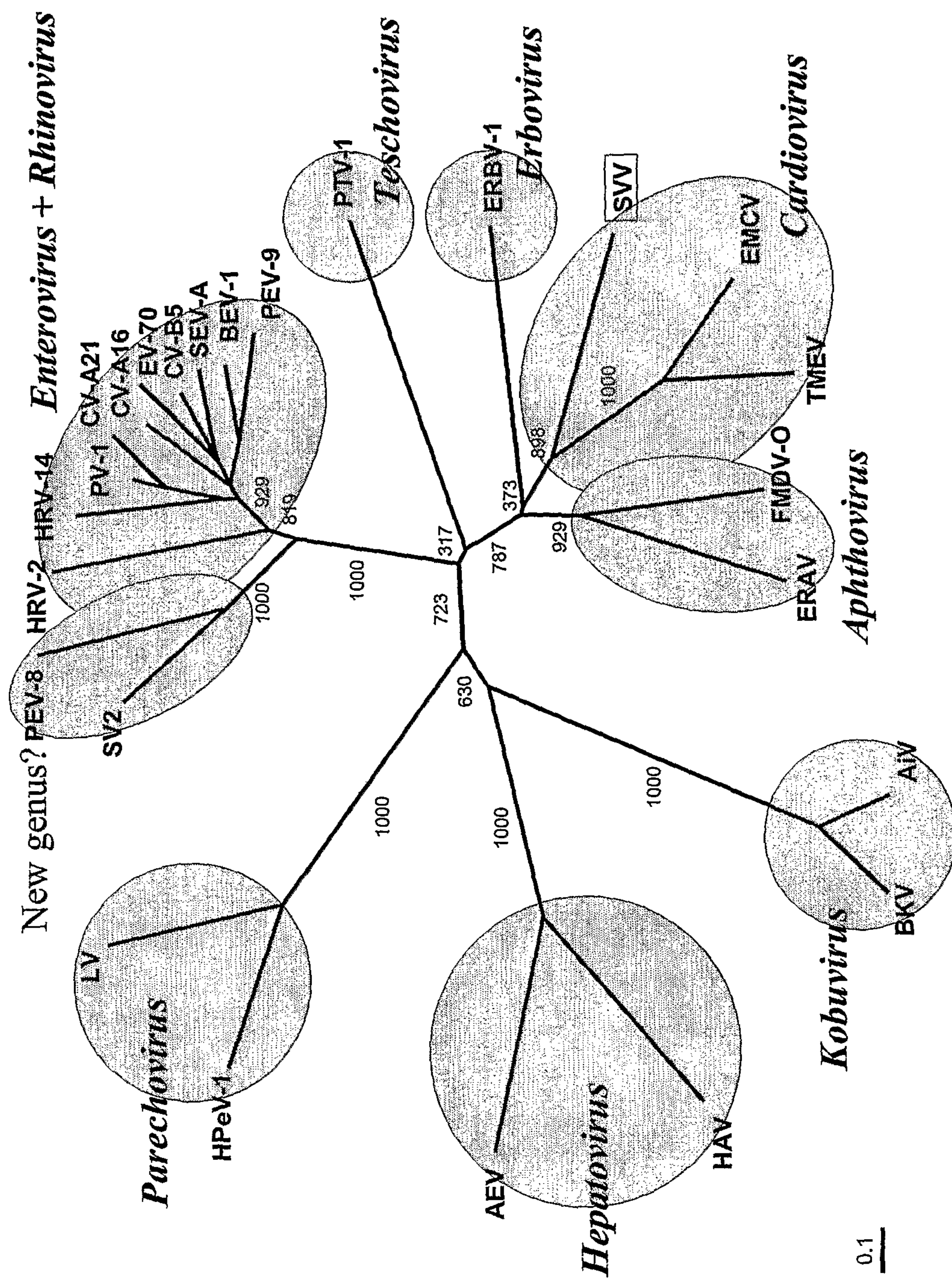


FIG. 35

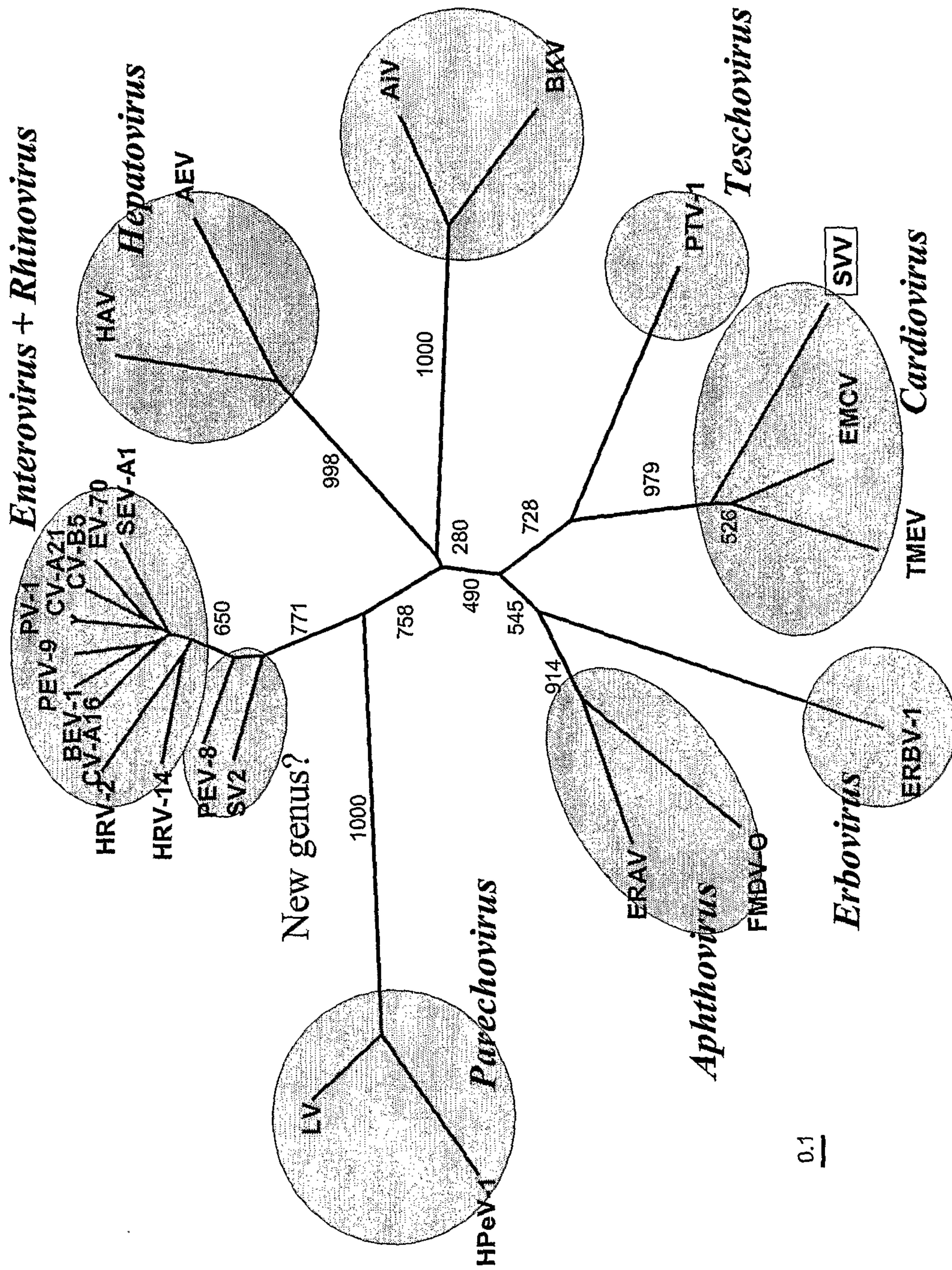


FIG. 36

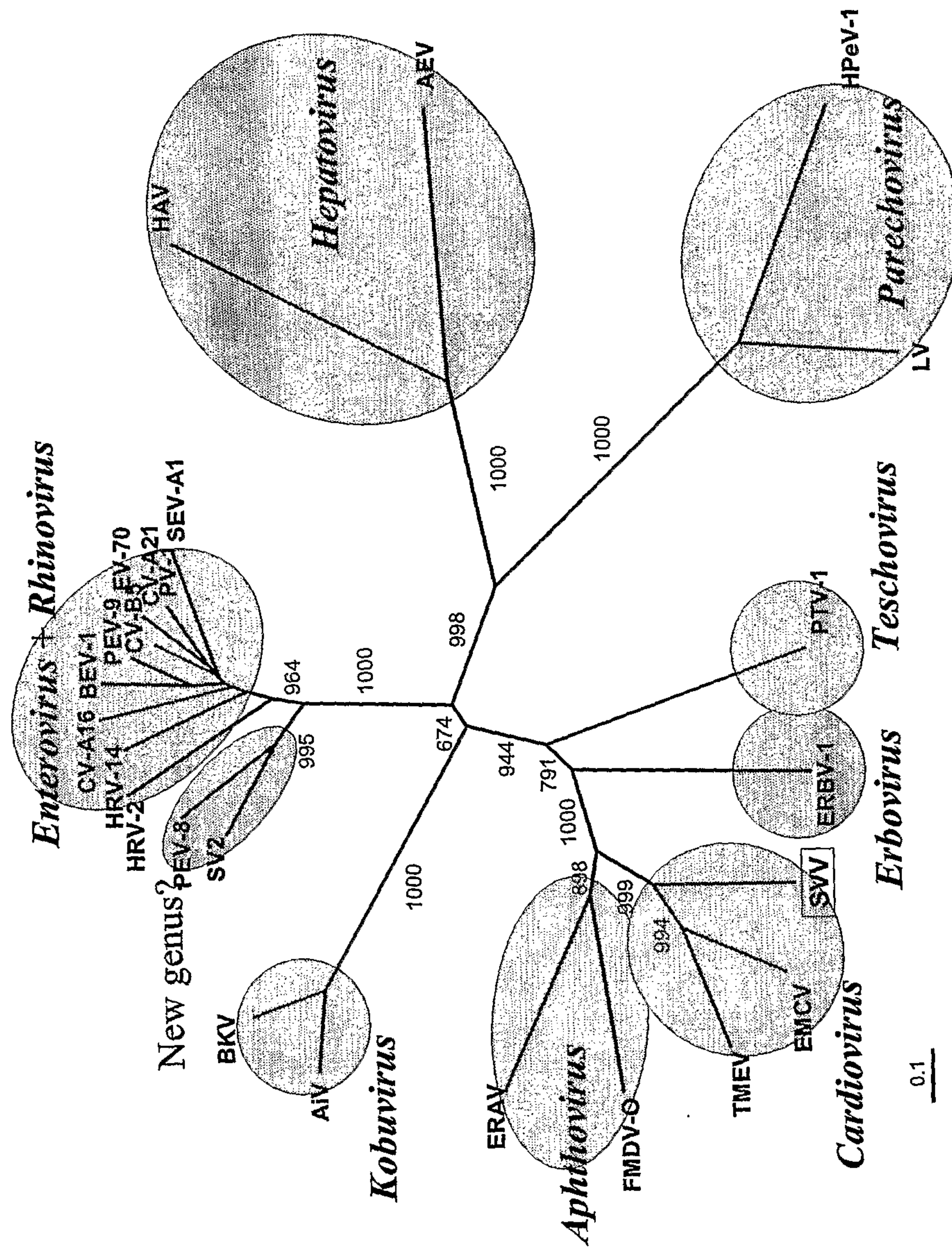


FIG. 37

	Cardiovirus			Aphthovirus		Erbo	Tescho	Enterovirus							Rhinovirus		Entero?	Kobu		Hepato		Parecho			
	SVV	EMCV	TMEV	FMDV-O	ERAV			ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1		PEV-9	HRV-14	HRV-2	SV2	PEV-8	AIV	BKV	HAV
SVV	100	33	35	24	26	30	24	24	24	29	29	30	35	25	27	26	25	31	31	27	21	20	19	18	18
EMCV	33	100	70	32	35	47	29	32	30	31	34	30	34	34	31	34	33	40	37	20	21	25	25	22	21
TMEV	35	70	100	33	34	45	31	35	34	30	32	31	34	33	31	31	28	36	37	21	21	25	25	23	21
FMDV-O	24	32	33	100	31	35	31	27	26	30	27	25	28	28	25	31	25	32	29	15	17	24	24	19	17
ERAV	26	35	34	31	100	35	36	32	31	28	35	30	31	28	27	29	30	39	34	22	22	20	24	18	22
ERBV	30	47	45	35	35	100	29	26	28	34	31	31	33	30	31	33	32	36	35	21	21	26	28	20	21
PTV-1	24	29	31	31	36	29	100	30	31	27	29	26	30	30	30	30	26	28	30	18	22	25	22	19	22
PV-1	24	32	35	27	32	26	30	100	71	52	52	51	55	54	48	53	51	42	44	21	23	19	21	18	20
CV-A21	24	30	34	26	31	28	31	71	100	51	53	52	52	53	47	54	55	43	46	19	23	23	22	20	19
CV-A16	29	31	30	30	28	34	27	52	51	100	52	53	54	54	52	52	48	49	48	18	19	22	22	19	17
CV-B5	29	34	32	27	35	31	29	52	53	52	100	55	54	53	49	56	51	51	48	20	17	23	22	20	18
EV-70	30	30	31	25	30	31	26	51	52	53	55	100	57	63	62	52	53	46	49	20	20	25	23	18	17
SEV-A	35	34	34	28	31	33	30	55	52	53	54	57	100	55	54	47	50	43	45	21	20	21	22	22	19
BEV-1	25	34	33	28	28	30	30	54	53	54	53	63	55	100	67	54	52	45	46	20	23	21	21	16	16
PEV-9	27	31	31	25	27	31	30	48	47	52	49	62	54	67	100	53	49	45	45	21	23	24	25	20	18
HRV-14	26	34	31	31	29	33	30	53	54	52	56	52	47	54	53	100	61	46	49	23	21	22	21	19	21
HRV-2	25	33	28	25	30	32	26	51	55	48	51	53	50	52	49	61	100	46	46	22	21	22	24	20	22
SV2	31	40	36	32	39	36	28	42	43	49	51	46	43	45	45	46	100	74	23	22	25	27	20	19	
PEV-8	31	37	37	29	34	35	30	44	46	48	48	49	45	46	45	49	46	74	100	24	22	25	26	18	21
AIV	27	20	21	15	22	21	18	21	19	18	20	20	21	20	21	23	22	23	24	100	56	19	18	16	16
BKV	21	21	21	17	22	21	22	23	23	19	17	20	20	23	23	21	21	22	22	55	100	16	18	16	16
HAV	20	25	25	24	20	26	25	19	23	22	23	25	21	21	24	22	22	25	25	19	16	100	57	18	23
AEV	19	25	25	24	24	28	22	21	22	22	22	23	22	21	25	21	24	27	26	18	18	57	100	18	23
HPeV-1	18	22	23	19	18	20	19	18	20	19	20	18	22	16	20	19	20	20	18	16	16	18	18	100	53
LV	18	21	21	17	22	21	22	20	19	17	18	17	19	16	18	21	22	19	21	16	16	23	23	53	100

Fig. 38

	Cardiovirus			Aphthovirus		Erbo	Tescho	Enterovirus							Rhinovirus		Entero?	Kobu		Hepato		Parecho				
	SVV	EMCV	TMEV	FMDV-O	ERAV	ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1	PEV-9	HRV-14	HRV-2	SV2	PEV-8	AEV	HAV	BKV	HPeV-1	LV		
SVV	100	42	39	32	34	43	31	32	30	25	29	26	30	25	29	28	28	35	30	26	28	19	17	17	19	
EMCV	42	100	67	29	38	39	35	28	31	29	31	31	29	31	30	26	31	31	35	27	26	19	18	17	18	
TMEV	39	67	100	31	37	35	33	29	29	31	29	27	27	27	28	26	30	31	35	27	28	18	17	17	20	
FMDV-O	32	29	31	100	39	31	23	26	23	26	27	25	26	25	24	26	23	30	32	20	25	14	15	14	17	
ERAV	34	38	37	39	100	38	25	25	24	20	27	23	23	23	25	24	27	31	32	25	24	20	15	15	19	
ERBV	43	39	35	31	38	100	30	27	29	27	28	27	30	29	28	28	28	34	34	27	29	21	19	18	17	
PTV-1	31	35	33	23	25	30	100	23	22	24	24	23	27	25	28	22	27	28	27	24	23	16	16	19	18	
PV-1	32	28	29	26	25	27	23	100	75	42	55	42	46	41	43	43	50	36	36	24	29	17	17	15	15	
CV-A21	30	31	29	23	24	29	22	75	100	43	61	42	46	42	45	48	53	36	35	24	26	20	13	13	13	
CV-A16	25	29	31	26	20	27	24	42	43	100	48	51	50	55	54	40	41	40	38	24	26	13	14	14	14	
CV-B5	29	31	29	27	27	28	24	55	61	48	100	47	48	49	49	51	52	40	36	24	27	16	17	14	15	
EV-70	26	31	27	25	23	27	23	42	42	61	47	100	49	49	53	45	44	32	38	25	27	16	15	15	15	
SEV-A	30	29	27	26	23	30	27	46	46	50	48	49	100	48	53	41	43	37	37	28	28	13	14	14	15	
BEV-1	25	31	27	25	23	29	25	41	42	55	49	49	48	100	63	45	45	40	40	23	25	11	12	13	17	
PEV-9	29	30	28	24	25	28	28	43	45	54	49	53	53	63	100	43	48	39	39	22	27	15	12	14	13	
HRV-14	28	26	26	26	24	28	22	43	48	40	51	45	41	45	43	100	52	33	33	26	27	16	17	14	19	
HRV-2	28	31	30	23	27	28	27	50	53	41	52	44	43	45	48	52	100	35	38	25	28	19	16	14	17	
SV2	35	31	31	30	31	34	28	36	36	40	40	32	37	40	39	33	35	100	63	25	29	18	16	21	21	
PEV-8	30	35	35	32	32	34	27	36	35	38	36	38	37	40	39	33	38	63	100	24	30	18	18	18	18	
AEV	26	27	27	20	25	27	24	24	24	24	24	25	28	23	22	26	25	25	24	100	58	16	17	19	19	
BKV	28	26	28	25	24	29	23	29	26	26	27	27	28	25	27	27	28	29	30	58	100	17	20	16	19	
HAV	19	19	18	14	20	21	16	17	16	13	16	16	13	11	15	16	19	18	18	16	17	100	53	18	19	
AEV	17	18	17	15	15	19	16	17	20	14	17	15	14	12	12	17	16	16	17	18	20	53	100	20	20	
HPeV-1	17	17	17	14	15	18	19	15	13	13	14	15	14	13	14	14	14	16	18	17	16	18	20	55	55	
LV	19	18	20	17	19	17	18	15	13	14	15	15	15	17	13	19	17	21	18	19	19	19	20	100	55	100

Fig. 39

	Cardiovirus			Aphthovirus		Erbo	Teschol	Enterovirus					Rhinovirus		Entero?		Kobu		Hepato		Parecho			
	SVV	EMCV	TMEV	FMDV-O	ERAV	ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1	PEV-9	HRV-14	HRV-2	SV2	PEV-8	AiV	BKV	HAV	AEV	HPeV-1
SVV	100	20	21	12	13	13	11	17	16	16	16	14	17	13	13	18	16	14	13	13	9	10	11	8
EMCV	20	100	48	11	15	16	15	16	16	14	14	15	18	17	17	17	15	16	16	14	13	12	12	11
TMEV	21	48	100	12	15	15	14	15	16	16	13	13	17	14	15	16	15	16	15	17	14	15	13	9
FMDV-O	12	11	12	100	23	13	9	11	13	12	11	11	11	10	11	10	13	9	12	12	8	11	10	10
ERAV	13	15	15	23	100	18	12	13	14	16	14	11	14	14	12	14	14	10	14	11	9	9	9	11
ERBV	13	16	15	13	18	100	13	13	13	14	15	15	13	12	11	12	12	12	13	17	12	10	12	13
PTV-1	11	15	14	9	12	13	100	19	18	21	19	19	18	17	17	18	17	17	17	16	12	12	9	13
PV-1	17	16	15	11	13	13	19	100	67	34	46	34	41	36	32	46	36	23	21	16	16	10	10	14
CV-A21	16	16	16	13	14	13	18	67	100	36	43	37	39	33	35	42	40	24	24	15	13	10	14	11
CV-A16	16	14	16	12	16	14	21	34	36	100	37	38	42	43	40	35	37	27	24	15	15	12	13	8
CV-B5	16	14	13	11	14	15	19	46	43	37	100	42	40	38	32	38	42	27	25	14	16	13	12	9
EV-70	14	15	13	11	11	15	19	34	37	38	42	100	37	39	33	38	37	24	23	13	13	16	12	13
SEV-A	17	18	17	11	14	13	18	41	39	42	40	37	100	39	40	39	40	25	25	13	13	13	13	14
BEV-1	13	17	14	10	14	12	17	35	33	43	38	39	39	100	46	37	35	25	25	15	14	15	11	12
PEV-9	13	17	15	11	12	11	17	32	35	40	32	33	40	45	100	36	34	23	22	13	13	12	11	12
HRV-14	18	17	16	10	14	12	18	46	42	35	38	38	39	37	36	100	42	23	22	15	16	11	13	14
HRV-2	16	15	15	13	14	12	17	36	40	37	42	37	40	35	34	42	100	24	21	16	14	13	14	9
SV2	14	16	16	9	10	12	17	23	24	27	27	24	25	25	23	23	24	100	46	15	12	11	12	11
PEV-8	13	16	15	12	14	13	17	21	24	24	25	23	25	25	22	22	21	46	100	14	12	13	12	8
AiV	13	14	17	12	11	17	16	16	15	15	14	13	13	15	13	15	16	15	14	100	28	11	13	11
BKV	13	13	14	8	9	12	12	16	13	15	16	13	13	14	13	16	14	12	12	28	100	11	11	10
HAV	9	12	15	11	9	10	12	10	10	12	13	16	13	15	12	11	13	11	13	11	11	100	43	15
AEV	10	12	13	10	9	12	9	10	14	13	12	12	13	11	11	13	14	12	12	13	11	43	100	18
HPeV-1	11	11	9	11	9	13	13	14	14	13	13	13	14	12	12	14	14	12	13	11	13	15	18	43
LV	8	11	11	10	11	11	12	10	11	8	9	12	12	10	9	10	9	11	8	10	10	14	14	100

Fig. 40

	Cardiovirus			Aphthovirus		Erbo	Tescho	Enterovirus					Rhinovirus		Entero?	Kobu		Hepato	Parecho						
	SVV	EMCV	TMEV	FMDV-O	ERAV	ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1	PEV-9	HRV-14	HRV-2	SV2	PEV-8	AIV	BKV	HAV	AEV	HPeV-1	LV
SVV	100	34	33	26	27	30	22	23	22	22	24	22	24	21	22	22	21	25	24	22	22	15	15	14	12
	34	100	59	25	28	31	25	22	21	22	24	22	23	23	22	21	23	24	25	21	21	15	16	15	14
	33	59	100	27	29	30	24	23	22	24	21	21	22	22	22	22	22	24	26	24	24	15	15	14	14
FMDV-O	26	25	27	100	33	27	20	21	20	21	20	20	22	19	18	22	19	22	23	17	19	13	12	13	12
ERAV	27	28	29	33	100	32	23	21	20	17	23	19	20	20	20	21	21	25	25	22	20	16	15	13	15
ERBV	30	31	30	27	32	100	24	22	23	23	24	23	24	23	22	22	21	26	26	24	21	16	16	15	13
PTV-1	22	25	24	20	23	24	100	19	20	20	19	20	22	19	22	19	19	20	20	20	20	15	13	13	14
PV-1	23	22	23	21	21	22	19	100	66	39	50	40	44	40	39	44	43	29	30	18	22	14	14	11	11
CV-A21	23	21	22	20	20	23	20	66	100	40	51	42	42	40	40	44	44	29	32	18	20	14	15	10	11
CV-A16	22	22	24	21	17	23	20	39	40	100	43	47	46	49	46	40	40	33	32	19	20	12	11	11	11
CV-B5	24	24	21	20	23	24	19	50	51	43	100	46	44	44	41	45	46	35	35	19	20	14	14	12	10
EV-70	22	22	21	20	19	23	20	40	42	47	46	100	45	47	45	42	41	30	32	20	20	14	12	13	11
SEV-A	24	23	22	22	20	24	22	44	42	46	44	45	100	46	48	41	41	32	33	21	21	12	12	12	11
BEV-1	21	23	22	19	20	23	19	40	40	49	44	47	46	100	56	42	42	33	33	19	20	11	11	11	13
PEV-9	22	22	22	18	20	22	22	39	40	46	41	45	48	56	100	41	42	32	32	18	20	12	10	12	10
HRV-14	22	21	22	22	21	22	19	44	44	40	45	42	41	42	41	100	47	30	29	20	20	14	14	12	13
HRV-2	21	23	22	19	21	21	19	43	44	40	46	41	41	42	42	47	100	30	30	20	20	14	12	11	12
SV2	25	24	24	22	25	26	20	29	29	33	35	30	32	33	32	30	30	100	57	19	21	15	14	14	14
PEV-8	24	25	26	23	25	26	20	30	32	32	35	32	33	33	32	29	30	57	100	18	22	16	15	14	13
AiV	22	21	24	17	22	24	20	18	18	19	19	20	21	19	18	20	20	19	18	100	46	14	14	14	14
BKV	22	21	24	19	20	21	20	22	20	20	20	20	21	20	20	20	20	21	22	46	100	13	15	13	13
HAV	15	15	15	13	16	16	15	14	14	12	14	14	12	11	12	14	14	15	16	14	13	100	49	16	15
AEV	15	16	15	12	15	16	13	14	15	11	14	12	12	11	10	14	12	14	15	49	100	13	15	18	17
HPeV-1	14	15	14	13	13	15	13	11	10	11	12	13	12	11	12	12	11	14	14	14	13	16	18	100	50
V	12	14	14	12	15	13	14	11	11	11	10	11	11	13	10	13	12	14	13	14	13	15	17	50	100

* not including VP4 or part of VP2

Fig. 41

	Cardiovirus			Aphthovirus		Erbo	Tescho	Enterovirus							Rhinovirus		Enterov?	Kobu		Hepato		Parech		
	SVV	EMCV	TMEV	FMDV-O	ERAV	ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1	PEV-9	HRV-14	HRV-2	SV2	PEV-8	AIV	BKV	HAV	AEV	HPeV-1
VV	100	41	39	35	34	35	32	29	28	30	29	30	26	26	26	29	27	28	29	26	23	25	26	21
MCV	41	100	60	37	36	35	30	27	27	29	28	28	27	27	27	25	27	26	25	23	24	27	25	20
MEV	39	60	100	36	38	35	31	28	26	26	29	28	26	25	24	27	26	29	25	23	23	26	25	20
MDV-O	35	37	36	100	47	38	30	31	28	32	30	32	30	31	27	31	28	31	31	27	28	25	24	20
RAV	34	36	38	47	100	37	35	31	28	27	29	31	28	28	27	27	28	28	30	25	25	24	24	22
RBV	35	35	35	38	37	100	27	29	27	30	30	29	30	27	30	28	26	27	27	23	23	27	24	23
TV-1	32	30	31	30	35	27	100	27	28	30	30	29	28	29	27	27	29	28	28	27	26	21	22	21
V-1	29	27	28	31	31	29	27	100	79	64	63	62	58	59	58	61	49	45	39	24	22	20	23	24
V-A21	28	27	26	28	28	27	28	79	100	58	59	56	55	56	56	56	43	41	38	24	21	19	22	23
V-A16	30	29	26	32	27	30	30	64	58	100	66	60	63	64	58	55	48	44	40	24	23	22	24	24
V-B5	29	28	29	30	29	30	30	63	59	66	100	67	71	63	59	57	46	43	37	23	23	22	24	26
V-70	30	28	28	32	31	29	29	62	56	60	67	100	65	59	56	53	45	43	39	24	23	21	22	25
EV-A	26	27	26	30	28	30	28	58	55	53	71	65	100	61	60	54	45	42	40	21	22	21	22	25
BEV-1	26	27	25	31	28	27	29	59	56	64	63	59	61	100	67	53	47	44	40	23	23	21	21	24
PEV-9	26	27	24	27	27	30	27	58	56	58	59	56	60	67	100	52	42	41	38	22	21	22	22	23
HRV-14	29	25	27	31	27	28	27	61	56	55	57	53	54	53	52	100	47	42	40	21	20	21	19	22
HRV-2	27	27	26	28	28	26	29	49	43	48	46	45	45	47	42	47	100	40	37	22	21	21	20	22
SV2	28	26	29	31	28	27	28	45	41	44	43	43	42	44	41	42	40	100	52	22	22	22	20	21
PEV-8	29	25	25	31	30	27	28	39	38	40	37	39	40	40	38	40	37	52	100	21	20	21	20	21
AIV	26	23	23	27	25	23	27	24	24	24	23	24	21	23	22	21	22	22	21	100	69	24	22	22
BKV	23	24	23	28	25	23	26	22	21	23	23	23	22	23	21	20	21	22	20	69	100	25	23	22
HAV	25	27	26	25	24	27	21	20	19	22	22	21	21	21	22	21	21	22	21	24	25	100	41	20
AEV	26	25	25	24	24	24	22	23	22	24	24	22	22	21	22	19	20	20	20	22	23	41	100	22
HPeV-1	21	20	20	20	22	23	21	24	23	24	26	25	25	24	23	22	22	21	21	22	22	20	22	100
LV	20	21	21	21	20	24	24	23	21	24	25	23	25	24	21	23	24	21	20	22	22	21	23	50

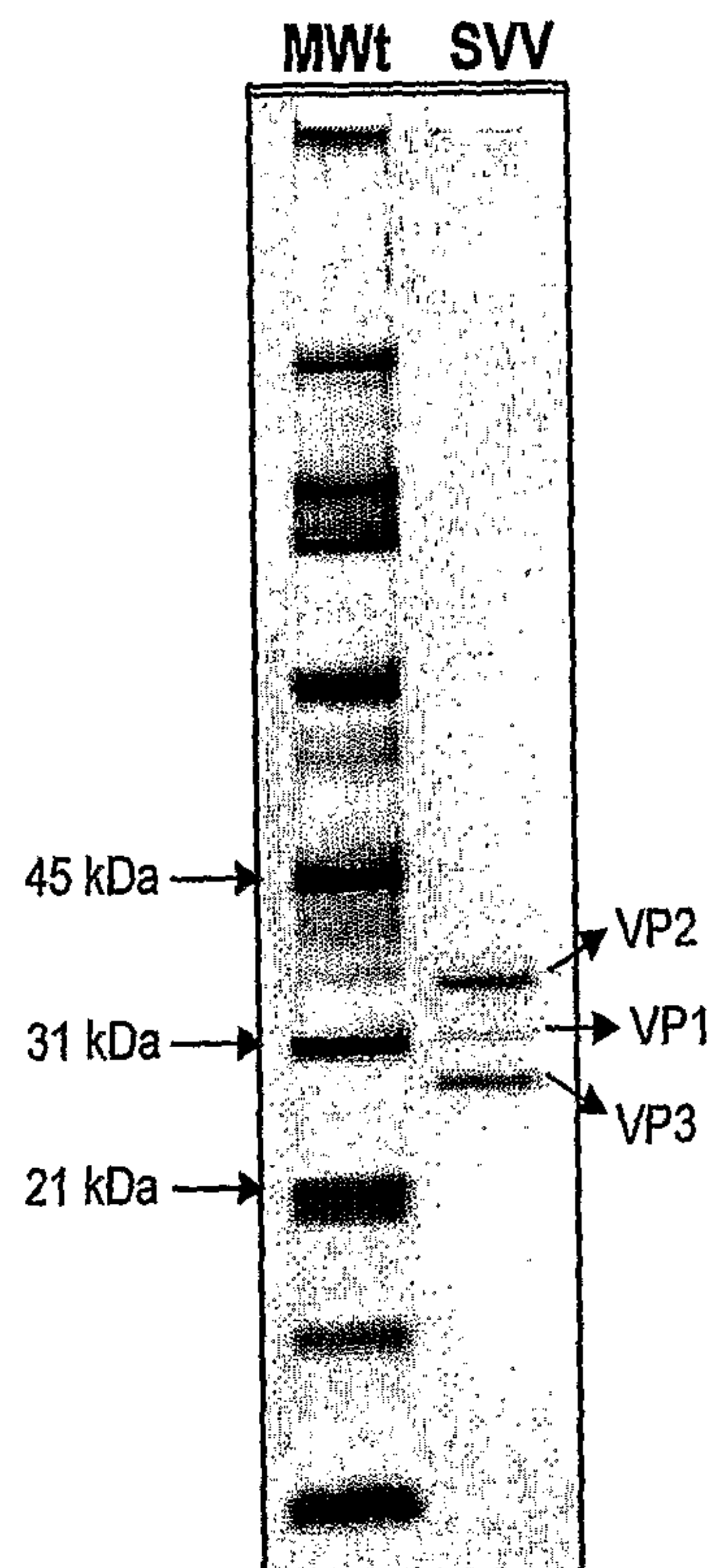
Fig. 42

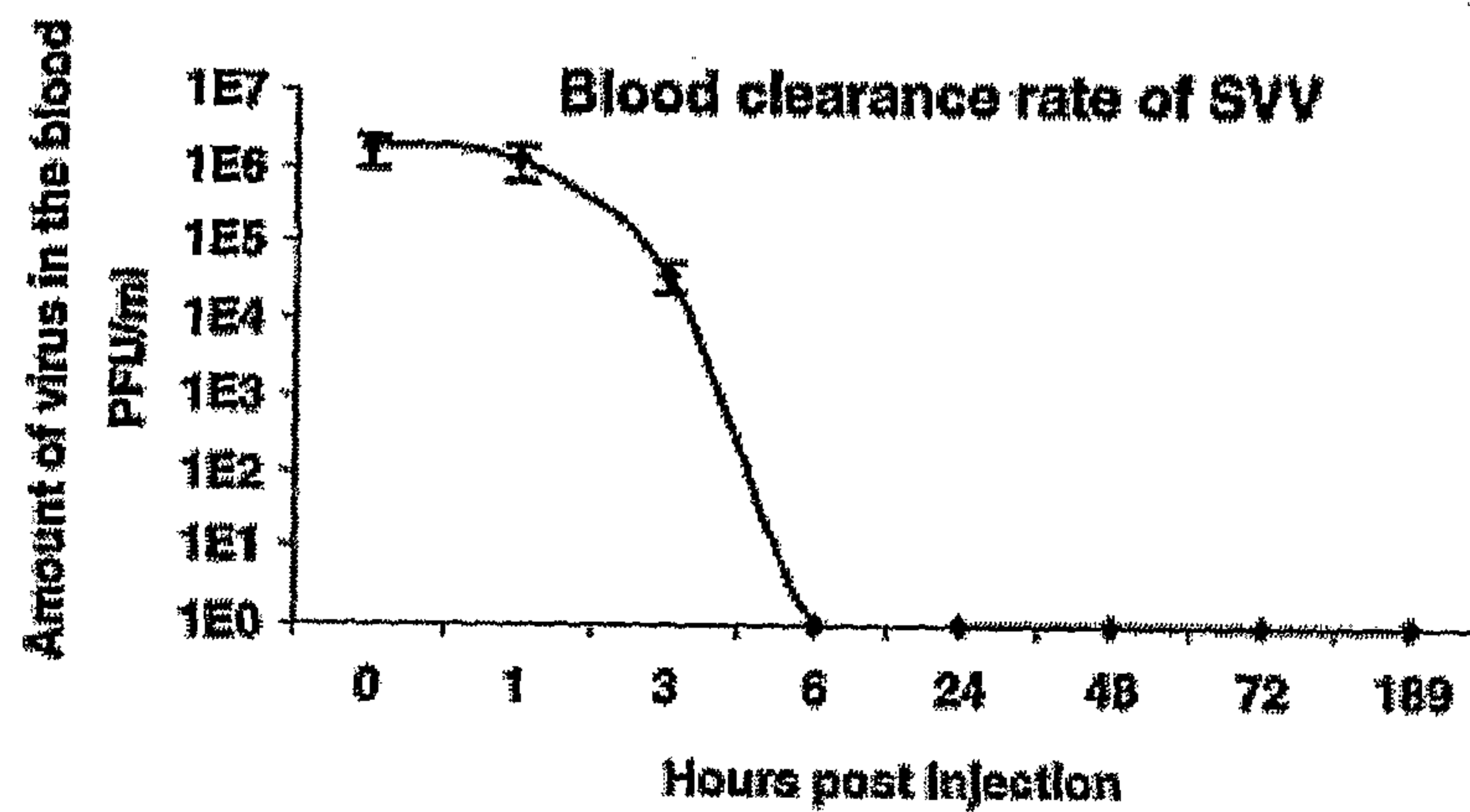
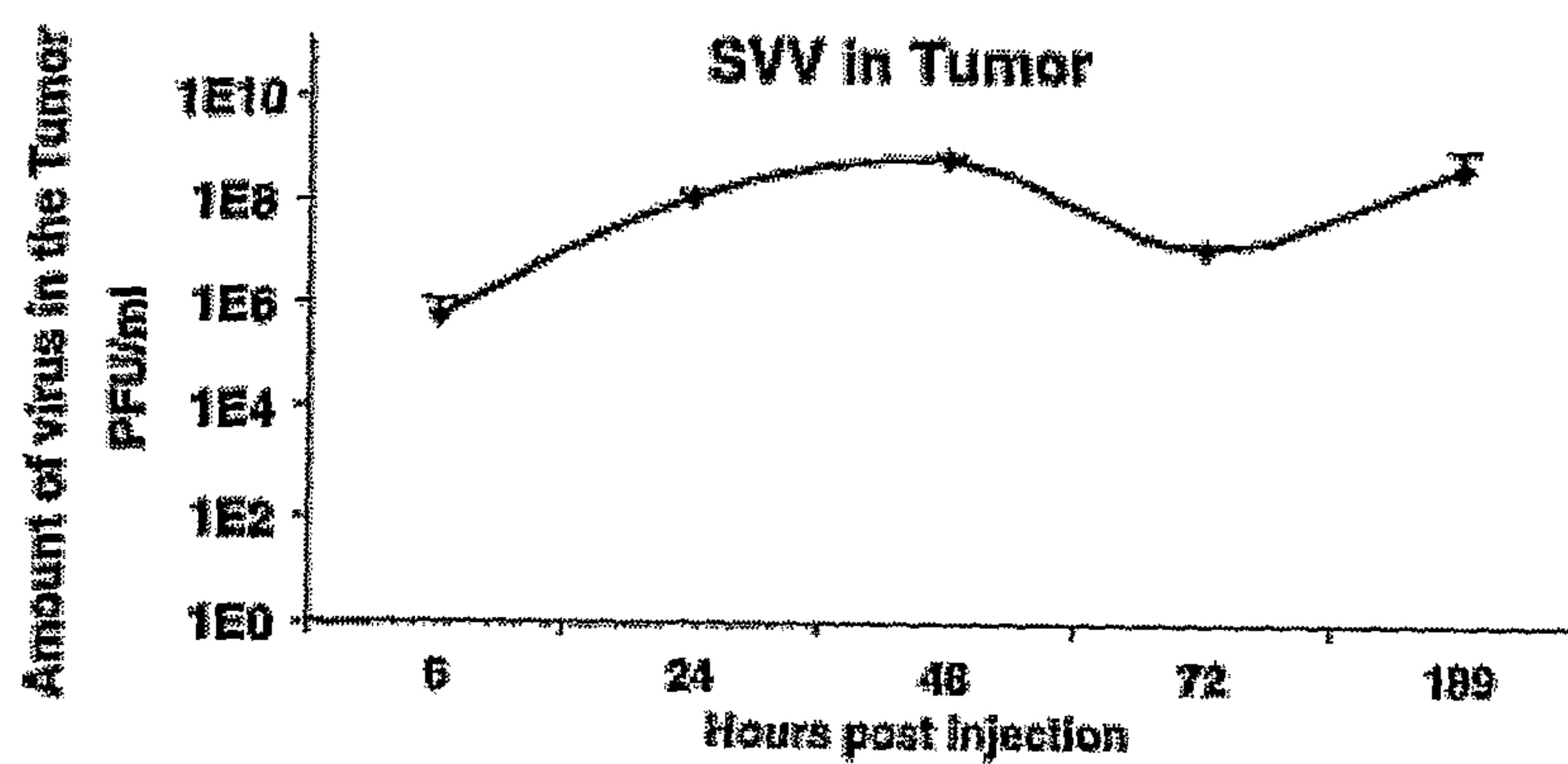
Cardiovirus			Aphthovirus		Erbo	Tescho	Enterovirus					Rhinovirus		Entero?	Kobu		Hepato	Parecho						
SVV	EMCV	TMEV	FMDV-O	ERAV	ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1	PEV-9	HRV-14	HRV-2	SV2	PEV-8	AiV	BKV	HAV	AEV	HPeV-1	LV
100	38	37	24	21	19	27	19	18	14	16	17	14	18	19	18	15	17	16	16	14	17	16	13	14
38	100	48	27	27	18	24	18	18	18	20	20	19	17	20	19	20	19	19	15	16	22	18	17	19
37	48	100	20	28	20	25	19	20	20	18	20	16	19	18	15	18	17	17	16	17	17	16	16	19
24	27	20	100	40	23	20	19	19	22	17	19	17	22	23	18	20	20	20	16	13	20	20	13	15
21	27	28	40	100	25	22	21	20	20	20	21	22	20	18	19	20	21	22	19	20	22	22	15	17
19	18	20	23	25	100	18	19	20	16	17	17	17	20	16	18	14	20	17	18	20	15	20	14	16
27	24	25	20	22	18	100	20	19	20	19	20	19	20	20	22	21	18	21	16	17	20	15	13	14
19	18	19	19	21	19	20	100	95	55	60	60	56	57	60	46	44	43	47	21	17	18	18	18	18
18	18	20	19	20	20	19	95	100	56	59	57	57	57	60	46	44	42	46	22	17	18	18	19	18
14	18	20	22	20	16	20	55	56	100	56	54	57	57	60	51	45	46	50	17	18	19	18	20	22
16	20	18	17	20	17	19	60	59	56	100	64	55	58	56	52	49	46	52	20	19	19	19	19	20
17	20	20	19	21	17	20	60	57	54	64	100	57	54	55	51	48	43	51	20	20	21	18	14	17
14	19	16	17	22	17	19	56	57	57	55	57	100	54	55	53	46	40	49	20	17	19	18	17	19
18	17	19	22	20	20	20	57	57	57	58	54	54	100	63	47	47	41	44	16	16	20	20	19	16
19	20	18	23	18	16	20	60	60	60	56	55	55	63	100	50	45	45	50	18	14	19	18	18	21
18	19	15	18	19	18	22	46	46	51	52	51	53	47	50	100	50	42	49	20	20	20	20	16	18
15	20	18	20	20	14	21	44	44	45	49	48	46	47	45	50	100	43	43	20	23	18	19	17	18
17	19	17	20	21	20	18	43	42	46	46	43	40	41	45	42	43	100	60	16	18	18	19	22	24
16	19	17	20	22	17	21	47	46	50	52	51	49	44	50	49	43	60	100	16	17	19	20	20	19
16	15	16	16	19	18	16	21	22	17	20	20	20	16	18	20	20	16	16	100	48	19	17	13	15
14	16	17	13	20	20	17	17	17	18	19	20	17	16	14	20	23	18	17	48	100	18	18	13	14
17	22	17	20	22	15	20	18	18	19	19	21	19	20	19	20	18	18	19	19	18	100	40	16	15
16	18	16	20	22	20	15	18	18	18	19	18	18	20	18	20	19	19	20	17	18	40	100	14	16
13	17	16	13	15	14	13	18	19	20	19	14	17	19	18	16	17	22	20	13	13	16	14	100	48
14	19	19	15	17	16	14	18	18	22	20	17	19	16	21	18	18	24	19	15	14	15	16	48	100

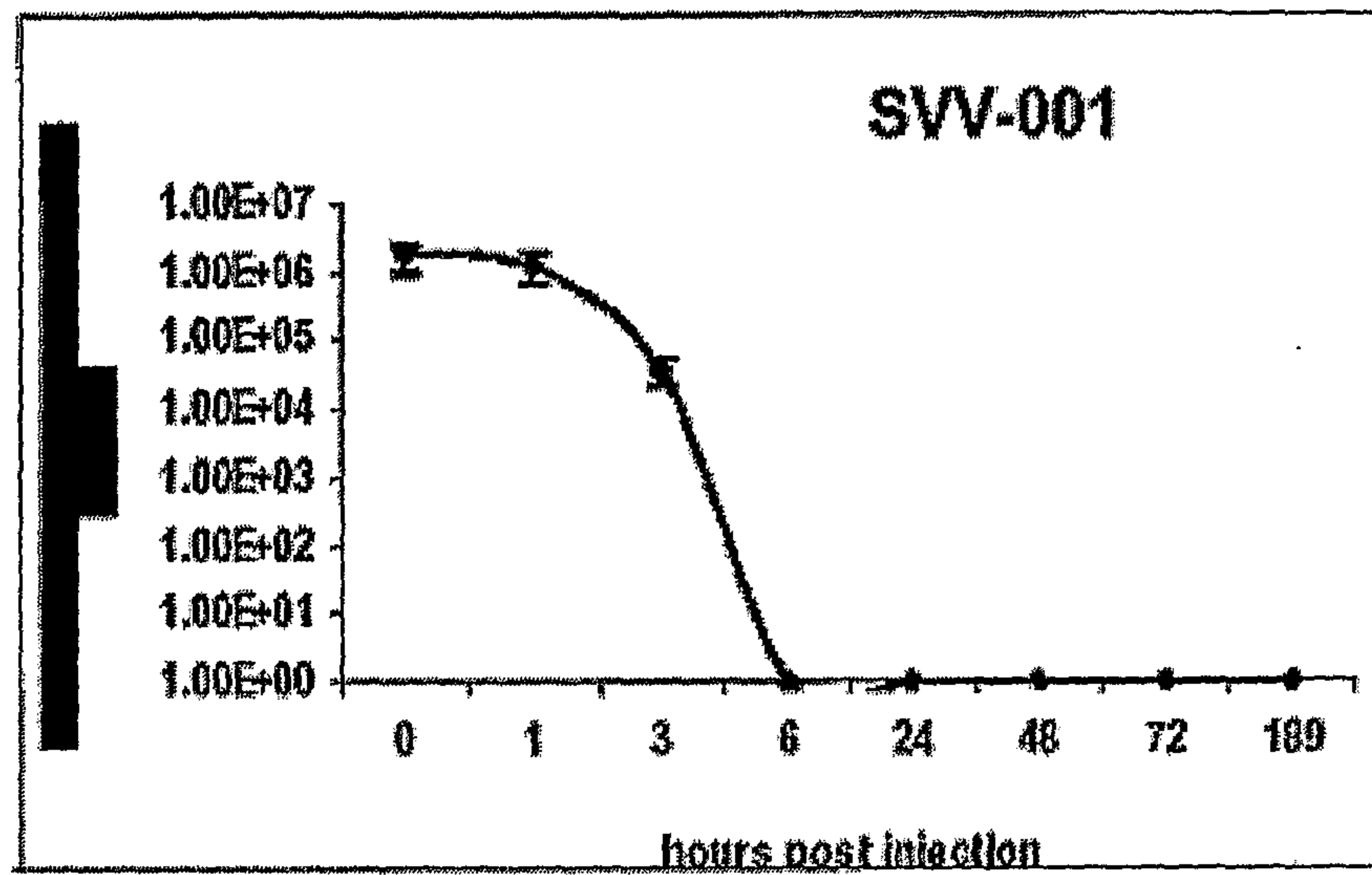
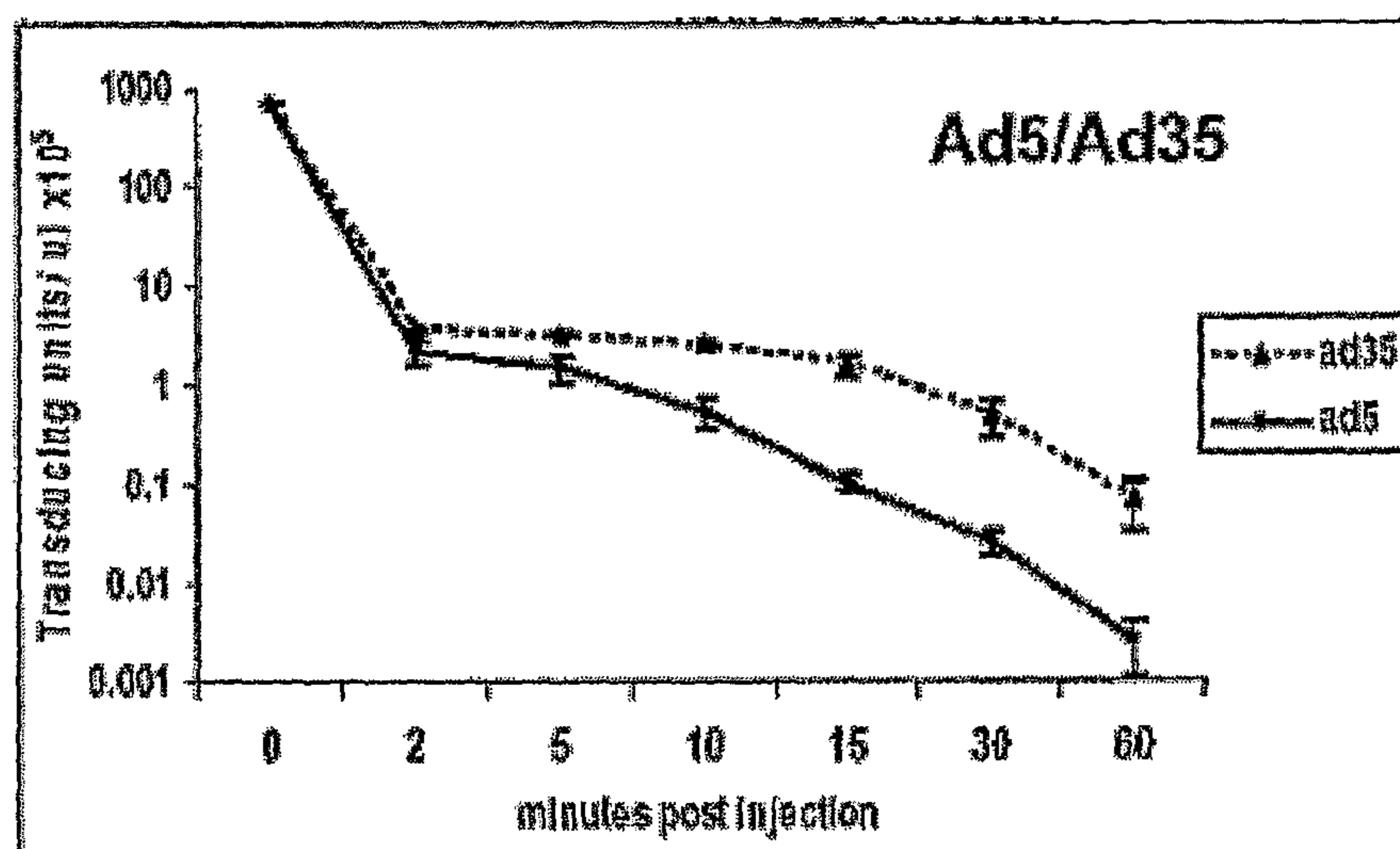
Fig. 43

	Cardiovirus			Aphthovirus		Erbo	Tescho	Enterovirus							Rhinovirus		Entero?	Kobu		Hepato		Parecho			
	SVV	EMCV	TMEV	FMDV-O	ERAV			ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1		PEV-9	HRV-14	HRV-2	SV2		PEV-8	AIv	BKV
SVV	100	58	58	47	48	42	40	33	32	30	30	32	31	31	32	34	31	32	31	33	32	24	24	23	23
	58	100	65	43	46	46	38	31	30	29	30	29	31	30	31	32	32	31	31	35	33	24	25	21	24
	58	65	100	42	47	42	38	30	29	28	30	30	30	30	29	31	31	30	32	34	32	24	26	22	25
FMDV-O	47	43	42	100	50	37	39	31	31	30	30	31	32	28	31	32	31	31	31	35	35	22	24	21	23
ERAV	48	46	47	50	100	41	37	29	29	29	29	31	31	29	29	31	30	29	30	34	35	25	24	22	24
ERBV	42	46	42	37	41	100	40	33	33	34	34	34	34	33	35	35	35	34	35	33	34	21	25	23	26
PTV-1	40	38	38	39	37	40	100	34	34	32	34	34	35	31	35	34	34	36	35	31	31	27	23	22	23
PV-1	33	31	30	31	29	33	34	100	98	69	75	76	67	68	71	64	57	56	55	33	32	27	27	23	27
CV-A21	32	30	29	31	29	33	34	98	100	68	75	75	66	68	71	64	57	56	55	33	32	27	26	23	27
CV-A16	30	29	28	30	29	33	32	69	68	100	67	65	59	62	66	61	54	55	53	33	35	27	26	23	26
CV-B5	30	30	30	30	29	34	34	75	75	67	100	76	69	70	71	66	57	57	55	32	32	25	25	23	27
EV-70	32	29	30	31	31	34	34	76	75	65	76	100	65	67	68	63	58	58	55	33	35	25	26	22	26
SEV-A	31	31	30	32	31	34	35	67	66	59	69	65	100	64	65	58	56	55	53	33	33	25	27	25	26
BEV-1	31	30	30	28	29	33	31	68	68	62	70	67	64	100	75	60	57	54	53	31	31	25	26	25	27
PEV-9	32	31	29	31	29	35	35	71	71	66	71	68	65	75	100	64	58	58	54	32	33	29	27	23	28
HRV-14	34	32	31	32	31	35	34	64	64	61	66	63	58	60	64	100	56	53	54	34	34	27	28	24	27
HRV-2	31	32	31	31	30	35	34	57	57	54	57	58	56	57	58	56	100	53	54	31	32	25	27	23	27
SV2	32	31	30	31	29	34	36	56	56	55	57	58	55	54	58	53	53	100	67	35	36	27	28	21	26
PEV-8	31	31	32	31	30	35	35	55	55	53	55	55	53	53	54	54	54	67	100	33	33	26	28	26	28
AIv	33	35	34	35	34	33	31	33	33	33	32	33	33	31	32	34	31	35	33	100	75	25	27	23	23
BKV	32	33	32	35	35	34	31	32	32	35	32	35	33	31	33	34	32	36	33	75	100	25	27	23	24
HAV	24	24	24	22	25	21	27	27	27	27	25	25	25	25	29	27	25	27	26	25	25	100	40	24	23
AEV	24	25	26	24	24	25	23	27	26	26	25	26	27	26	27	28	27	28	28	27	27	40	100	26	24
HPeV-1	23	21	22	21	22	23	22	23	23	23	23	22	25	25	23	24	23	21	26	23	23	24	26	100	50
LV	23	24	25	23	24	26	23	27	27	26	27	26	26	27	28	27	27	26	28	23	24	23	24	50	100

Fig. 44

**Fig. 45**

**FIG. 46A****FIG. 46B**

**Fig. 46C****Fig. 46D**

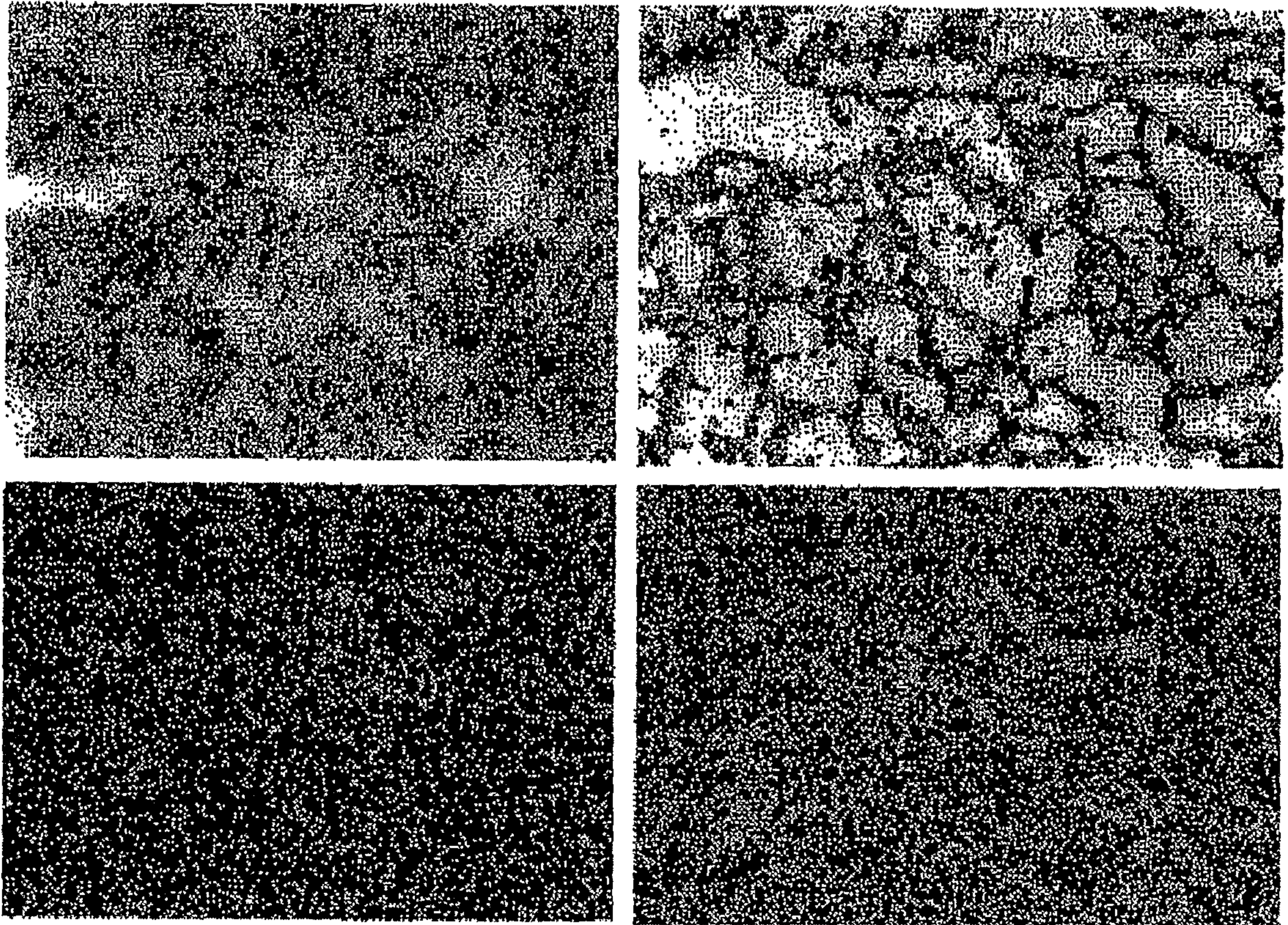
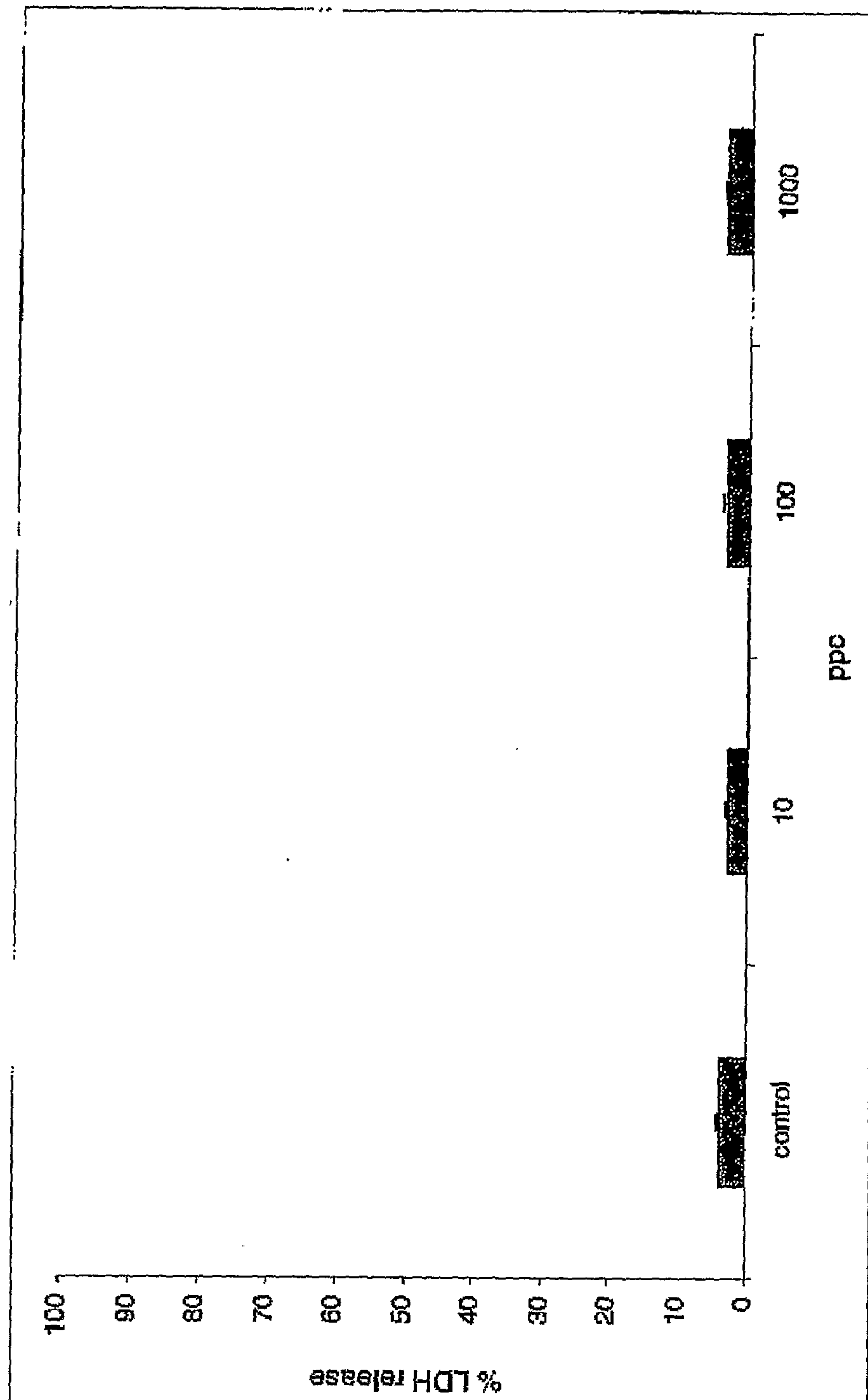


Fig. 47

**Fig. 48**

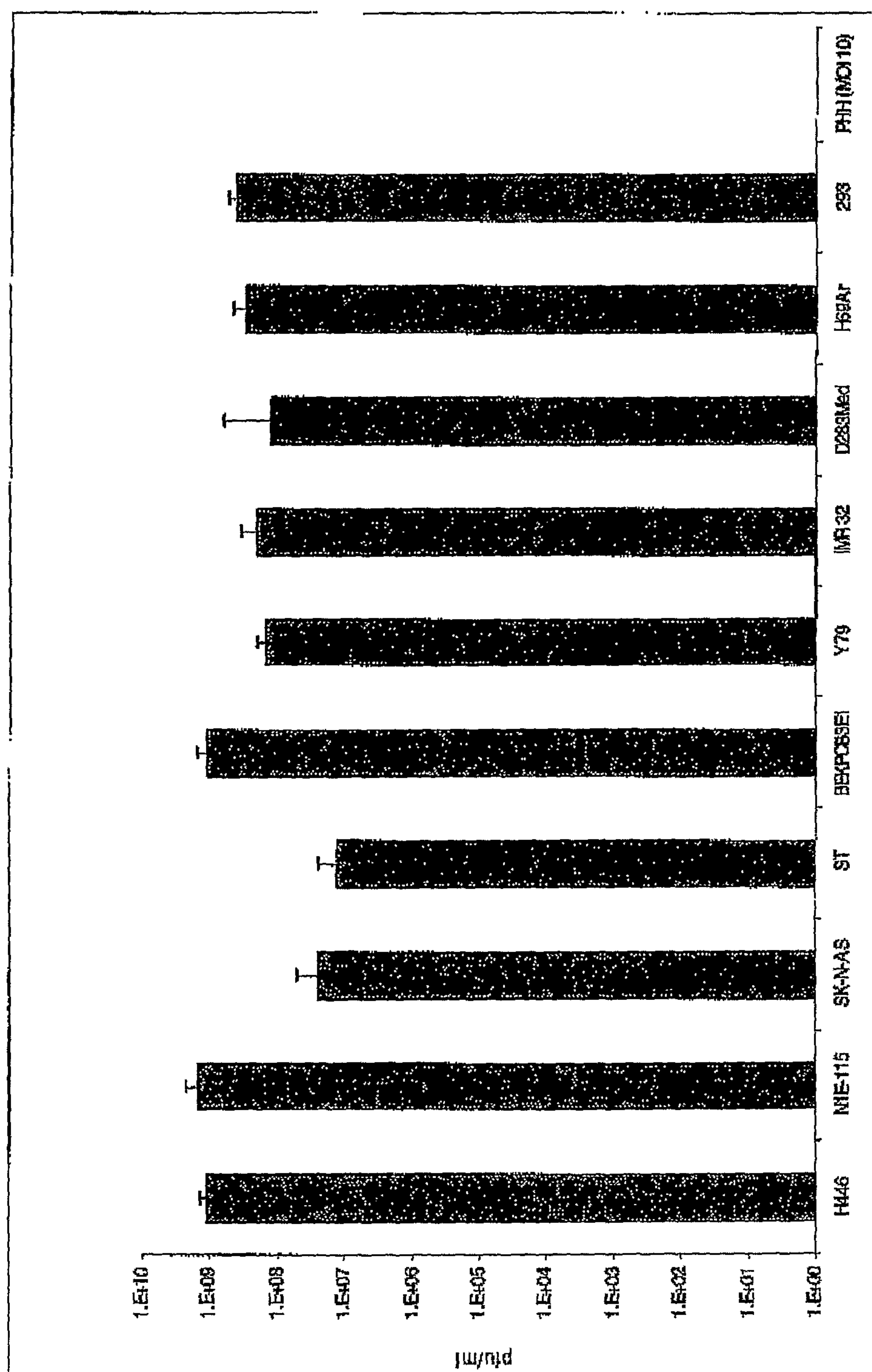


Fig. 49

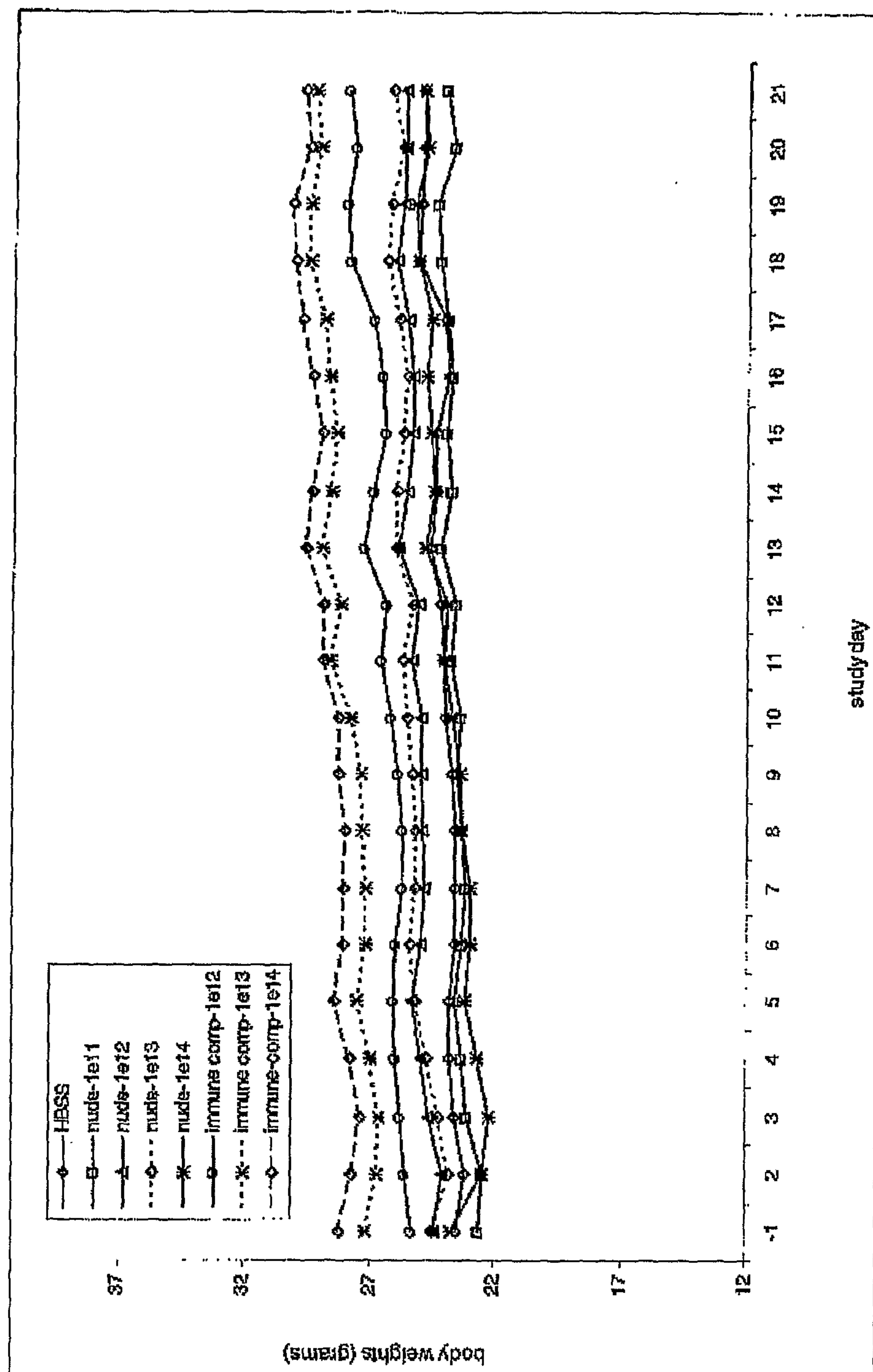


Fig. 50

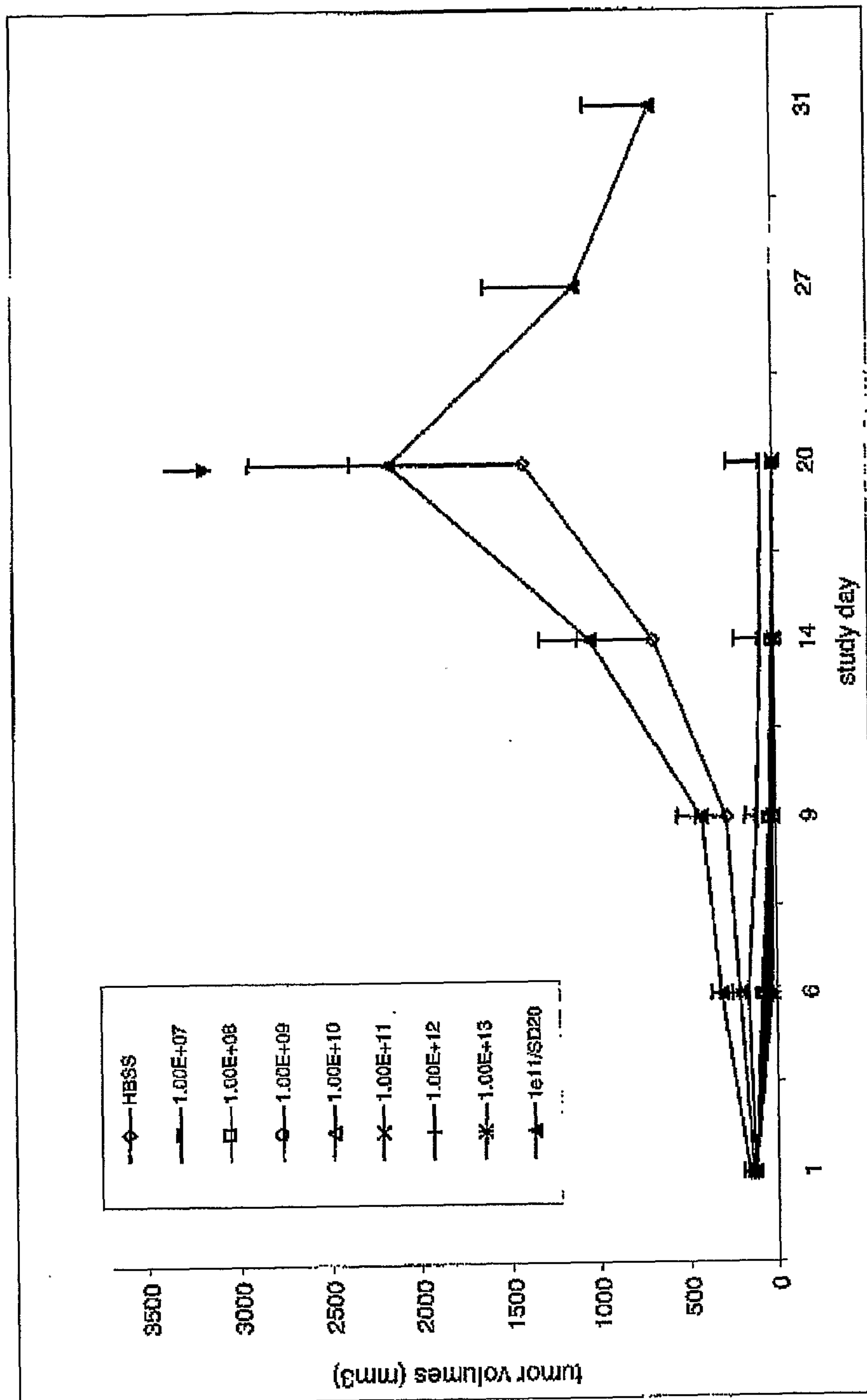


Fig. 51

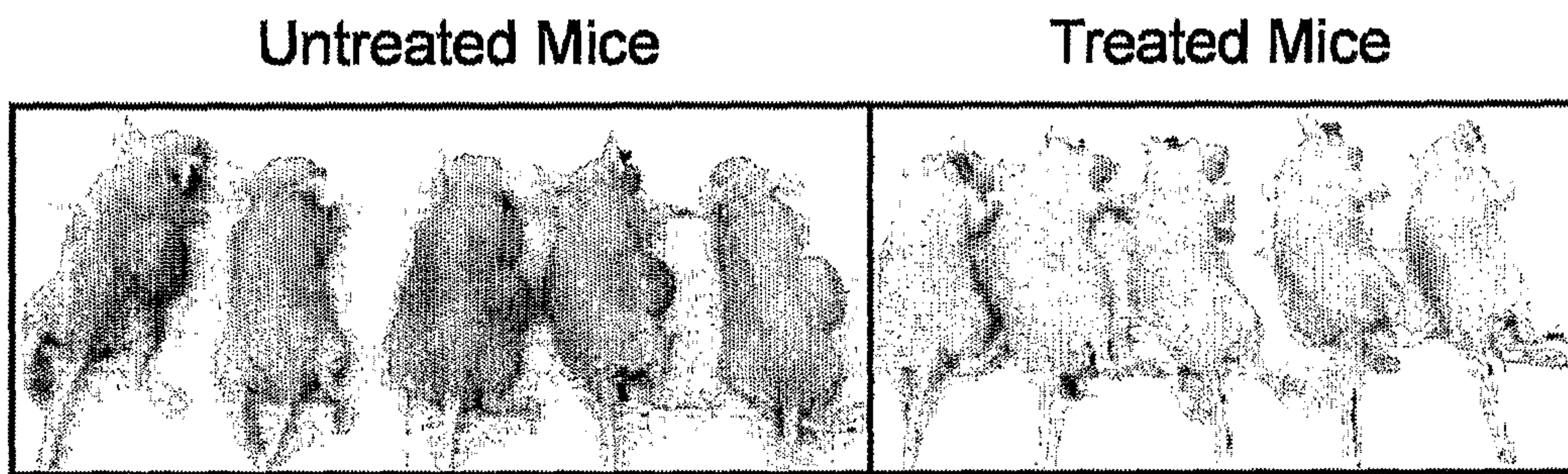
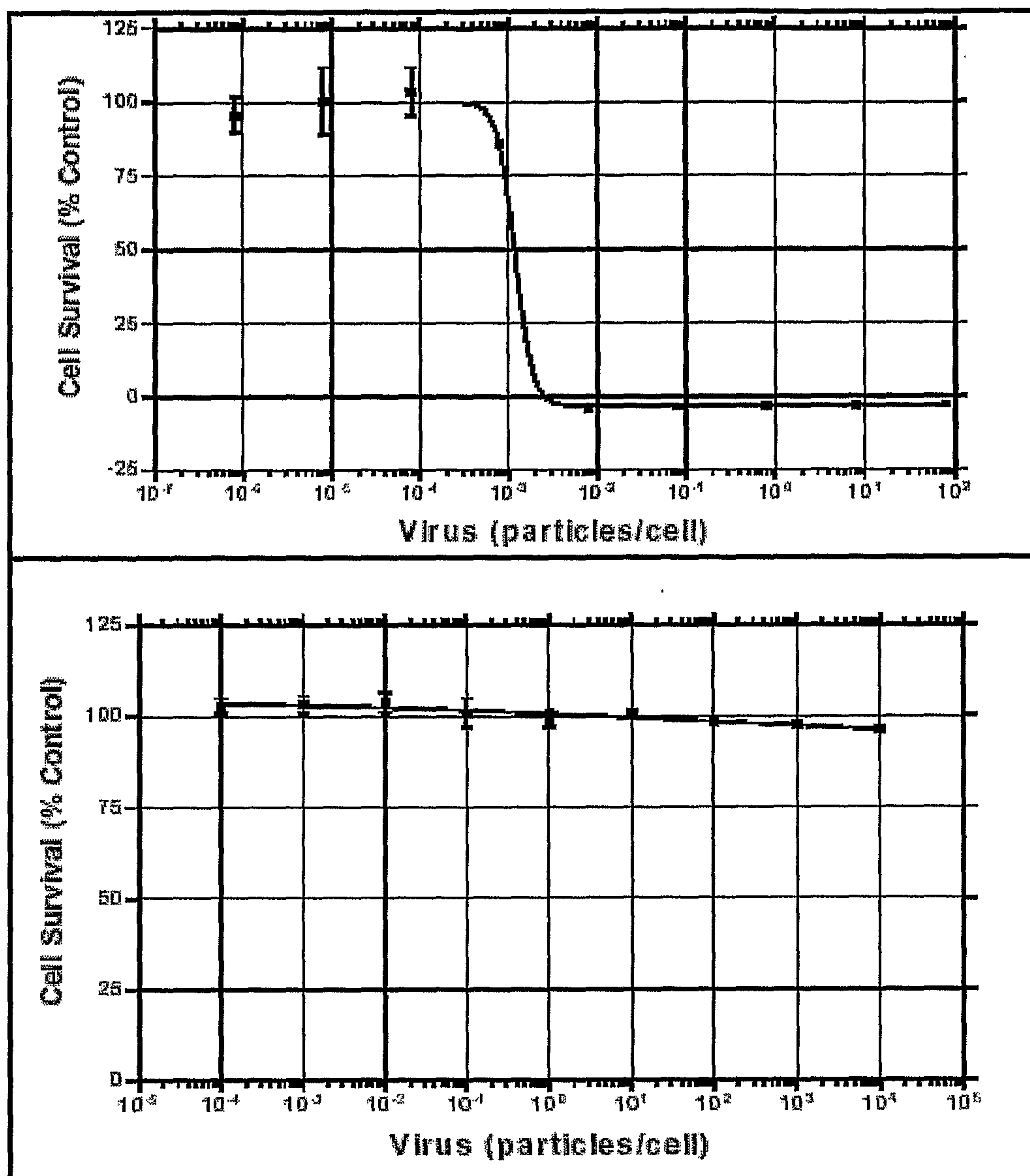
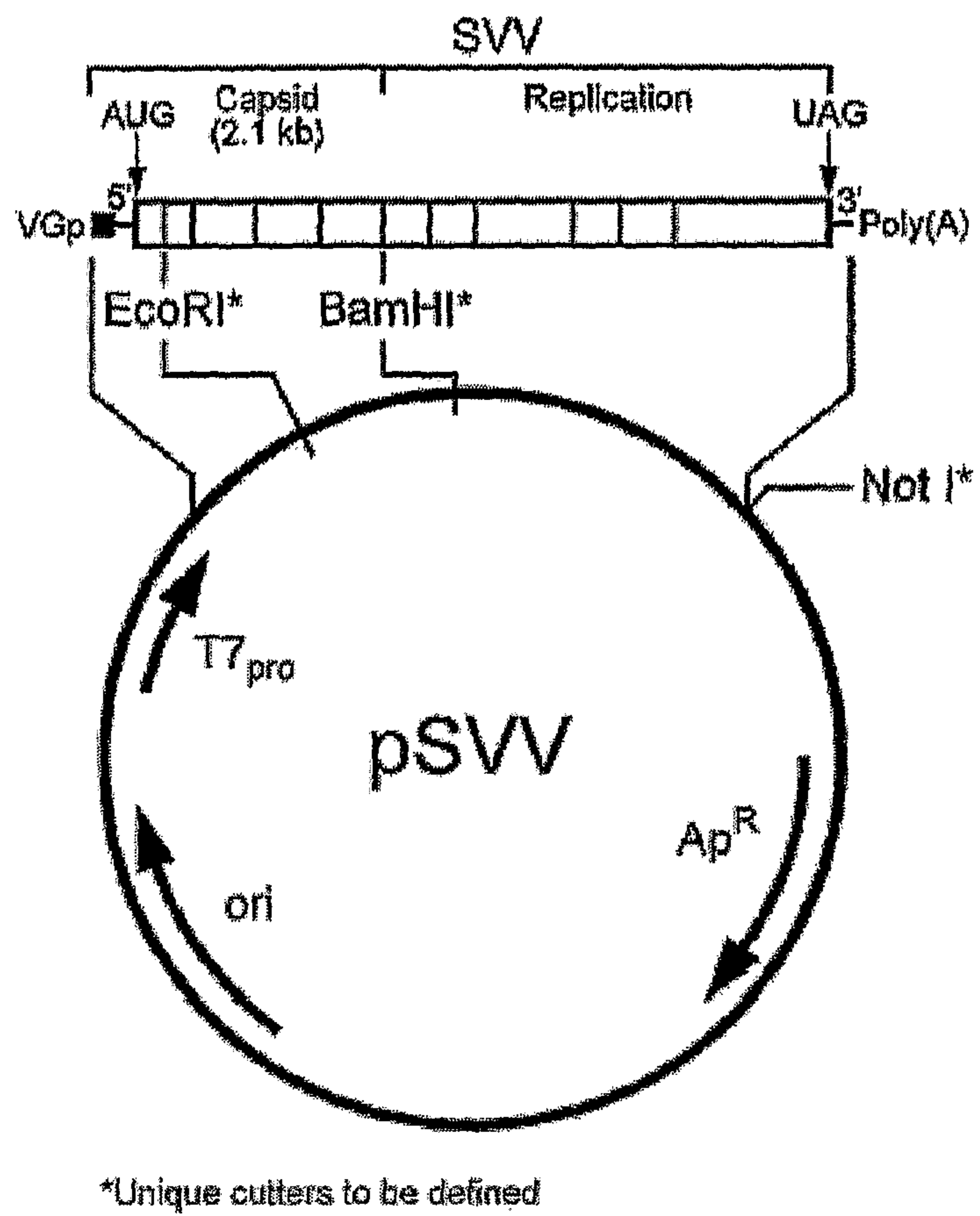


Fig. 52

**Fig. 53**

**Fig. 54**

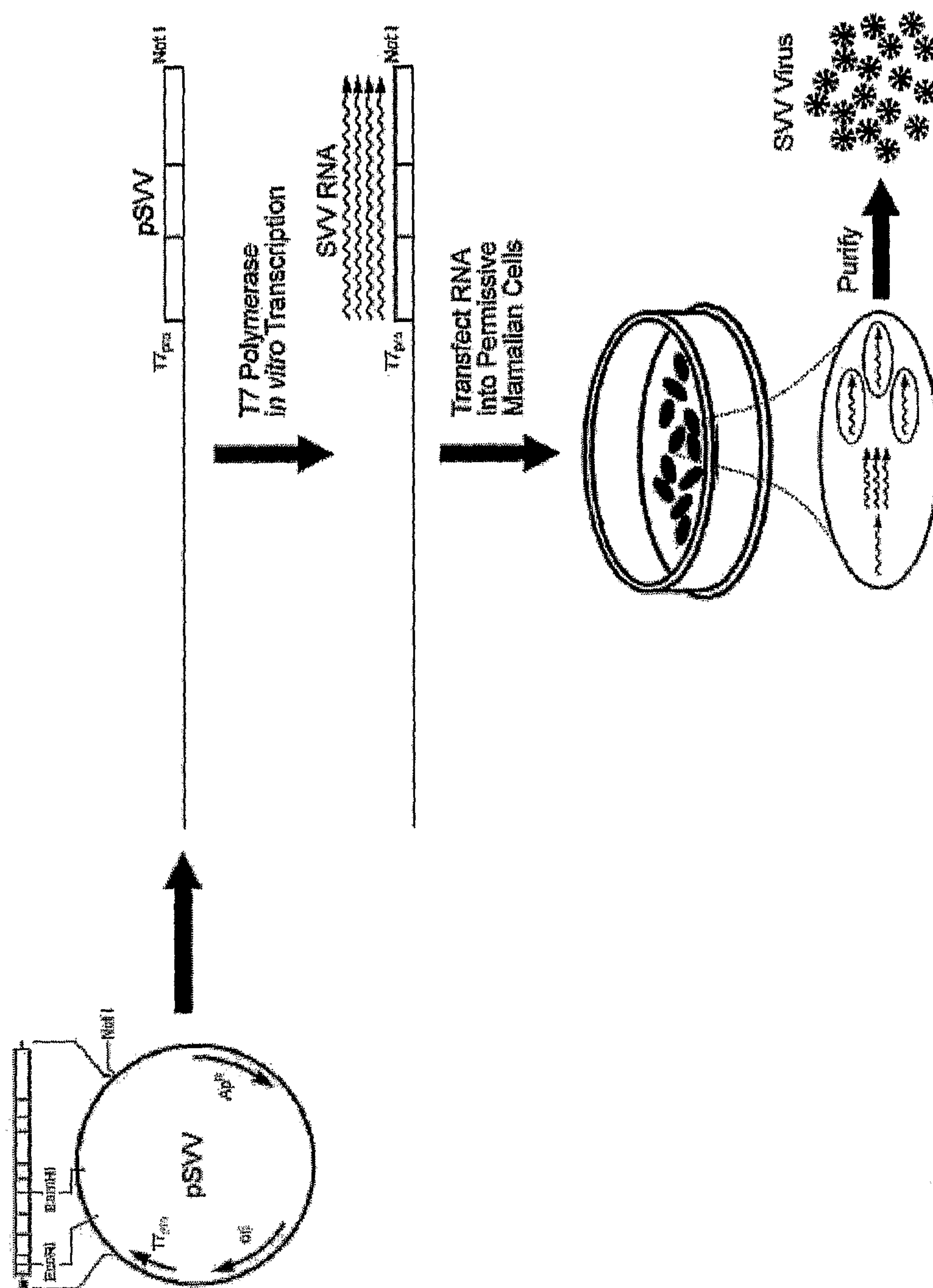


Fig. 55

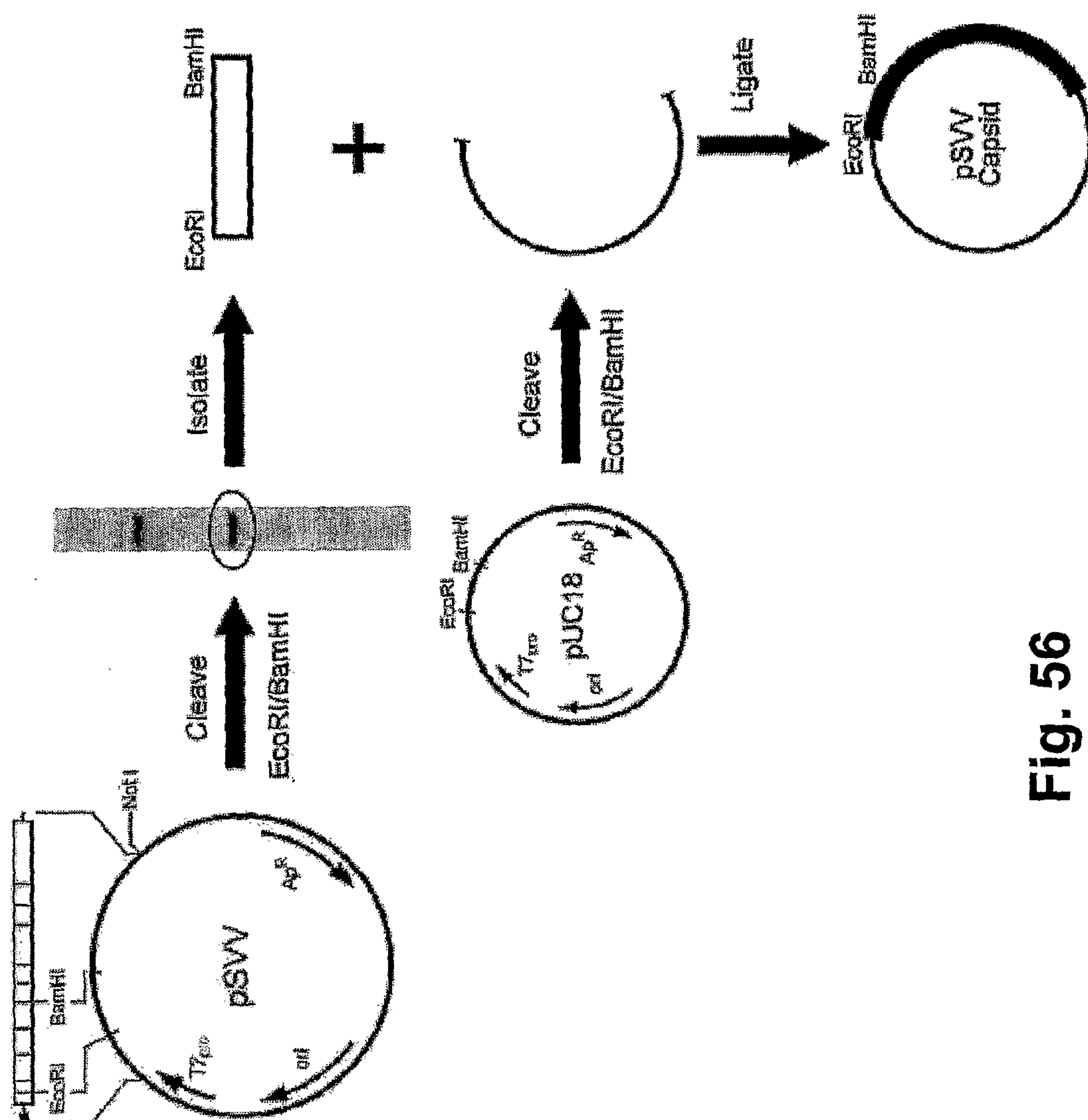


Fig. 56

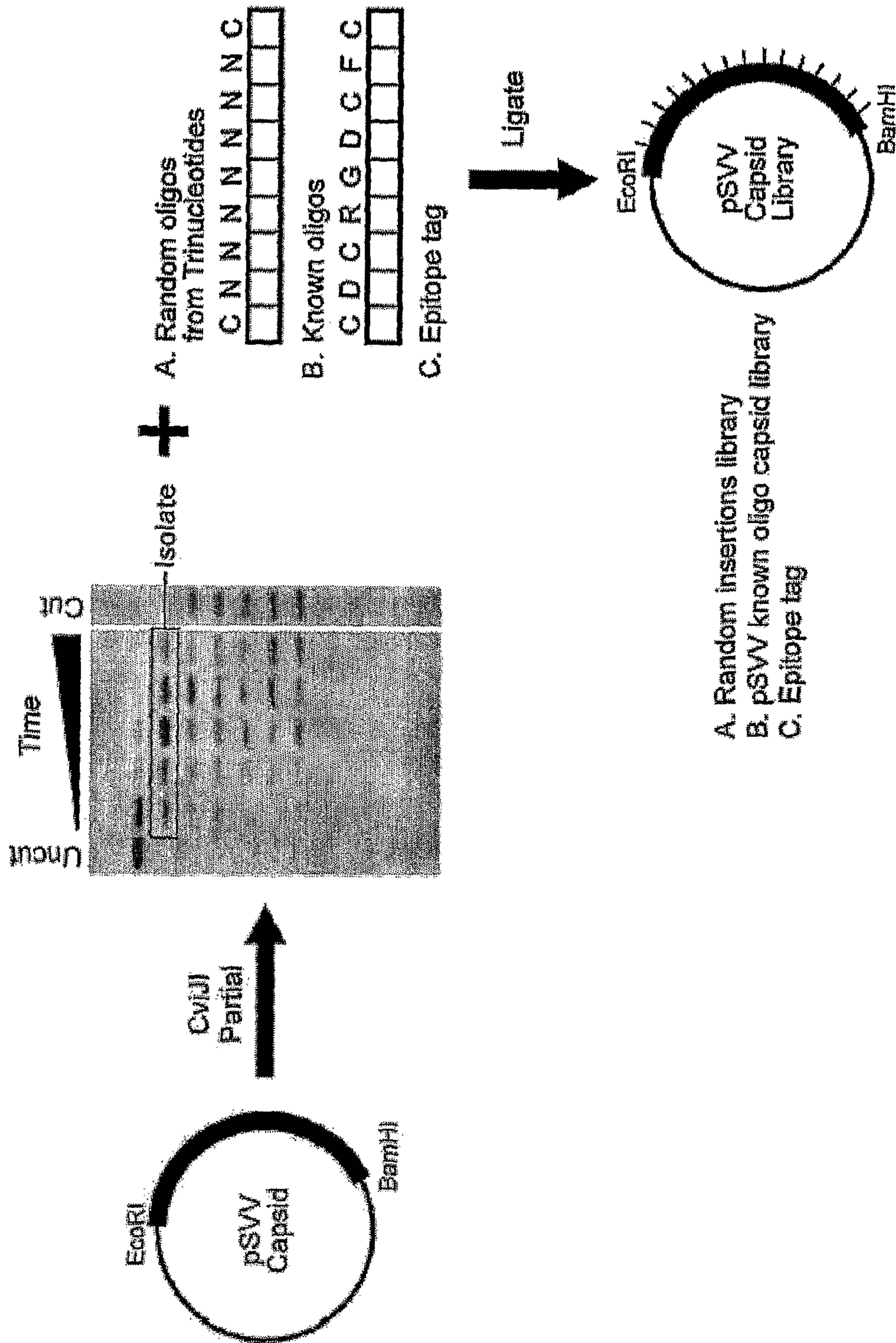


Fig. 57

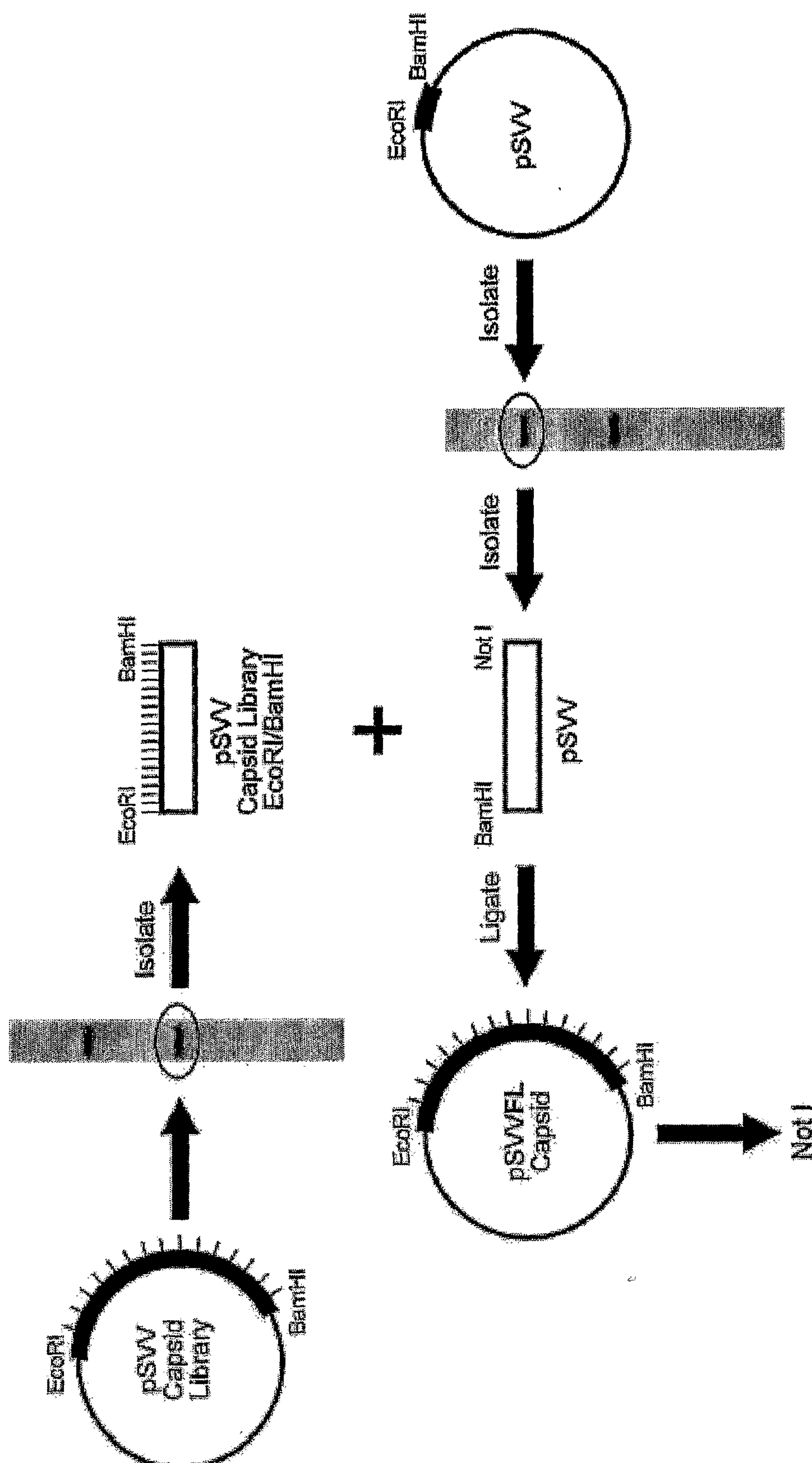


Fig. 58

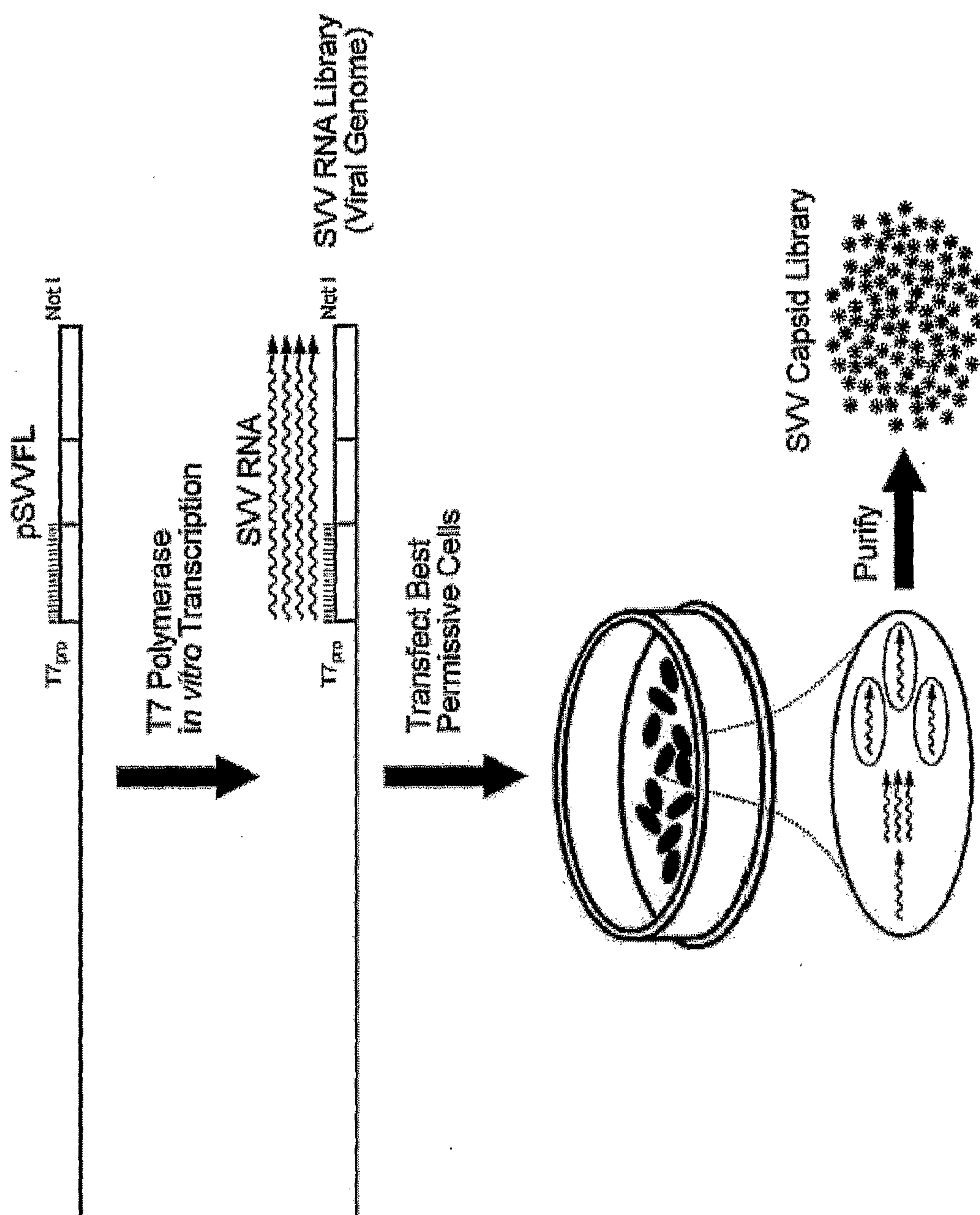


Fig. 59

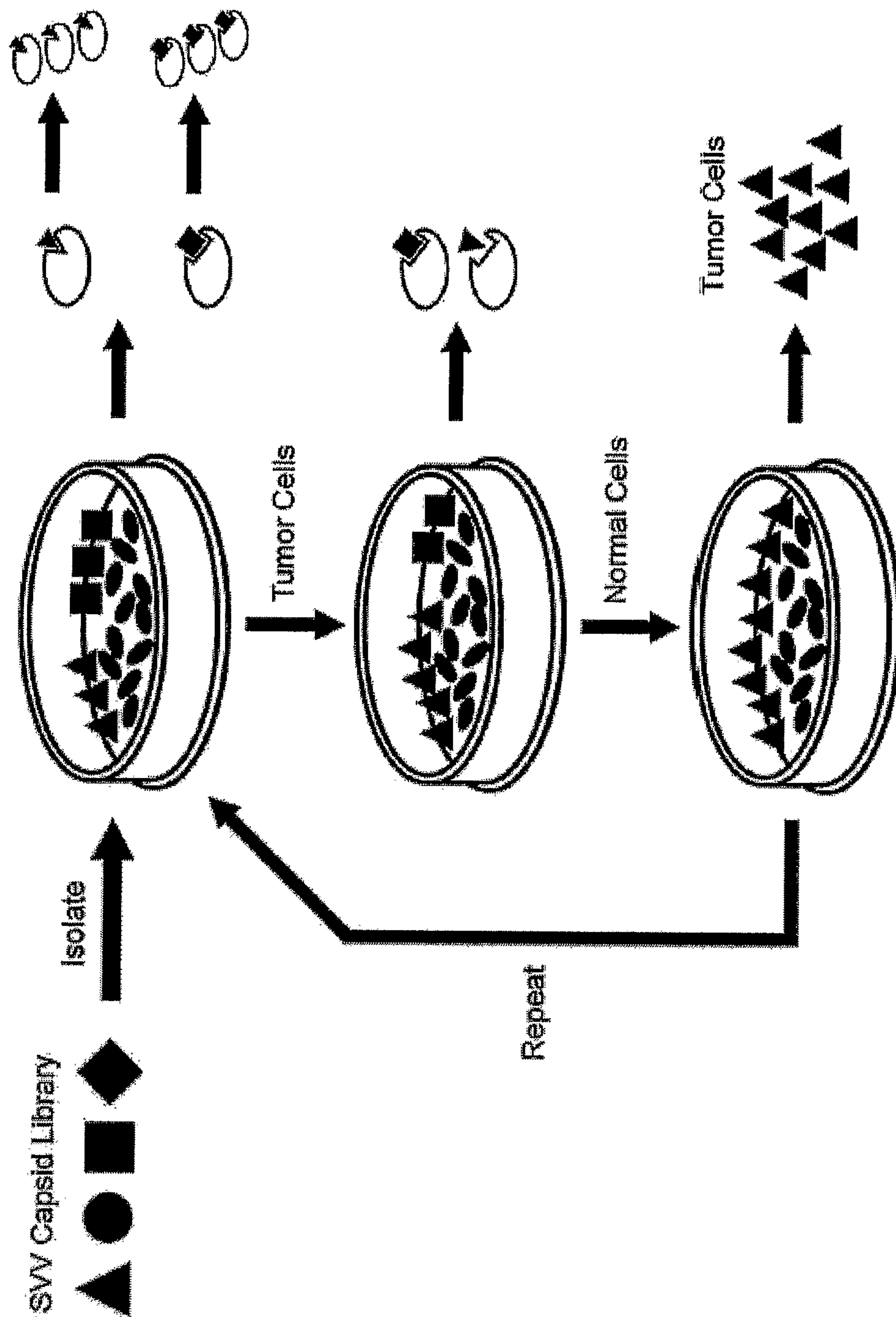


Fig. 60

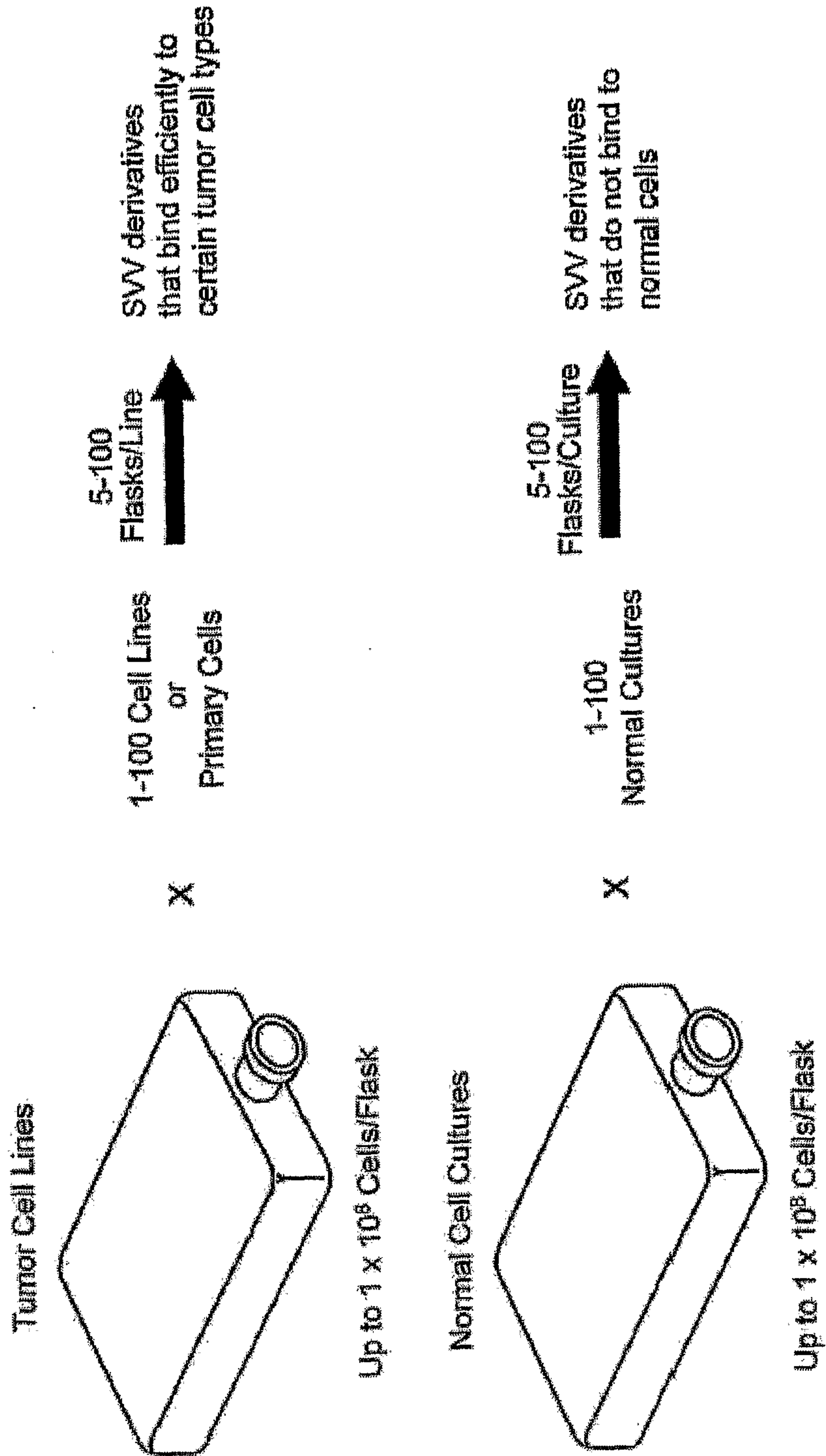


Fig. 61

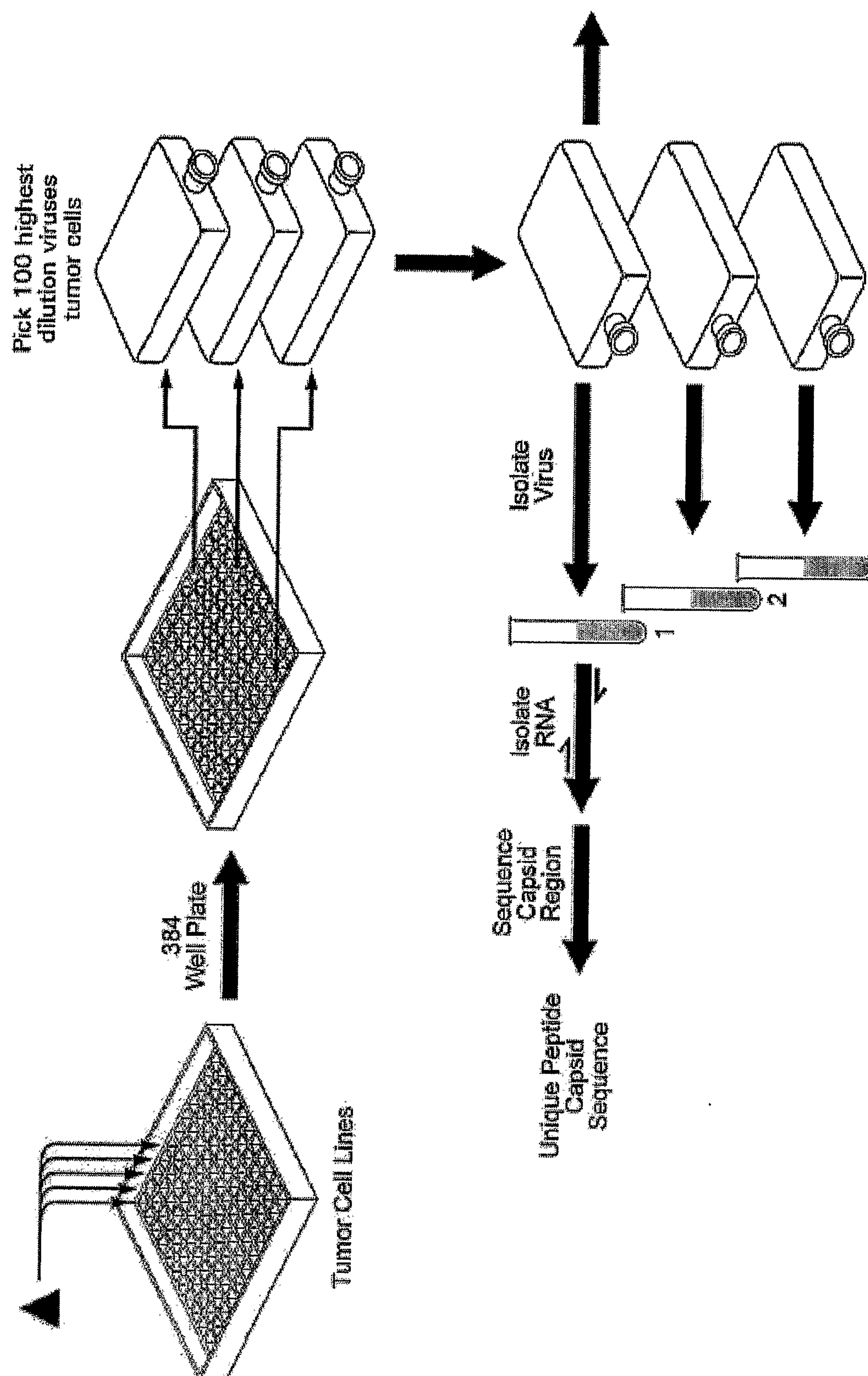


Fig. 62

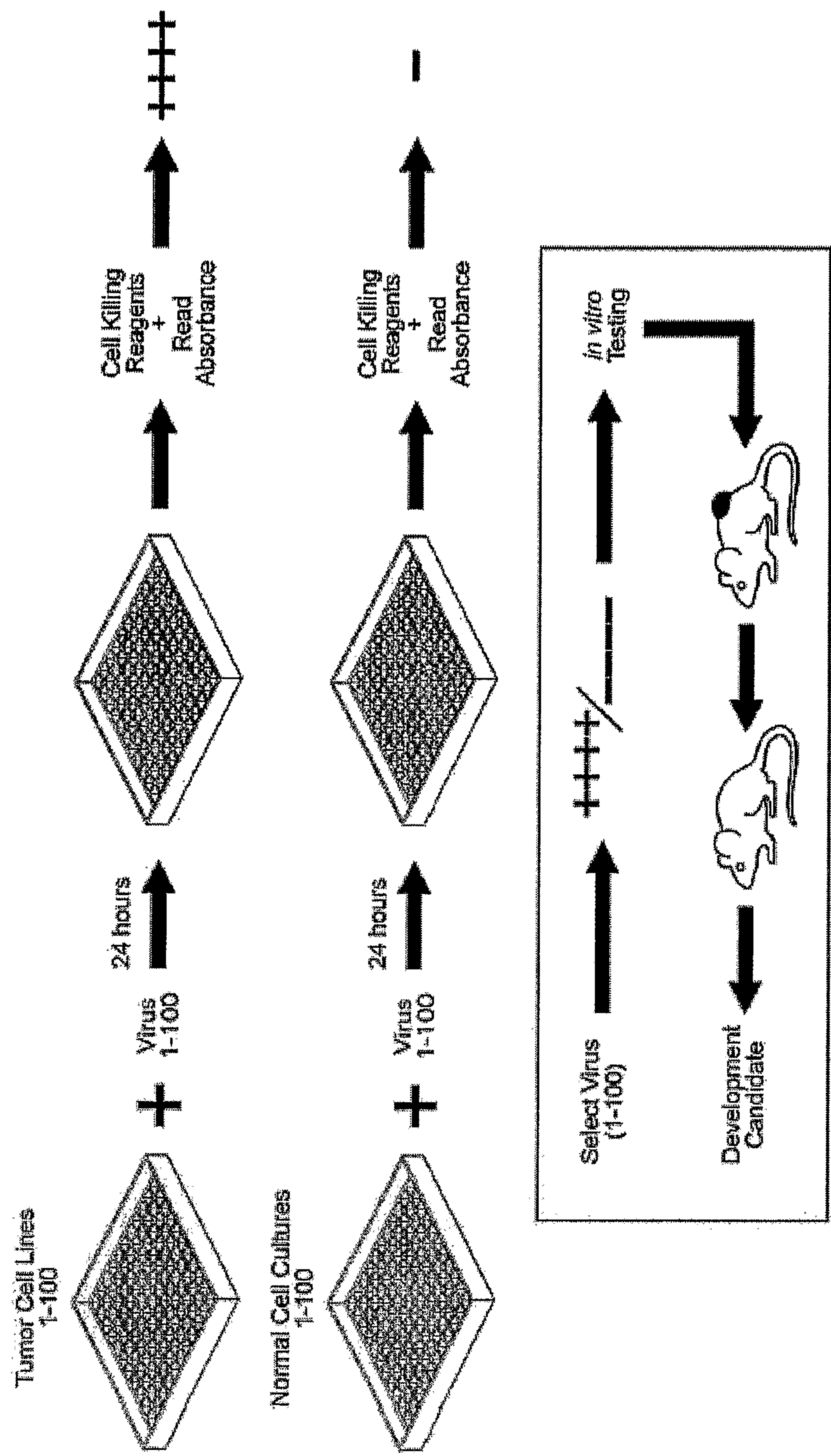


Fig. 63

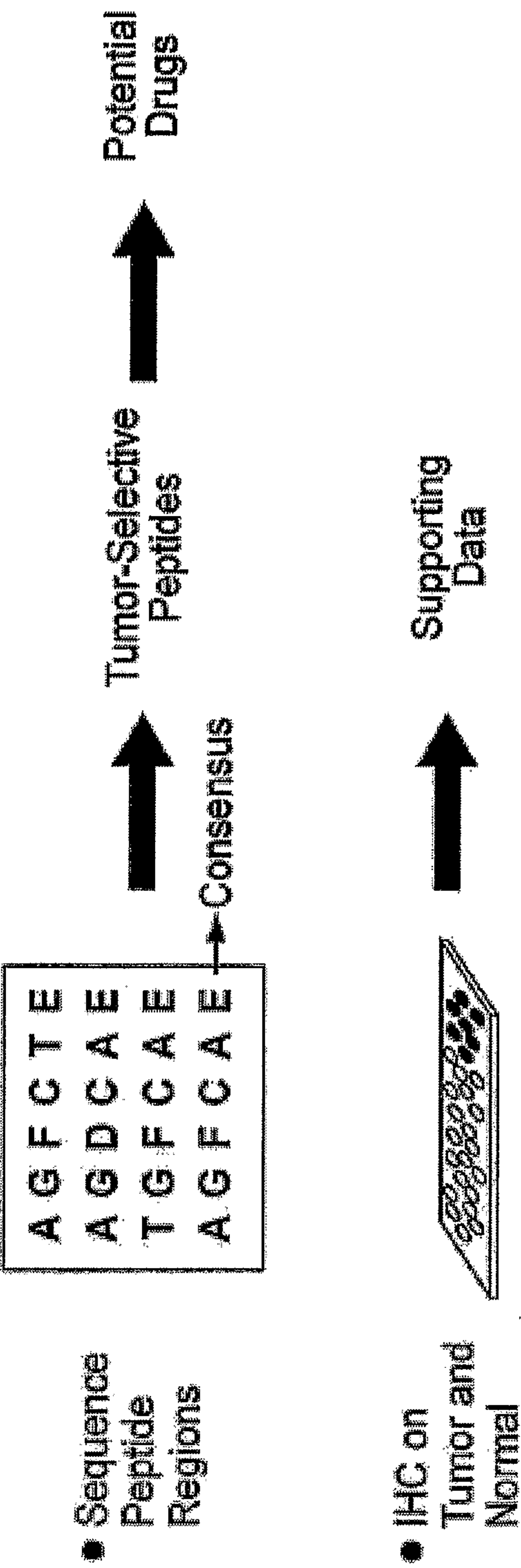
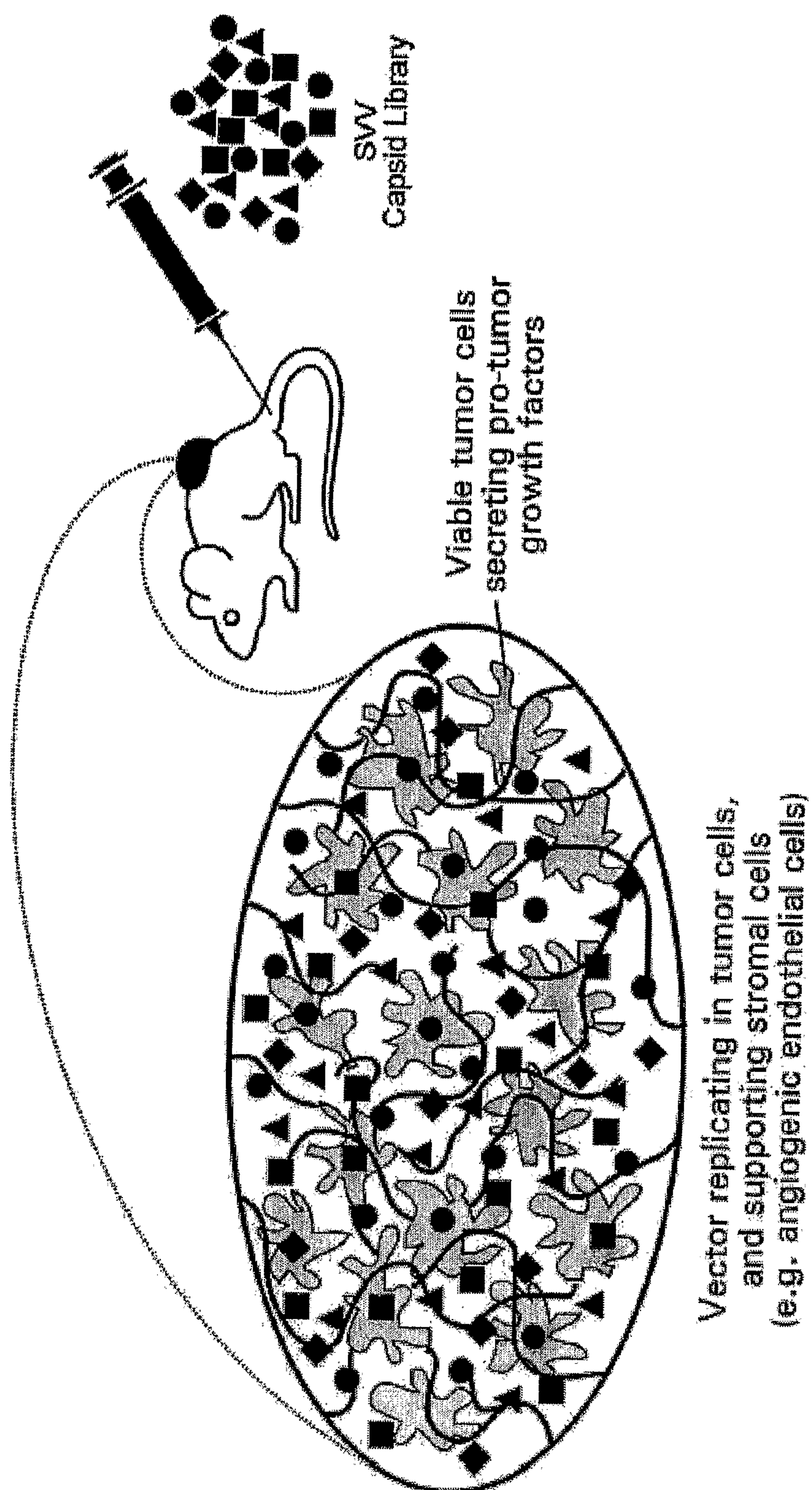


Fig. 64

**Fig. 65**

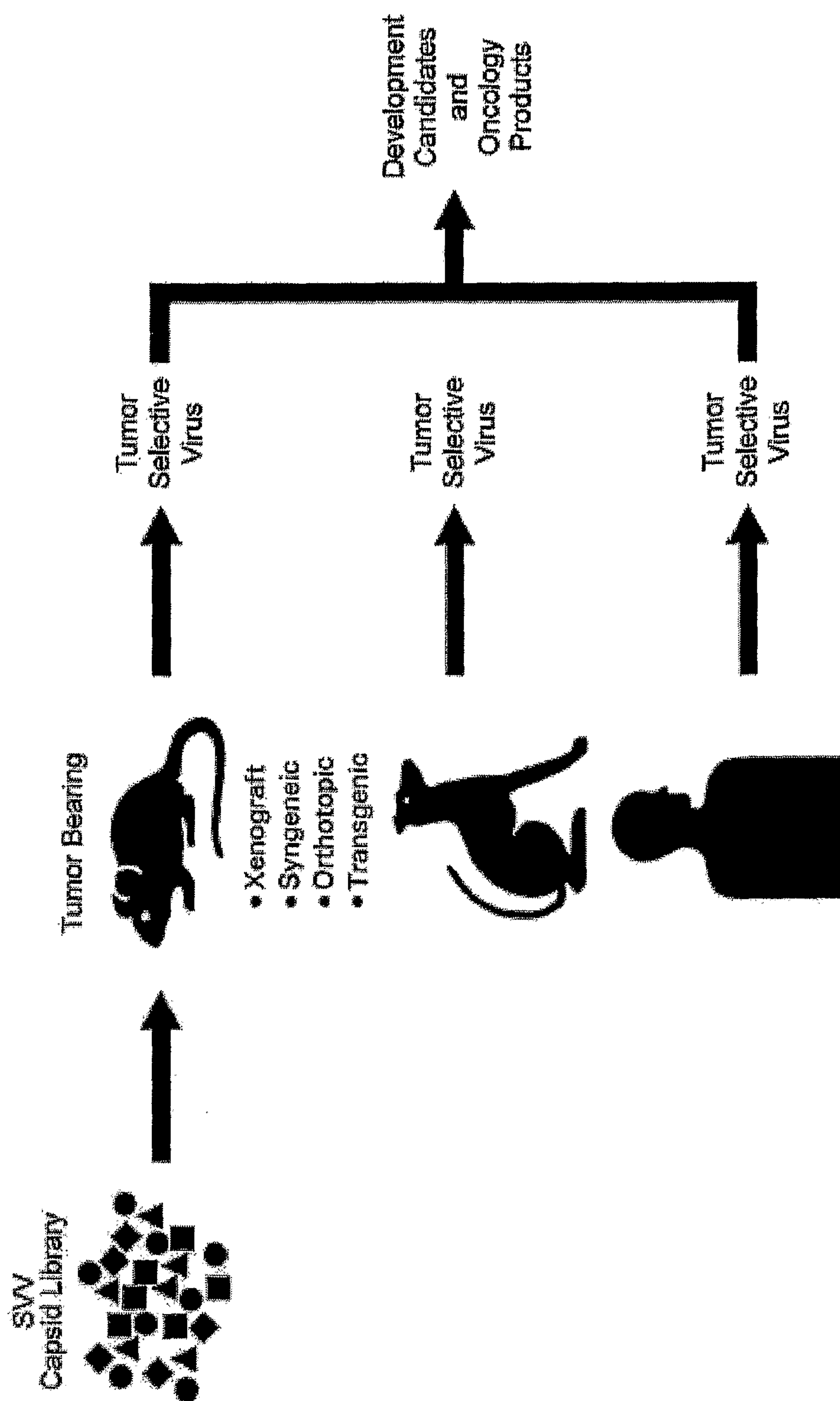


Fig. 66

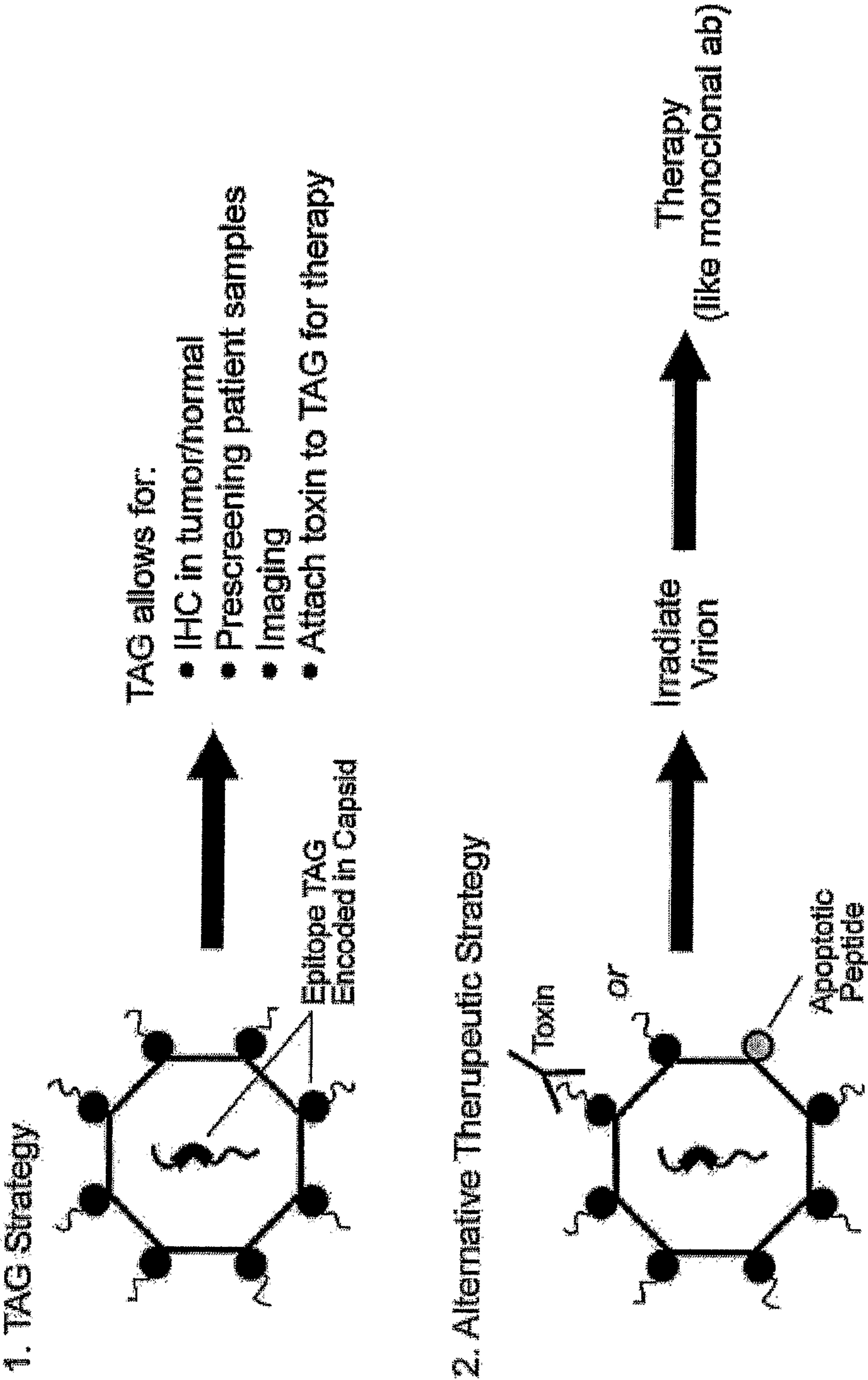
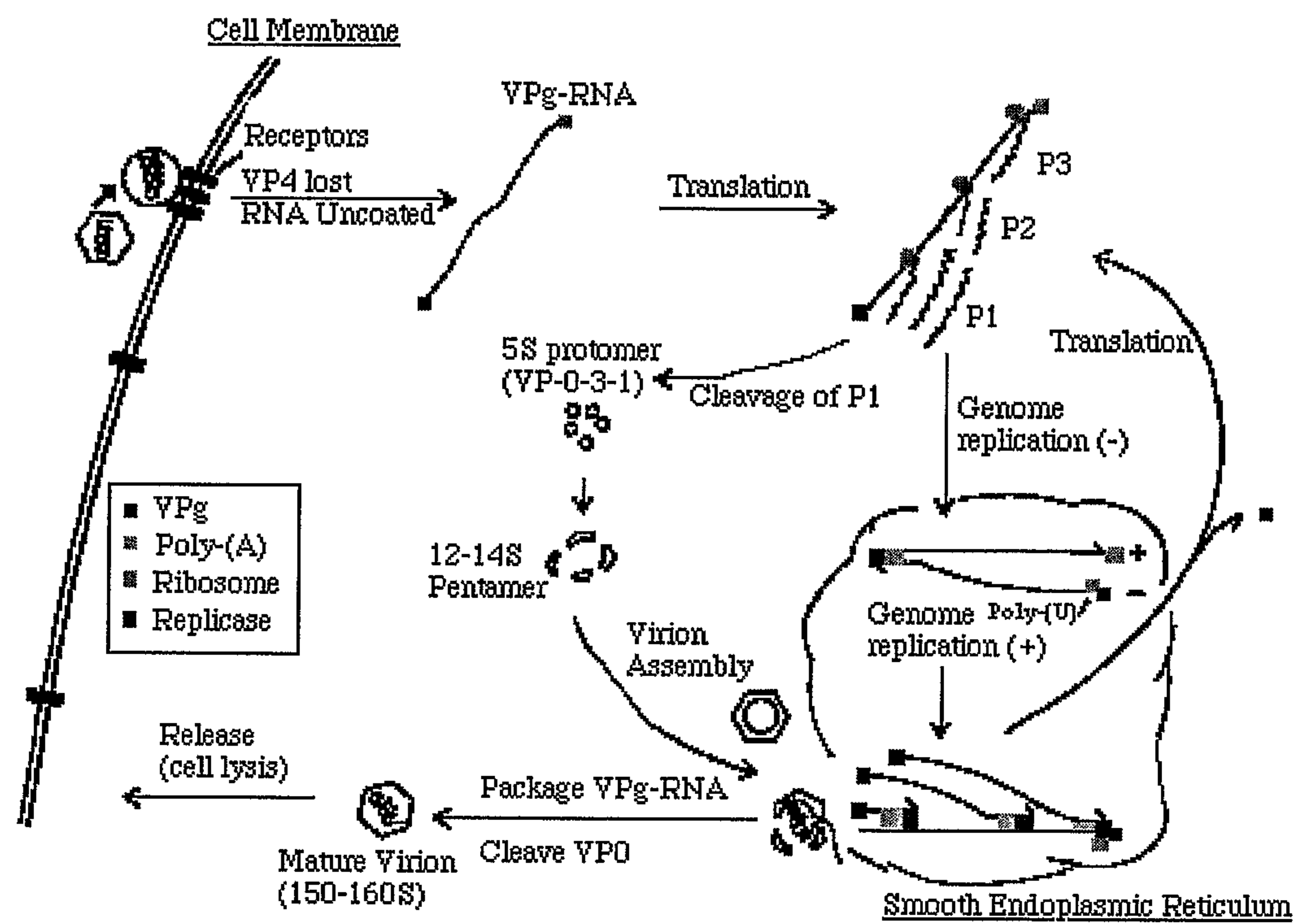


Fig. 67

**Fig. 68**

Polypeptide lengths of Seneca Valley virus compared to other picornaviruses

	SVV	EMCV	TMEV	ERBV-1	FMDV-O	ERAV	PTV-1	PV-1	HRV-1B	SV2	PEV-8	DPV	HAV	AEV	AIv	BKV	HPeV-1	LV
Polyprotein	?	2292		2590	2332	2249		2209	2157				2227	2134	2432	2463	2180	2253
P1	?	834		880	736	784		881	857				791	757	846	857	776	820
P2	465	618		616	488	467		575	559				631	590	636	634	598	606
P3	785	773		875	907	789		753	741				805	787	780	785	806	827
Leader	?	67		219	201	209		-	-				-	-	170	187	-	-
VP0	?	326		327	303	310		341	332				245	242	370	367	289	259
VP4	?	70		71	85	80		69	69				23	19	-	-	-	-
VP2	?	256		256	218	230		272	263				222	223	-	-	-	-
VP3	239	231		229	220	226		238	238				246	245	223	223	253	244
VP1	259	277		324	211	248		302	287				300	270	253	267	234	317
2A	14	143		16	18	16		149	142				45	13	136	134	147	135
2B	128	150		283	154	136		97	95				251	251	165	165	122	138
2C	323	325		317	318	315		329	322				335	326	335	335	329	333
3A	90	88		133	153	95		87	77				74	65	95	94	117	130
3B	22	20		21	23-24†	24		22	21				23	21	27	30	20	29
3C	211	205		252	213	205		183	183				219	215	190	192	200	198
3D	462	460		469	470	465		461	460				489	486	468	469	469	470

*, assuming that the 20 aa peptide ending in NPG is the C-terminus of VP1.

†, FMDV has three copies of VPg in tandem.

na, these individual polypeptides do not exist in these viruses.

Fig. 69

	10	20
PTV-1_Teschen-Konratice [AF231		
PTV-2_T80 [AF296087]	GPGATNFSLLKQAGDVEENPGE	
PTV-3_O2b [AF296088]	GPGATNFSLLKQAGDVEENPGE	
PTV-4_PS36 [AF296089]	GPGASSFSLLKQAGDVEENPGE	
PTV-4_Vir_2500/99 [AF296113]	GPGASNFSLLKQAGDVEENPGE	
PTV-5_F26 [AF296090]	GPGATNFSLLKQAGDVEENPGE	
PTV-6_PS37 [AF296091]	GPGAANFSLLKQAGDVEENPGE	
PTV-7_F43 [AF296092]	GPGATNFSLLKQAGDVEENPGE	
PTV-8_UKG/173/74 [AF296093]	GPGATNFSLLKQAGDVEENPGE	
PTV-9_Ger-2899/84 [AF296094]	GPGATNFSLLKQAGDVEENPGE	
PTV-10_Vir_461/88 [AF296119]	GPGATNFSLLKQAGDVEENPGE	
PTV-11_Dresden [AF296096]	GPGATNFSLLKQAGDVEENPGE	
AY593829_FMDV-O6/UK/1/24_(iso5	---TLNFDLLKLAGDVEENPGE	
AY593828_FMDV-O5/India/62_(iso	---LLNFDLLKLAGDVEENPGE	
AY593774_FMDV-A2/Spain/43_(iso	---LLNFDLLKLAGDVEENPGE	
AY593759_FMDV-A1/Bavaria/GER/4	---LLNFDLLKLAGDVEENPGE	
AY593810_FMDV-C/UK/149/34_(iso	---LLNFDLLKLAGDVEENPGE	
AY593807_FMDV-C3/Resende/BRA/5	---LSNFDLLKLAGDVEENPGE	
AY593795_FMDV-Asia1/PAK/1/54_(	---LLNFDLLKLAGDVEENPGE	
AY593796_FMDV-Asia1/ISR/3/63_(	---TLNFDLLKLAGDVEENPGE	
AY593797_FMDV-Asia1/Kimron_(is	---ALNFDLLKLAGDVEENPGE	
AY593798_FMDV-Asia1/LEB/89_(is	---VLNFDLLKLAGDVEENPGE	
AY593839_FMDV-SAT1/RV/11/37_(i	---MCSFDLLKLAGDVEENPGE	
AY593838_FMDV-SAT1/BEC/1/48_(i	---LCNFDLLKLAGDVEENPGE	
AY593841_FMDV-SAT1/SR/2/58_(is	---LCNFDLLMLAGDVEENPGE	
AY593844_FMDV-SAT1/ISR/14/62_(	---MANFALLKLAGDVEENPGE	
AY593845_FMDV-SAT1/BOT/1/68_(i	---LSNFDLLKLAGDVEENPGE	
AY593847_FMDV-SAT2/RHO/1/48_(i	---LFNFDLLKLAGDVEENPGE	
AY593848_FMDV-SAT2/SA/106/59_(	---LCNCDLLKLAGDVEENPGE	
AY593849_FMDV-SAT2/KEN/11/60_(	---LLNFDLLKLAGDVEENPGE	
AY593850_FMDV-SAT3/SA/57/59_(i	---LCNFDLLKLAGDVEENPGE	
AY593853_FMDV-SAT3/BEC/1/65_(i	---MCNFDLLKLAGDVEENPGE	
ERAV	NKQCTNYALLKLAGDVEENPGE	
ERBV-1	SEGATNFSLLKLAGDVEENPGE	
ERBV-2	SQGATNFDLLKLAGDVEENPGE	
EMCV-R	NAHYAGYFADLLIEDVEENPGE	
EMCV-BC	NAHYAGYFADLLIEDVEENPGE	
Mengo	ETHYAGYFSDLLIEDVEENPGE	
SVV	PFRSYKQKMLMQSGDIEDVEENPGE	
TMEV-BeAn	RGYHADYYRQRLIEDVEENPGE	
TMEV-GD7	RGYHADYYKQRLIEDVEENPGE	
TMEV-DA	RAYHADYYKQRLIEDVEENPGE	
Rat TLV	REYHAAYYKQRLMEDVEENPGE	
LV_87-012	EMDFAGGKFLNQCGDVEENPGE	
LV_174F	EMDFAGGKFLNQCGDVEENPGE	
LV_145SL	EMDYSGGKFLNQCGDVEENPGE	
LV_M1146	DMDYAGGKFLNQCGDVEENPGE	
SVV	PFRSYKQKMLMQSGDIEDVEENPGE	
Trypanosoma_brucei	ALSSIIRTKMLLSGDVEENPGE	
Trypanosoma_cruzi	AVCDAQRQKLLLSGDIEDVEENPGE	
BoRotavirus_C_strain_Shintoku	ANSKFQIDRILISGDIEDVEENPGE	
HuRotavirus_C_strain_Bristol	ANSKFQIDKILISGDIEDVEENPGE	
PoRotavirus_C_strain_Cowden	ANAKFQIDKILISGDVEENPGE	
Drosophila_C_virus	KQEAARQMLLLSGDVEENPGE	
Cricket paralysis virus	RAFLRKRTQLLMSGDVEENPGE	
Acute bee paralysis virus	HCGSWTDIILLLSGDVEENPGE	
Cricket paralysis virus	TLTRAEIEDELIRAGIESNPGE	
Perina nuda picorna-like virus	VTAQGWVPDITVDGDVEENPGE	
Ectropis obliqua picorna-like	VTAQGWAPDITQDGDVEENPGE	
Perina nuda picorna-like virus	NIIGGGQKDLTQDGDIEDVEENPGE	
Ectropis obliqua picorna-like	NIIGGGQRDLTQDGDIEDVEENPGE	
Deformed wing virus	NLLQLSNFVQAKPEMDNEENPGE	
Kakugo virus	NLLQLSNFVQAKPEMDNEENPGE	

Fig. 70

1 MATTMEQETC AHSLTFEECP KCSALQYRNG FYLLKYDEEW YPEELLTDGE DDVFDPELDM
61 EVVFELQGNS TSSDKNNSSS EGNIEGVIINN FYSNQYQNSI DLSANAAGSD PPRTYGQFSN
121 LFSGAVNAFS NMLPLLADQN TEEMENLSDR VSQDTAGNTV TNTQSTVGRL VGYGTVHDGE
181 HPASCADTAS EKILAVERY Y TFKVNDWTST QKPFYIRIP LPHVLSGEDG GVFGAALRRH
241 YLVKTGWRVQ VQCNASQFHA GSVLVFMAPE YPTLDAFAMD NRWSKDNLPN GTRTQTNKKG
301 PFAMDHQNFQ QWTLYPHQFL NLRNTNTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
361 YSTGASTSLD ITASIQPVRP VFNGLRHETL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
421 KTPVAPSNYM VGEYKDFLEI AQIPTFIGNK IPNAVPIYEA SNTAVKTQPL ATYQVTLSCS
481 CLANTFLAAL SRNFAQYRGS LVYTFVFTGT AMMKGKFLIA YTPPGAGKPT SRDQAMQATY
541 AIWDLGLNSS YSFTVPPFISP THFRMVGTDQ VNITNADGWV TVWQLTPLY PPGCPTSAKI
601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE KGVNTENTNAT ADFVAQPVYL PENQTKVAFF
661 YNRSSPIGAF TVKSGSLESG FAPFSNGTCP NSVILTPGPQ FDPAYDQLRP QRLTEIWNGG
721 NEETSKVFPL KSKQDYSFCL FSPFVYYKCD LEVTLSPHTS GNHGLLVRWC PTGTPTKPTT
781 QVLHEVSSLS EGRTPOVYSA GPGISNQISF VVPYNSPLSV LSAVWYNGHK RFDNTGSLGI
841 APNSDFGTLF FAGTKPDIKF TVYLRYKNKR VFCPRPTVFF PWPTSGDKID MTPRAGVLMML
901 ESPNALDISR TYPTLHVLIQ FNHRGLEVRL FRHGHFWAET RADVILRSKT KQVSFSLNGN
961 YPSMDSRAPW NPWKNTYQAV LRAEPCRVTM DIYYKRVPRF RLPLVQKEWP VREENVFGLY
1021 RIFNAHYAGY FADLLIHDIE TNPFPFMFRP RKQVFQTOGA AVSSMAQTLL PNDLASKAMG
1081 SAFTALLDAN EDAQKAMKII KTLSSLSDAW ENVKETLNNP EFWKQLLSRC VQLIAGMTIA
1141 VMHPDPLTLL CLGTLTAAEI TSQTSLECEI AAKFKTIFIT PPPREPTISL FQQQSPLKQV
1201 NDIFSLAKNL DWAVKTVEKV VDWFGTWIVQ EEKEQTLQDL LQRFPEHAKR ISDLRNGMAA
1261 YVECKESFDF FEKLYNQAVK EKRTGIAAVC EKFRQKHDHA TARCEPVVIV LRGDAGQGKS
1321 LSSQVIAQAV SKTIFGRQSV YSLPPDSDFD DGYENQFAAI MDDLQONPDG SDFTTFCQMV
1381 STTNFLPNMA SLERKGTFFT SQLVVATTNL PEFRPVTIAH YPAVERRITE DYSVSAGPVC

Fig. 71A

1441 SKTEAGYKVL DVERAFRPTG EAPLPCFQNN CLFLEKAGLQ FRDNRTKEII SLVDVIERAV
1501 ARIERKKKVL TTVQTLVAQG PVDEVSFHSV VQQLKARQQA TDEQLEELQE AFAKVQERNNS
1561 VFSDWLKISA MLCAATLALS QVVKMAKAVK QMVKPDLVRV QLDEQEQQPY NETARVKPKT
1621 LQLLDIQGPN PVMDFEKYVA KHVTAFIGFV YPTGVSTQTC LLVRGRTLTV NRHMAESDWT
1681 SIVVRGVTHA RSTVKILAIA KAGKETDVSF IRLSSGPLFR DNTSKFVKAG DVLPTGAAPV
1741 TGIMNTDIPM MYTGTFKAG VSVPVETGQT FNHCIHYKAN TRKGWCGSAL LADLGGSKKI
1801 LGIHSAGSMG IAAASIVSQE MIRAVVNAFE PQGALERLPD GPRIHVPRKT ALRPTVARQV
1861 FQPAYAPAVL SKFDPRTEAD VDEVAFSKHT SNQESLPPVF RMVAKEYANR VFTLLGKDNG
1921 RLTVKQALEG LEGMDPMDRN TSPGLPYTAL GMRRTDVVDW ESATLIPFAA ERLRKMNEGD
1981 FSEVVYQTFK KDELRPKEKV QAAKTRIVDV PPFHCILGR QLLGKFASKF QTQPGLELGS
2041 AIGCDPDVHW TAFGVAMQGF ERVYDVDYSN FDSTHSVAMF RLLAEFFFTP ENGFDPLTRE
2101 YLESLAISTH AFEEKRFLIT GGLPSGCAAT SMLNTIMNNI IIRAGLYLTY KNFEEDDVKV
2161 LSYGDDLLVA TNYQLDFDKV RASLAKTGYK ITPANKTSTF PLNSTLEDVV FLKRKFKEG
2221 PLYRPVMNRE ALEAMLSYYR PGTLSKLTIS ITMLAVHSGK QEYDRLFAPF REVGVVVPSF
2281 ESVEYRWRSI FW (SEQ ID NO:23)

Fig. 71B

1 MATTMEQETC AHSLTFEECP KCSALQYRNG FYLLKYDEEW YPEELLTDGE DDVFDPELDM
61 EVVFELQGNS TSSDKNNSSS EGNFVVIINN FYSNQYQNSI DLSANAAGSD PPRTYGQFSN
121 LFSGAVNAFS NMLPLLADQN TEEMENLSDR VSQDTAGNTV TNTQSTVGRL VGYGTVHDGE
181 HPASCADTAS EKILAVERYY TFKVNDWTST QKPFEYIRIP LPHVLSGEDG GVFGAALRRH
241 YLVKTGWRVQ VQCNASQFHA GSKLVFMAPE YPTLDTFVMD NRWSKDNLPN GARTQTNKKG
301 PFAMDHQNFW QWTLYPHQFL NLRTNTTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
361 YSTGASTSLD ITASIQPVRP VFNGLRHETL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
421 KTPVAPSNYM VGEYKDFLEI AQIPTFIGNK IPNAVPIYIEA SNTAVKTQPL ATYQVTLSCS
481 CLANTFLAAL SRNFAQYRGS LVYTFVFTGT AMMKGKFLIA YTPPGAGKPT SRDQAMQATY
541 AIWDLGLNSS YSFTVPFISP THFRMVGTQD VNITNADGWV TVWQLTPLTY PPGCPTSAKI
601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE KGV TENADAT ADFVAQPVYL PENQTKVAFF
661 YDRSSPIGAF TVQSGSLESG FAPFSNKTCP NSVILTPGPQ FDPAYDQLRP QRLTEIWGNG
721 NEETSKVFPL KSKQDYSFCL FSPFVYYKCD LEVTLSPHTS GNHGLLVRWC PTGTPTKPTT
781 QVLHEVSSLS EGRTPQVYSA GPGISNQISF VVPYNSPLSV LPAVWYNGHK RFDNTGSLGI
841 APNSDFGTLF FAGTKPDIKF TVYLRYKNMR VFCPRPTVFF PWPTSGDKID MTPRAGVLMML
901 ESPNALDISR TYPTLHVLIQ FNHRGLEVRL FRHGQFWAET RADVILRSKT KQVSFSLNGN
961 YPSMDSRAPW NPWKNTYQAV LRAEPCRVTM DIYYKRVPRF RLPLVQKEWR VREENVFGLY
1021 RIFNAHYAGY FADLLIHDIE TNP GPFMFRP RKQVFQTQGA AVSSMAQTLL PNDLASKAMG
1081 SAFTALLDAN EDARKAMKII KTLSSLSDAW ENVKETLNNP EFWKQLLSRC VQLIAGMTIA
1141 VMHPDPLTLL CLGTLTAAEI TSQTS LCEEI AAKFKTIFIT PPRFPTISL FQQQSPLKQV
1201 NDFFS LAKNL DWAVKTVEKV VDWFEGTWIVQ EEKEQTL DQL LQRFPEHAKR ISDLRNGMAA
1261 YVECKESFDF FEKLYNQAVK EKRTGIAAVC EKFRQKHDHA TARCEPVVIV LRGDAGQGKS
1321 LSSQVIAQAV SKTIFGRQSV YSLPPDSDFE DGYENQFAAI MDDL GQNP DG SDFTTFCQMV

Fig. 72A

1381 STTNFLPNMA SLERKGTFFT SQLVVATTNL PEFRPVTIAH YPAVERRITF DYSVSAGPVC
1441 SKTEAGYKVL DVERAFRPTG EAPLPCFQNN CLFLEKAGLQ FRDNRTKEII SLVDVIERAV
1501 ARIERKKKVL TTVQTLVAQA PVDEVSEFSV VQQLKARQEA TDEQLEELQE AFAKVQERNS
1561 VFSDWLKISA MLCAATLALS QVVKMAKAVK QMVKPDLVRV QLDEQEQGPY NETARAKPKT
1621 LQLLDIQGPN PVMDFEKYVA KHVTAPIDFV YPTGVSTQTC LLVRGRTLAV NRHMAESDWT
1681 SIVVRGVTHA RSTVKILAIA KAGKETDVSE IRLSSGPLFR DNTSKFVKAG DVLPTGAAPV
1741 TGIMNTDIPM MYTGTFKAG VSVPVETGQT FNHCIHYKAN TRKGWCGSAL LADLGGSKKI
1801 LGIHSAGSMG IAAASIVSQE MIRAVVNAFE PQGALERLPD GPRIHVPRKT ALRPTVARQV
1861 FQPAYAPAVL SKFDP RTEAD VDEVAFSKHT SNQESLPPVF RMVAKEYANR VFTLLGKDNG
1921 RLTVKQALEG LEGMDPMDRN TSPGLPYTAL GMRRTDVVDW ESATLIPFAA ERLRKMNEGD
1981 FSEVVYQTFL KDEL RPIEKV QAAKTRIVDV PPFEHCILGR QLLGKFASKF QTQPGLELGS
2041 AIGCDPDVHW TAFGVAMQGF ERVYDVDYSN FDSTHSVAME RLLAEFFFTP ENGFDPLTRE
2101 YLESLAISTH AFEEKREFLIT GGLPSGCAAT SMLNTIMNNI IIRAGLYLTY KNFEFDDVKV
2161 LSYGDDLVA TNYQLDFDKV RASLAKTGYK ITPANKTSTF PLNSTLEDVV FLKRKFKEG
2221 PLYRPVMNRE ALEAMLSYYR PGTLSSEKLT ITMLAVHSGK QEYDRLEAPF REVGVVVPSF
2281 ESVEYRWRS LFW (SEQ ID NO:24)

Fig. 72B

1 MATTMEQEIC AHSITLKGCP KCSALQYRNG FYLLKYDEEW YPEELLTDGE DDVFDPELDM
61 EVVFELQGNS TSSDKNNSSS DNEGVIINN FYSNQYQNSI DLSANATGSD PPRTYGQFSN
121 LLSGAVNAFS NMIPLLADQN TEEMENLSDR VLQDTAGNTV TNTQSTVGRL VGYGAVHDGE
181 HPASCADTAS EKILAVEYYY TFKVNDWTST QKPFYIRIP LPHVLSGEDG GVFGAALRRH
241 YLVKTGWRVQ VQCNASQFHA GSLLVFMAPE YPTLDAFAMD NRWSKDNLPN GTKTQTNRKG
301 PFAMDHQNEF QWTLYPHQFL NLRNTTTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
361 YSTGASTSLD ITASIQPVRP VFENGLRHETL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
421 KTPVAPANYM VGEYKDFLEI AQIPTFIGNK IPNAVPHYEA SNTAVKTQPL ATYQVTLSCS
481 CLANTFLAAL SRNFAQYRGS LVYTFVFTGT AMMKGKFLIA YTPPGAGKPT SRDQAMQATY
541 AIWDLGLNSS YSFTVPFISP THERMVGTDQ VNITNVDGWV TVWQLTPLY PPGCPTSAKI
601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE RGVTEDTDAT ADFVAQPVYL PENQTKVAFF
661 YDRSSPIGAF TVKSGSLESG FTPFSNQTCP NSVILTPGPQ FDPAYDQLRP QRLTEIWGNG
721 NEETSKVFPL KSKQDYSFCL FSPFVYYKCD LEVTLSPHTS GNHGLLVRWC PTGTPTKPTT
781 QVLHEVSSLS EGRTPOVYSA GPGITNQISF VVPYNSPLSV LPAVWYNGHK RFDNTGSLGI
841 APNSDFGTLF FAGTKPDIKF TVYLRYKNMR VFCPRPTVFF PWPSSGDKID MTPRAGVLML
901 ESPNALDISR TYPTLHILIQ FNHGGLEIRL FRHGMFWAEA HADVILRSRT KQISFLNNGS
961 FPSMDARAPW NPWKNTYHAV LRAEPYRVTM DVYHKRIRPF RLPLVQKEWN VREENVFGLY
1021 GIFNAHYAGY FADLLIHDIE TNP GP FMAKP KKQVFQTQGA AVSSMAQTLL PNDLASKVMG
1081 SAFTALLDAN EDAQKAMRII KTLSSLSDAW ENVKETLNNP EFWKQLLSRC VQLIAGMTIA
1141 VMHPDPLTLL CLGTLTAAEI TSQTS LCEEI VAKFKKIFTT PPPRFPTISL FQQQSPLKQV
1201 NDVFS LAKNL DWAVKTVEKV VDWFGTWVVQ EEKEQTL DQL LQRFPEHAKR ISDLRNGMSA
1261 YVECKESFDF FEKLYNQAVK EKRTGIAAVC EKFRQKHDHA TARCEPVVIV LRGDAGQGKS
1321 LSSQVIAQAV SKTIFGRQSV YSLPPDSDEF DGYENQFAAI MDDLQNP DG SFTTFCQMV

Fig. 73A

1381 STTNFLPNMA SLERNGTPFT SQIVVATTNL PEFRPVTIAH YPAVERRITF DYSVSAGPVC
1441 SKTEAGYKVL DVERAFRPTG DAPLPCFQNN CLFLEKAGLQ FRDNRTKEIL SLVDVIERAV
1501 ARIERKKKVL TTVQTLVAQA PVDEVSFHSV VQQLKARQEA TDEQLEELQE AFAKTQERS
1561 VFSDWMKISA MLCAATLALS QVVKMAKTVK QMVRPDLVRV QLDEQEQQPY NEAVRAKPKT
1621 LQLLDIQGPN PVMDFEKYVA KFVTAPIDFV YPTGVSTQTC LLVKGRTLAV NRHMAESDWS
1681 SIVVRGVTHA RSTVRILAIA KAGKETDVSF IRLSSGPLFR DNTSKFVKAD DVLPTSAPV
1741 IGIMNTDIPM MFTGTFLKAG VSVPVETGQT FNHCIHYKAN TRKGWCGSAL LADLGGKKKI
1801 LGMHSAGSMG RTAASIVSQE MICAVVSAFE PQGALERLPD GPRIHVPRKT ALRPTVARRV
1861 FQPAYAPAVL SKFDP RTEAD VDEVAFSKHT SNQESLPPVF RMVAKEYANR VFTLLGRDNG
1921 RLTVKQALEG LEGMDPMDKN TSPGLPYTAL GMRRTDVVDW ESATLIPYAA DRLKKMNEGD
1981 FSDIVYQTFL KDEL RPVEKV QAAKTRIVDV PPFEHCILGR QLLGRFASKF QTQPGLELGS
2041 AIGCDPDVHW TAFGVAMQGF ERVYDVDYSN FDSTHSVAMF RLLAEEFFTP ENGFDPLVKE
2101 YLESLAISTH AFEEKRYLIT GGLPSGCAAT SMLNTIMNNI IIRAGLYLTY KNFEEDDVKV
2161 LSYGDDLLVA TNYQLNFDKV RASLAKTGYK ITPANKTSTF PLDSTLEDVV FLKRKFKEG
2221 PLYRPVMNRE ALEAMLSYYR PGTLSSEKLS ITMLAVHSGK PEYDRLEFAPF REVGVVVPSF
2281 ESVEYRWRS L FW (SEQ ID NO:25)

Fig. 73B

1 MATTMEQEIC AHSITFKGCP KCSALQYRNG FYLLKYDEEW YPEELLTDGE DDVFDPELDM
61 EVVFELQGNS TSSDKNNSSS DGNFVVIINN FYSNQYQNSI DLSANATGSD PPRTYGQFSN
121 LLSGAVNAFS NMIPLLDQON TEEMENLSDR VLQDTAGNTV TNTQSTVGRL VGYGAVHDGE
181 HPASCADTAS EKILAVERYY TFKVNDWTST QKPFEYIRIP LPHVLSGEDG GVFGAALRRH
241 YLVKTGWRVQ VQCNASQFHA GSKLVFMAPE YPTLDAFAMD NRWSKDNLPN GTKTQTNRKG
301 PFAMDHQNEF QWTLYPHQFL NLRNTTTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
361 YSTGASTSLD ITASIQPVRP VFNGLRHETL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
421 KTPVAPANYM VGEYKDFLEI AQIPTFIGNK IPNAVPIYIEA SNTAVKTQPL ATYQVTLSCS
481 CLANTFLAAL SRNFAQYRGS LVYTFVFTGT AMMKGKFLIA YTPPGAGKPT SRDQAMQATY
541 AIWDLGLNSS YSFTVPFISP THERMVGTDQ VNITNVDGWV TVWQLTPLY PPGCPTSAKI
601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE RGVTEDTDAT ADFVAQPVYL PENQTKVAFF
661 YDRSSPIGAF AVKSGSLESG FAPFSNETCP NSVILTPGPQ FDPAYDQLRP QRLTEIWGNG
721 NEETSKVFPL KSKQDYSFCL FSPFVYYKCD LEVTLSPHTS GNHGLLVRWC PTGTPAKPTT
781 QVLHEVSSLS EGRTPOVYSA GPGISNQISF VVPYNSPLSV LPAVWYNGHK RFDNTGSLGI
841 APNSDFGTLF FAGTKPDIKF TVYLRYKNMR VFCPRPTVFF PWPSSGDKID MTPRAGVLMML
901 ESPNALDISR TYPTLHILIQ FNHGGLEIRL FRHGMFWAEA HADVILRSRT KQISFLNNGS
961 FPSMDARAPW NPWKNTYHAV LRAEPYRVTM DVYHKRIRPF RLPLVQKEWN VREENVFGLY
1021 GIFNAHYAGY FADLLIHDIE TNPFPFMAKP KKQVFQTOGA AVSSMAQTLL PNDLASKVMG
1081 SAFTALLDAN EDAQKAMRII KTLSSLSDAW ENVKETLNNP EFWKQLLSRC VQLIAGMTIA
1141 VMHPDPLTLL CLGTLTAAEI TSQTSLECEI VAKFKKIFTT PPRFPTISL FQQQSPLKQV
1201 NDVFSLAKNL DWAVKTVEKV VDWFGTWVVQ EEKEQTLQDL LQRFPEHAKR ISDLRNGMSA
1261 YVECKESFDF FEKLYNQAVK EKRTGIAAVC EKFRQKHDHA TARCEPVVIV LRGDAGQGKS
1321 LSSQVIAQAV SKTIFGRQSV YSLPPDSDEF DGYENQFAAI MDDLQONPDG SDFTTFCQMV

Fig. 74A

1381 STTNFLPNMA SLERNGTPFT SQIVVATTNL PEFRPVTIAH YPAVERRITF DYSVSAGPVC
1441 SKTEAGYKVL DVERAFRPTG DAPLPCFQNN CLFLEKAGLQ FRDNRTKEIL SLVDVIERAV
1501 ARIERKKKVL TTVQTLVAQA PVAEVSFHSV VQQLKARQEA TDEQLEELQE AFAKTQERS
1561 VFSDWMKISA MLCAATLALS QVVKMAKTVK QMVRPDLVRV QLDEQEOPGY NEAVRAKPKT
1621 LQLLDIQGN PVMDFEKYVA KFVTAPIDFV YPTGVSTQTC LLVKGRTLAV NRHMAESDWS
1681 SIVVRGVTHA RSTVRILAIA KAGKETDVSF IRLSSGPLFR DNTSKFVKAD DVLPTSAPV
1741 IGIMNTDIPM MFTGTFLKAG VSVPVETGQT FNHCIHYKAN TRKGWCGSAL LADLGKKKI
1801 LGMHSAGSMG RTAASIVSQE MICAVVSAFE PQGALERLPD GPRIHVPRKT ALRPTVARRV
1861 FQPAYAPAVL SKFDPRTDAD VDEVAFSKHT SNQESLPPVF RMVAKEYANR VFTLLGRDNG
1921 RLTVKQALEG LEGMDPMDKN TSPGLPYTAL GMRRTDVVDW ESATLIPYAA DRLKKMNEGD
1981 FSDIVYQTFE KDELRPVEKV QAAKTRIVDV PPFEHCILGR QLLGRFASKF QTQPGLELGS
2041 AIGCDPDVHW TAFGVAMQGF ERVYDVEDYN FDSTHSVAMF RLLAEFFFTP ENGFDPLVKE
2101 YLESLAISTH AFEEKRYLIT GGLPSGCAAT SMLNTIMNNI IIRAGLYLTY KNFEEDDVKV
2161 LSYGDDLLVA TNYQLNFDKV RASLAKTGYK ITPANKTSTF PLDSTLEDVV FLKRKFKEG
2221 PLYRPVMNRE ALEAMLSYYR PGTLSKLTIS ITMLAVHSGK PEYDRLEAPF REVGVVVPSF
2281 ESVEYRWRSF FW (SEQ ID NO:26)

Fig. 74B

1 MATTMEQEIC AHSLTFKGCP KCSALQYRNG FYLLKYDEEW YPEELLTDGE DDVFDPELDM
61 EVVFELQGNS TSSDKNNSSS DGNIEGVIINN FYSNQYQNSI DLSANATGSD PPTYGQFSN
121 LLSGAVNAFS NMIPLLDQON TEEMENLSDR VLQDTAGNTV TNTQSTVGRL VGYGAVHDGE
181 HPASCADTAS EKILAVEYYY TFKVNDWTST QKPFYIRIP LPHVLSGEDG GVFGAALRRH
241 YLVKTGWPVQ VQCNASQFHA GSVLVFMAPE YPTLDAFAMD NRWSKDNLPN GTKTQTNRKG
301 PFAMDHQNEF QWTLYPHQFL NLRNTTTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
361 YSTGASTSLD ITASIQPVRP VFNGLRHETL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
421 KTPVAPANYM VGEYKDFLEI AQIPTFIGNK IPNAVPIYIEA SNTAVKTQPL ATYQVTLSCS
481 CLANTFLAAL SRNFAQYRGS LVYTFVFTGT AMMKGKFLIA YTPPGAGKPT SRDQAMQATY
541 AIWDLGLNSS YSFTVPFISP THFRMVGTDO VNITNVGDGWV TVWQLTPLTY PPGCPTSAKI
601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE RGVTEDTDAT ADFVAQPVYL PENQTKVAFF
661 YDRSSPIGAF TVKSGSLESG FAPFSNETCP NSVILTPGPQ FDPAYDQLRP QRLTEIWGNG
721 NEETSKVFPL KSKQDYSFCL FSPFVYYKCD LEVTLSPHTS GNHGLLVRWC PTGTPAKPTT
781 QVLHEVSSLS EGRTPQVYSA GPGVSNQISF VVPYNSPLSV LPAVWYNGHK RFDNTGSLGI
841 APNSDFGTLF FAGTKPDIKF TVYLRYKNMR VFCPRPTVFF PWPSSGDKID MTPRAGVLMML
901 ESPNALDISR TYPTLHILIQ FNHGGLEIRL FRHVQFWAEA HADVILRSRT KQISFLNNGS
961 FPSMDARAPW NPWKNTYHAV LRAEPYRVTM DVYHKRIRPF RLPLVQKEWN VREENVFGLY
1021 GIFNAHYAGY FADLLIHDIE TNPGPFMAKP KKQVFQQTGA AVSSMAQTLL PNDLASKVMG
1081 SAFTALLDAN EDAQKAMRII KTLSSLSDAW ENVKETLNNP EFWKQLLSRC VQLIAGMTIA
1141 VMHPDPLTLL CLGTLTAAEI TSQTSLCEEI VAKFKKIETT PPPRFPTISL FQQQSPLKQV
1201 NDVFSLAKNL DWAVKTVEKV VDWFGTWVVQ EEKEQTLDOQ LQRFPEHAKR ISDLRNGMSA
1261 YVECKESFDF FEKLYNQAVK EKRTGIAAVC EKFRQKHDHA TARCEPVVIV LRGDAGQGKS

Fig. 75A

1321 LSSQVIAQAV SKTIFGRQSV YSLPPDSDFE DGYENQFAAI MDDLGNPDG SDFTTFCQMV
1381 STTNFLPNMA SLERNGTPFT SQLVVATTNL PEFRPVTIAH YPAVERRITF DYVSAGPVC
1441 SKTEAGYKVL DVERAFRPTG DAPLPCFQNN CLFLEKAGLQ FRDNRTKEIL SLVDVIERAV
1501 ARIERKKKVL TTVQTLVAQA PVAEVSFHSV VQQLKARQEA TDEQLEELQE AFAKTQERS
1561 VFSDWMKISA MLCAATLALT QVVKMAKTVK QMVRPDLVRV QLDEQEQQPY NEAVRAKPKT
1621 LQLLDIQGN PVMDFEKYVA KFVTAPIDFV YPTGVSTQTC LLVKGRTLAV NRHMAESDWS
1681 SIVVRGVTHA RSTVRILAIA KAGKETDVSF IRLSSGPLFR DNTSKFVKAD DVLPTSAPV
1741 IGIMNTDIPM MFTGTFLKAG VSPVETGQT FNHCIHYKAN TRKGWCGSAL LADLGKKKI
1801 LGMHSAGSMG VAAASIVSQE MICAVVSAFE PQGALERLPD GPRIHVPRKT ALRPTVARQV
1861 FQPAYAPAVL SKFDPRTEAD VDEVAFSKHT SNQESLPPVF RMVAKEYANR VFTLLGRDNG
1921 RLTVKQALEG LEGMDPMDKN TSPGLPYTAL GMRRTDVVDW ESATLIPYAA DRLKKMNEGD
1981 FSDIVYQTFE KDELRPVEKV QAAKTRIVDV PPFEHCILGR QLLGRFASKF QTQPGLELGS
2041 AIGCDPDVHW TAFGVAMQGF ERVYDVDYSN FDSTHSVAMF RLLAEFFFTP ENGFDPLVKE
2101 YLESLAISTH AFEEKRYLIT GGLPSGCAAT SMLNTIMNNI IIRAGLYLTY KNFEFDDVKV
2161 LSYGDDLLVA TNYQLNFDKV RASLAKTGYK ITPANKTSTF PLDSTLEDVV FLKRKFKEG
2221 PLYRPVMNRE ALEAMLSYR PGTLSKLT ITMLAVHSGK PEYDRLFAPF REVGVVVPSF
2281 ESVEYRWRSF FW (SEQ ID NO:27)

Fig. 75B

1 MATTMEQEIC AHSITFKGCP KCSALQYRNG FYLLKYDEEW YPEELLTDGE DDVFDPELDM
61 EVVFELQGNS TSSDKNNSSS DNEGVIINN FYSNQYQNSI DLSANATGSD PPRTYGQFSN
121 LLSGAVNAFS NMIPLLADQN TEEMENLSDR VLQDTAGNTV TNTQSTVGRL VGYGAVHDGE
181 HPASCADTAS EKILAVEYYY TFKVNDWTST QKPFYIRIP LPHVLSGEDG GVFGAALRRH
241 YLVKTGWRVQ VQCNASQFHA GSLLVFMAPE YPTLDAFAMD NRWSKDNLPN GTKTQTNRKG
301 PFAMDHQNEF QWTLYPHQFL NLRNTTTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
361 YSTGASTSLD ITASIQVVRP VFNGLRHETL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
421 KTPVAPANYM VGEYKDFLEI AQIPTFIGNK IPNAVPIYEA SNTAVKTQPL ATYQVTLSCS
481 CLANTFLAAL SRNFAQYRGS LVYTFVETGT AMMKGKFELIA YTPPGAGKPT SRDQAMQATY
541 AIWDLGLNSS YSFTVPFISP THERMVGTDQ VNITNVDGWV TVWQLTPLTY PPGCPTSAKI
601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE RGVTEDTDAT ADFVAQPVYL PENQTKVAFF
661 YDRSSPIGAF TVKSGSLESG FAPFSNKTCP NSVILTPGPQ FDPAYDQLRP QRLTEIWGNR
721 NEETSKVFPL KSKQDYSFCL FSPFVYYKCD LEVTLSPHTS GNHGLLVVWC PTGTPAKPTT
781 QVLHEVSSLS EGRTPOVYSA GPGISNQISF VVPYNSPLSV LPAVWYNGHK RFDNTGSLGI
841 APNSDFGTLF FAGTKPDIKF TVYLRYKNMR VFCPRPTVEF PWPSSGDKID MTPRAGVLML
901 ESPNALDISR TYPTLHILIQ FNHGGLEIRL FRHGQFWAEA HADVILRSRT KQISFLNNGS
961 FPSMDARAPW NPWKNTYHAV LRAEPYRVTM DVYHKRIRPF RLPLVQKEWN VREENVFGLY
1021 SIFNAHYAGY FADLLIHDIE TNPGPFMAKP KKQVFQTOGA AVSSMAQTLL PNDLASKVMG
1081 SAFTALLDAN EDAQKAMRII KTLSSLSDAW ENVKETLNNP EFWKQLLSRC VQLIAGMTIA
1141 VMHPDPLTLL CLGTLTAAEI TSQTSLCEEI VAKFKKIETT PPRFPTISL FQQQSPLKQV
1201 NDVFSLAKNL DWAVKTVEKV VDWFGTWVVQ EEKEQTLTDL LQRFPEHAKR ISDLRNGMSA
1261 YVECKESFDF FEKLYNQAVK EKRTGIAAVC EKFRQKHDHA TARCEPVVIV LRGDAGQGKS
1321 LSSQVIAQAV SKTIFGRQSV YSLPPDSDFD DGYENQFAAI MDDLGNPDG SDFTTFCQMV

Fig. 76A

1381 STTNFLPNMA SLERKGTPTFT SQLVVATTNL PEFRPVTIAH YPAVERRITF DYSVSAGPVC
1441 SKTEAGYKVL DVERAFRPTG DAPLPCFQNN CLFLEKAGLQ FRDNRTKEIL SLVDVIERAV
1501 ARIERKKKVL TTVQTLVAQA PVDEVSFHSV VQQLKARQEA TDEQLEELQE AFAKTQERS
1561 VFSDWMKISA MLCAATLALT QVVKMAKTVK QMVRPDLVRV QLDEQEQQPY NEAVRAKPKT
1621 LQLLDIQGPN PVMDFEKYVA KEVTAPIDFV YPTGVSTQTC LLVKGRTLAV NRHMAESDWS
1681 SIVVRGVTHA RSTVRILAIA KAGKETDVSF IRLSSGPLFR DNTSKFVKAD DVLPTSAPV
1741 IGIMNTDIPM MFTGTFLKAG VSPVETGQT FNHCIHYKAN TRKGWCGSAL LADLGKKKI
1801 LGMHSAGSMG RTAASIVSQE MICAVVSAFE PQGALERLPD GPRIHVPRKT ALRPTVARQV
1861 FQPAYAPAVL SKFDPRTDAD VDEVAFSKHT SNQESLPPVF RMVAKEYANR VFTLLGRDNG
1921 RLTVKQALEG LEGMDPMDKN TSPGLPYTAL GMRRTDVVDW ESATLIPYAA DRLKKMNEGD
1981 FSDIVYQTFL KDELRPVEKV QAAKTRIVDV PPFEHCILGR QLLGRFASKF QTQPGLELGS
2041 AIGCDPDVHW TAFGVAMQGF ERVYDVDSYN FDSTHSVAMF RLLAEFFFTP ENGFDPLVKE
2101 YLESLAISTH AFEEKRYLIT GGLPSGCAAT SMLNTIMNNI IIRAGLYLTY KNFEFDDVKV
2161 LSYGDDLLVA TNYQLNFDKV RASLAKTGYK ITPANKTSTF PLDSTLEDVV FLKRKFKKEG
2221 PLYRPVMNRE ALEAMLSYYR PGTLSSEKLT ITMLAVHSGK PEYDRLEFAPF REVGVVVPSF
2281 ESVEYRWRSL FW (SEQ ID NO:28)

Fig. 76B

1 MATTMEQEIC AHSMTFEECP KCSALQYRNG FYLLKYDEEW YPEESLTDGE DDVFDPDLDLDM
61 EVVFETQGNS TSSDKNNSSS EGNIEGVIINN FYSNQYQNSI DLSANATGSD PPKTYGQFSN
121 LLSGAVNAFS NMLPLLADQN TEEMENLSDR VSQDTAGNTV TNTQSTVGRL VGYGTVHDGE
181 HPASCADTAS EKILAVEERY TFKVNDWTST QKPFYIRIP LPHVLSGEDG GVFGATLRRH
241 YLVKTGWRVQ VQCNASQFHA GSLLVFMAPE YPTLDVFAMD NRWSKDNLPN GTRTQTNRKG
301 PFAMDHQNFQ QWTLYPHQFL NLRNTNTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
361 YSTGASTSLD ITASIQPVRP VFENGLRHEVL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
421 KTPVAPANYM VGEYKDFLEI AQIPTFIGNK VPNAVPIEA SNTAVKTQPL AVYQVTLSCS
481 CLANTFLAAL SRNFAQYRGS LVTFTVFTGT AMMKGKFLIA YTPPGAGKPT SRDQAMQATY
541 AIWDLGLNSS YSFTVPPFISP THFRMVGTDQ ANITNVDGWV TVWQLTPLTY PPGCPTSARI
601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE KGVNTENTDAT ADFVAQPVYL PENQTKVAFF
661 YDRSSPIGAF AVKSGSLESG FAPFSNKACP NSVILTPGPQ FDPAYDQLRP QRLTEIWGNG
721 NEETSEVFPL KTKQDYSFCL FSPFVYYKCD LEVTLSPHTS GAHGLLVVWC PTGTPTKPTT
781 QVLHEVSSLS EGRTPQVYSA GPGTSNQISF VVPYNSPLSV LPAVWYNGHK RFDNTGDLGI
841 APNSDFGTLF FAGTKPDIKF TVYLRYKNMR VFCPRPTVFF PWPTSGDKID MTPRAGVLMML
901 ESPNPLDVSK TYPTLHILLQ FNHRGLEARI FRHGQLWAET HAEVVLRSKT KQISFLSNGS
961 YPSMDATTPL NPWKSTYQAV LRAEPHRVTM DVIYHKRIRPF RLPLVQKEWR TCEENVFGLY
1021 HVFETHYAGY FSDLLIHDVE TNPGPFTFKP RQRPVFQTQG AAVSSMAQTL LPNDLASKAM
1081 GSAFTALLDA NEDAQKAMKI IKTLSSLSDA WENVKGTLLN PEFWKQLLSR CVQLIAGMTI
1141 AVMHPDPLTL LCLGVLTAEE ITSQTSLECE IAAKFKTIFT TPPPRFPVIS LFQQQSPLKQ
1201 VNDVFSLAKN LDWAVKTVEK VVDWFGTWVA QEEREQTLDO LLQRFPEHAK RISDLRNGMA
1261 AYVECKESFD FFEKLYNQAV KEKRTGIAAV CEKFRQKHDH ATARCEPVVI VLRGDAGQOK

Fig. 77A

1321 SLSSQIIAQA VSKTIFGRQS VYSLPPDSDF FDGYENQFAA IMDDLQNPDP GSDFTTFCQM
1381 VSTTNLLPNM ASLERKGTPF TSQLVVATTN LPEFRPVTIA HYPAVERRIT FDYSVSAGPV
1441 CSKTEAGCKV LDVERAFRPT GDAPLPCFQN NCLFLEKAGL QFRDNRSKEI LSLVDVIERA
1501 VTIERKKKV LTAVQTLVAQ GPVDEVSFYS VVQQLKARQE ATDEQLEELQ EAFARVQERS
1561 SVFSDWMKIS AMLCAATLAL TQVVKMAKAV KQMVRLDLVR VQLDEQEQQP YNETTRIKPK
1621 TLQLLDVQGP NPTMDFEKFV AKFVTAPIGF VYPTGVSTQT CLLVKGRTLA VNRHMAESDW
1681 TSIVVRGVSH TRSSVKIIAI AKAGKETDVS FIRLSSGPLF RDNTSKFVKA SDVLPHSSSP
1741 LIGIMNVDIP MMYTGTFLLA GVSVPVETGQ TFNHCIHYKA NTRKGWCGSA ILADLGGSKK
1801 ILGFHSAGSM GVAAASIISQ EMIDAVVQAF EPQGALERLP DGPRIHVPRK TALRPTVARQ
1861 VFQPAFAPAV LSKFDPRTDA DVDEVAFSKH TSNQETLPPV FRMVAREYAN RVFALLGRDN
1921 GRLSVKQALD GLEGMDPMDK NTSPGLPYTT LGMRRTDVVD WETATLIPFA AERLEKMNNK
1981 DFSDIVYQTF LKDELRIPIK VQAAKTRIVD VPPFEHCILG RQLLGKFASK FQTQPGLELG
2041 SAIGCDPDVH WTAFGVAMQG FERVYDVDYS NFDSTHSVAI FRLLAEFFFS EENGFDPLVK
2101 DYLESLAISK HAYEEKRYLI TGGLPSGCAA TSMLNTIMNN IIIRAGLYLT YKNFEFDDVK
2161 VLSYGDDLLV ATNYQLNFDR VRTSLAKTGY KITPANKTST FPLESTLEDV VFLKRKFKKE
2221 GPLYRPVMNR EALEAMLSYY RPYTLSEKLT SITMLAVHSG KQEYDRLFAP FREVGIVVPT
2281 FESVEYRWRS LFW (SEQ ID NO:29)

Fig. 77B

1 MACKHGYPDV CPICTAVDVT PGFEYLLLDAD GEWFPTDLLC VDLDDDVFWP SNSSNQSETM
61 EWTDLPLVRD IVMEPQGNAS SSDKSNSQSS GNEGVIINNF YSNQYQNSID LSASGGNAGD
121 APQNNGQLSN ILGGAANAFATMAPLLLDQN TEEMENLSDR VASDKAGNSA TNTQSTVGRL
181 CGYGEAHHGE HPASCADTAT DKVLAAERYT TIDLASWTTT QEAFSHIRIP LPHVLAGEDG
241 GVFGATLRRH YLCKTGWRVQ VQCNASQFHA GSLLVFMAPE FYTGKGTKTG DMEPTDPFTM
301 DTTWRAPQGA PTGYRYDSRT GFFAMNHQNO WQWTVYPHQI LNLRTNTTVD LEVPYVNIAP
361 TSSWTQHAW TLVVAVFSPL QYASGSSSDV QITASIQPVN PVFNGLRHET VIAQSPIAVT
421 VREHKGCFYS TNPDTTVPIY GKTISTPNDY MCGEFSDLLE LCKLPTFLGN PNSNNKRYPY
481 FSATNSVPTT SLVDYQVALS CSCMCNSMLA AVARNFNQYR GSLNFLFVFT GAAMVKGKFL
541 IAYTPPGAGK PTTRDQAMQA TYAIWDLGLN SSFVFTAPFI SPTHYRQTSY TSATIASVDG
601 WVTWVQLTPL TYPGAPVNS DILTIVSAGD DFTLRMPISP TKWAPQGSND AEKGKVSND
661 ASVDFVAEPV KLPENQTRVA FFYDRAVPIG MLRPGQNIES TFVYQENDLR LNCLLLTPLP
721 SFCPDSTSGP VKTKAPVQWR WVRSGGTTNF PLMTKQDYAF LCFSPFTYYK CDLEVTVSAL
781 GTDTVASVLR WAPTGAPADV TDQLIGYTPS LGETRNPBMW LVGAGNTQIS FVVPYNSPLS
841 VLPAAWFNGW SDFGNTKDFG VAPNADFGRL WIQGNTSASV RIRYKKMKVF CPRPTLFFPW
901 PVSTRSKINA DNPVPILELE NPAAFYRIDL FITFIDEFIT FDYKVHGRPVT LTRIPGFGL
961 TPAGRMLVCM GEKPAHGPFT SSRSLYHVIF TATCSSFSFS IYKGRYRSWK KPIHDELVDR
1021 GYTTFGEFFR AVRAYHADYY KQRLIHDVEM NPGPVQSVFQ PQGAVLTKSL APQAGIQNLL
1081 LRLLGIDGDC SEVSKAITVV TDLFAAWERA KTTLVSPFEW SKLILKTTKF IAASVLYLHN
1141 PDFTTTVCLS LMTGVDLLTN DSVFDWLKNK LSSFFRTPPP VCPNVLQPQG PLREANEGET
1201 FAKNIEWAMK TIQSIVNWLTSWFKQEEDHP QSKLDKFLME FPDHCRNIMD MRNGRKAYCE
1261 CTASFKYFDE LYNLAVTCKR IPLASLCEKF KNRHDHSVTR PEPVVVVLRG AAGQGKSVTS
1321 QIIAQSVSKM AFGRQSVYSM PPDSEYFDGY ENQFSVIMDD LGQNPDGEDF TVFCQMVST

Fig. 78A

1381 NFLPNMAHLE RKGTPFTSSF IVATTNLPKF RPVTVAHYPA VDRRITFDFT VTAGPHCTTS
1441 NGMLDIEKAF DEIPGSKPQL ACFSADCPLL HKRGVMFTCN RTKAVYNLQQ VVKMVNDTIT
1501 RKTENVKKMN SLVAQSPPDW EHFENILTCL RQNNALQDQ LDELQEAFAQ ARERSDFLSD
1561 WLKVSIIIFA GIASLSAVIK LASKFKESIW PSPVRVELSE GEQAAYAGRA RAQKQALQVL
1621 DIQGGGKVLA QAGNPVMDFE LFCAKNMVAP ITFYYPDKAE VTQSCLLLRA HLFVVNRHVA
1681 ETEWTAFKLK DVRHERDTVV TRSVNRSGAE TDLTFIKVTK GPLFKDNVNK FCSNKDDFPA
1741 RNDAVTGIMN TGLAFVYSGN FLIGNQPVNT TTGACFNHCL HYRAQTRRGW CGSAVICNVN
1801 GKKAVYGMHS AGGGGLAAAT IITRELIEAA EKSMLELEPQ GAIVDISTGS VVHVPRKTKL
1861 RRTVAHDVFQ PKFEPVLSR YDPRTDKDVD VVAFSKHTTN MESLPPVFDI VCDEYANRVF
1921 TILGKDNGLL TVEQAVLGLP GMDPMEKDTS PGLPYTQQGL RRTDLLNFNT AKMTPQLDYA
1981 HSKLVLGVYD DVVYQSFLKD EIRPLEKIHE AKTRIVDVPP FAHCIWGRQL LGRFASKFQT
2041 KPGLELGSAI GTDPDWDWTP YAAELSGFNY VYDVDYSNFD ASHSTAMFEC LIKNFFTEQN
2101 GFDRRIAAYL RSLAVSRHAY EDRRVLIRGG LLSGCAATSM LNTIMNNVII RAALYLTYSN
2161 FEFDDIKVLS YGDDLLIGTN YQIDFNLVKE RLAPFGYKIT PANKTTTFPL TSHLQDVTFL
2221 KRRFVRFNSY LFRPQMDAVN LKAMVSYCKP GTLKEKLMSI ALLAVHSGPD IYDEIFLPFR
2281 NVGIIVPTYS SMLYRWLSLF R (SEQ ID NO:30)

Fig. 78B

1 MACKHGYPDV CPICTAVDAT PDFEYLLMAD GEWEPTDLLC VDLDDDVFWP SDTSTQPQTM
61 EWTDVPLVCD TVMEPQGNAS SSDKSNSQSS GNEGVIINNF YSNQYQNSID LSASGGNAGD
121 APQNNQQLSS ILGGAANAFA TMAPLLMDQN TEEMENLSDR VASDKAGNSA TNTQSTVGRL
181 CGYGKSHHGE HPTSCADAAT DKVLAAERY Y TIDLASWTTS QEAFSHIRIP LPHVLAGEDG
241 GVFGATLRRH YLCKTGWRVQ VQCNASQFHA GSLLVFMAPE FYTGKGTKSG TMEPSDPFTM
301 DTTWRSPQSA PTGYRYDRQA GFFAMNHQNO WQWTVYPHQI LNLRTNTTVD LEVPYVNVAP
361 SSSWTQHAW TLVVAVLSPL QYATGSSPDV QITASLQPVN PVFNGLRHET VLAQSPIPVT
421 VREHQGCFYS TNPDTTVPIY GKTISTPSDY MCGEFSDLLE LCKLPTFLGN PSTDNKRYPY
481 FSATNSVPAT SLVDYQVALS CSCTANSMLA AVARNFNQYR GSLNFLEVFT GAAMVKGKFR
541 IAYTPPGAGK PTTRDQAMQA TYAIWDLGLN SSFNFTAPFI SPTHYRQTSY TSPTITSVDG
601 WVTWQLTPL TYPSTGTPHS DILTLVSAGD DFTLRMPISP TKWVPQIDN AEKGKVSND
661 ASVDFVAEPV KLPENQTRVA FFYDRAVPIG MLRPGQNMET TFSYQENDER LNCLLLTPLP
721 SYCPDSSSGP VRTKAPVQWR WVRSGGANGA NFPLMTKQDY AFLCFSPFTY YKCDLEVTVS
781 AMGAGTVSSV LRWAPTGAPA DVTDLQIGYT PSLGETRNP MWIVGSGNSQ ISFVVPYNP
841 LSVLPAAWFN GWSDFGNTKD FGVAPTSDFG RIWIQGNSSA SVRIRYKKMK VFCPRPTLFF
901 PWPTPTTTKI NADNPVPILE LENPASLYRI DLFITFTDEL ITFDYKVHGR PVLTFRIPGF
961 GLTPAGRMLV CMGAKPAHSP FTSSKSLYHV IFTSTCNSFS FTIYKGRYS WKKPIHDELV
1021 DRGYTTTREF FKAVERGYHAD YYKQRLIHDV EMNPGPVQSV FQPOGAVLTK SLAPQAGION
1081 ILLRLLGIEG DCSEVSKAIT VVTDLVAAWE KAKTTLVSPE FWSELILKTT KFIAASVLYL
1141 HNPDETTTVC LSLMTGVDLL TNDVFDWLK SKLSSFFRTP PPACPNVMQP QGPLREANEG
1201 FTFKNIEWA TKTIQSIVNW LTSWFKQED HPQSKLDKLL MEFPDHCRNI MDMRNGRKAY
1261 CECTASFKEY DDLYNLAVTC KRIPLASLCE KFKNRHDHSV TRPEPVAVL RGAAGQGKSV
1321 TSQIIAQSVS KMAFGRQSVY SMPPDSEYFD GYENQFSVIM DDLGQNPGE DFTVFCQMV
1381 STNFLPNMAH LERKGTPTS SFIVATTNLP KFRPVTVAHY PAVDRRITFD FTVTAGPHCK

Fig. 79A

1441 TPAGMLDIEK AFDEIPGSKP QLACFSADCP LLHKRGVMFT CNRTKTVYNL QQVVKMVNDT
1501 ITRKTENVKK MNSLVAQSPP DWQHFENILT CLRQNNALQ DQVDELQEAF TQARERSDFL
1561 SDWLKVSAIL FAGIVSLSAV IKLASKFKES IWPTPVRVEL SEGEQAAYAG RARAQKQALQ
1621 VLDIQGGGKV LAQAGNPVMD FELFCAKNMV SPITFYYPDK AEVTQSCLLL RAHLFVVNRH
1681 VAETEWTAfk LRDVRHERDT VVMRSVNRSG AETDLTFVKV TKGPLEKDNV NKFCSNKDDF
1741 PARNDTVTGI MNTGLAFVYS GNFLIGNQPV NTTTGACFNH CLHYRAQTRR GWCGSAIICN
1801 VNGKKAVYGM HSAGGGGLAA ATIIITRELIE AAEKSMLALE PQGAIVDIST GSVVHVPRKT
1861 KLRRTVAHDV FQPKFEPÄVL SRYDPRTDKD VDVVAFSKHT TNMESLPPIF DIVCGEYANR
1921 VFTILGKDNG LLTVEQAVLG LSGMDPMEKD TSPGLPYTQQ GLRRTDLLDF NTAKMTPQLD
1981 YAHSKLVLGV YDDVVYQSFL KDEIRPLEKI HEAKTRIVDV PPFACIWR GRQLLGRFASKF
2041 QTKPGFELGS AIGTDPDVDW TRYAAELSGF NYVYDVDYSN FDASHSTAMF ECLINNEFTE
2101 QNGFDRRIAE YLRSLAVSRH AYEDRRVLIR GGLPSGCAAT SMLNTIMNNV IIRAALYLT
2161 SNFEFDDIKV LSYGDDLLIG TNYQIDFNLV KERLAPFGYK ITPANKTTTF PLTSHLQDVT
2221 FLKRRFVRFN SYLFRPQMDA VNLKAMVSYC KPGTLKEKLM SIALLAHVSG PDIYDEIFLP
2281 FRNVGIVVPT YDSMLYRWLS LFR (SEQ ID NO:31)

Fig. 79B

1 MACKHGYPDV CPICTAVDAT PGFEYLLMAD GEWYPTDLLC VDLDDDVFWP SDTSNQSQTM
61 DWTDVPLIRD IVMEPQGNSS SSDKSNSQSS GNEGVIINNF YSNQYQNSID LSASGGNAGD
121 APQTNGQLSN ILGGAANAFA TMAPLLLDQN TEEMENLSDR VASDKAGNSA TNTQSTVGRL
181 CGYGKSHHGE HPASCADTAT DKVLAAERY Y TIDLASWTTS QEAFSHIRIP LPHVLAGEDG
241 GVFGATLRRH YLCKTGWRVQ VQCNAQOFHA GSELLVFMAPE FYTGKGTKTG TMEPSDPFTM
301 DTEWRSPQGA PTGYRYDSRT GFFATNHQNO WQWTVYPHQI LNLRTNTTVD LEVPYVNVAP
361 SSSWTQHAW TLVVAVLSPL QYATGSSPDV QITASLQPVN PVFNGLRHET VIAQSPIPVT
421 VREHKGCFYS TNPDTTVPIY GKTISTPSDY MCGEFSDLLE LCKLPTFLGN PNTNNKRYPY
481 FSATNSVPAT SMVDYQVALS CSCMANSM LA AVARNFNQYR GSLNFLFVFT GAAMVKGKFL
541 IAYTPPGAGK PTTRDQAMQS TYAIWDLGLN SSFNFTAPFI SPTHYRQTSY TSPTITSVDG
601 WVTVWKLTP L TYPSTPTNS DILTLVSAGD DFTLRMPISP TKWVPQGVND AEKGKVSND
661 ASVDFVAEPV KLPENQTRVA FFYDRAVPIG MLRPGQNMET TFNYQENDYR LNCLLLTPLP
721 SFCPDSSSGP QKTKAPVQWR WVRSGGVNGA NFPLMTKQDY AFLCFSPFTF YKCDLEVTVS
781 ALGMTRVASV LRWAPTGAPA DVTDQLIGYT PSLGETRNP MWLVGAGNSQ VSFVVPYNP
841 LSVLPAAWFN GWSDFGNTKD FGVAPNADEF RLWIQGNTSA SVRIRYKMK VFCPRPTLFF
901 PWPTPTTTKI NADNPVPILE LENPAALYRI DLFITFTDEF ITFDYKVHGR PVLTFRIPGF
961 GLTPAGRMLV CMGEQPAHGP FTSSRSLYHV IFTATCSSFS FSIYKGRYRS WKKPIHDELV
1021 DRGYTTFGEF FKAVERGYHAD YYRQRLIHDV ETNPGPVQSV FQPQGAULTK SLAPQAGION
1081 LLLRLLGIDG DCSEVSKAIT VVTDLVAAWE KAKTTLVSPE FWSKLILKTT KFIAASVLYL
1141 HNPDEFTTVC LSLMTGVDLL TNDVFDWLK QKLSSFFRTP PPACPNVMQP QGPLREANEG
1201 FTFKNIEWA MKTIQSVVNW LTSWFKQED HPQSKLDKLL MEFPDHCRI MDMRNGRKAY
1261 CECTASFKYF DELYNLAVTC KRIPLASLCE KFKNRHDHSV TRPEPVVVVL RGAAGQGKSV
1321 TSQIIAQSVS KMAFGRQSVY SMPDSEYFD GYENQFSVIM DDLGQNPDE DFTVFCQMV
1381 STNFLPNMAH LERKGTPTS SFIVATTNLP KERPVTVAHY PAVDRRITFD FTVTAGPHCK

Fig. 80A

1441 TPAGMLDVEK AFDEIPGSKP QLACFSADCP LLHKRGVMFT CNRTQTVYNL QQVVKMVNDT
1501 ITRKTENVKK MNSLVAQSPP DWEHFENILT CLRQNNALQ DQLDELQEAF AQARERSDFL
1561 SDWLKVSAIL FAGIASLSAV IKLASKFKES IWPTPVRVEL SEGEQAAYAG RARAQKQALQ
1621 VLDIQGGGKV LAQAGNPVMD FELFCAKNIV APITFYYPDK AEVTQSCLLL RAHLEVVNRH
1681 VAETDWTAFK LKDVRHERHT VALRSVNRSG AKTDLTFIKV TKGPLFKDNV NKFCSNKDDF
1741 PARNDTVTGI MNTGLAFVYS GNFLIGNQPV NTTTGACFNH CLHYRAQTRR GWCGSAIICN
1801 VNGKKAVYGM HSAGGGGLAA ATIITKELIE AAEKSMLALE PQGAIVDIAT GSVVHVPRKT
1861 KLRRTVAHDV FQPKFEPVL SRYDPRTDKD VDVVAFSKHT TNMESLPPIF DVVCGEYANR
1921 VFTILGKENG LLTVEQAVLG LPGMDPMEKD TSPGLPYTQQ GLRRTDLLNF ITAKMTPQLD
1981 YAHSKLVIGV YDDVVYQSFL KDEIRPIEKI HEAKTRIVDV PPFAHCIWGR QLLGRFASKF
2041 QTKPGLELGS AIGTDPDVDW TRYAVELSGF NYVYDVDYSN FDASHSTAMF ECLINNFFTE
2101 QNGFDRRIAE YLRSLAVSRH AYEDRRVLIR GGLPSGCAAT SMLNTIMNNV IIRAALYLT
2161 SNFDFDDIKV LSYGDDLLIG TNYQIDFNLV KERLAPFGYK ITPANKTTTF PLTSHLQDVT
2221 FLKRREFVRN SYLFRPQMDA VNLKAMVSYC KPGTLKEKLM SIALLAHVSG PDIYDEIFLP
2281 FRNVGIVVPT YSSMLYRWLS LER (SEQ ID NO:32)

Fig. 80B

1 MMACIHGYPS VCPICTAIDK SSDGMYLLLA DNEWFPADLL TMDLDDDVFW PNDES DVSET
61 MDWTDLPFIL DTIMEPQGNS TSSDKSNSQS SGNEGVIINN FYSNQYQNSI DLSANGGNAG
121 GAPKTEGQLG NILGNAANAF STMAPLLLDQ NTEEMENLSD RVDSDKAGNS AVNTQSSVGR
181 LCGYGMHHKG KHPASCADTA TDKVLSAERY YTIDLATWTT TLGTFSHIRI PLPHVLAGED
241 GGVFGSTLRR HYLCKCGWRI QVQCNASQFH AGSLLVFMAP EFYTGHTPVT GTTEPATPFT
301 MDSSWQTPQQ NPVGFRYDGR TGYFALNHQN YWQWMVYPHQ ILNLRTNTSV DLEVPFNTIA
361 PTSSWTQHAN WTLVVAVLTP LQYAAGSATD VQITASIQPV KPVFNGLRHE AVVPQSPIPV
421 TVREHQGTFFY STNPDTTVPI YGKTIATPSD YMCGEFSDLV ELCKLPTFLG NPANTSPAGG
481 RYPYFSATNS VPATALASYQ VALSCSCMSN SMLAAVARNF NQYRGSLNFL FVFTGTAMTK
541 GKFLIAYTPP GAGKPTTREQ AMQATYAIWD LGLNSSYNFT VPFISPTHYR QTSYTSTSIT
601 SVDGWLTWVQ LTPLTYPANT PPNADILTLV SAGDDEFTLRM PISPTKWIPQ GVDNAEKGKV
661 SNDDATVDFV AEPVKFPDNQ TKVSFFYDRS VPLGLLRPAQ GMEQDFAYAA NDSRANSILL
721 TPLPSYAPDS TTGPTETQAP IQWRWLRGTS DGSTTFPLMT KQDYAFLLFS PFTYYKADLE
781 VTLAISNSN NVTVVRWAPT GAPADISRQL SGYTPSIGDT RDPHLWFEVGA GNSQTSFVVP
841 YNSPLSVLPA AWFNGWSDFG NTKDFGVAPN ADFGRLWIQG NTSVAVRVRY KKMKVFCPRP
901 TLFLPWPSTT TTRIHADNPV SVMELQNPFS FYRVDLFITE TDELITFDYK VHGRPVLQYQ
961 VPGLGLTCAG RMLVCMGQMP NHAPFSTVRH LYHVVFETGSR NSFQVVIYYK RHRPWKKPLH
1021 EELHDYGFEC FSDFFKHVRE YHAAYYKQRL MHDVETNPGP PVQSVFRPQG GVLTKSQAPM
1081 SGIQNLFLRA LGIDADHGEF TRAVTMITDL CNTWEKAKNT LVSPEFWTVL IMKTVKFIAA
1141 SVLYLHNPDL TATICLSLMT GVDVLTNESI FNWLSNKLKSK LFHTPPPPTS PLLQAQSPLR
1201 EANDGFNLAK NIEWAIKTVQ KIVDWLMSWF KQEEAHPQAK LDKMLADFPE HCASILAMRN
1261 GRKAYTDCAG AFKYFEDLYN LAVQCKRIPL ATLCEKFKNK HDHAVARPEP VVVVLRGNAG
1321 QGKSVTSQII AQAVSKLAFG RQSVYSIPPD SDYLDGYENQ FSVIMDDLQ NPEDGEFKVF
1381 CQMVSSTNFL PNMAHLEKKG TPFTSNFIVA TTNLPKFRPV TVAHYPAVDR RITFDLTVEA

Fig. 81A

1441 GPACKTPTGM LDVEKAFQEI PGEPQLDCFS SDCALLHKRG VQFICNRTHK IYNLQQIVKM
1501 VKDTIDNKVA NLKKMNTLVA QSPNNGNDME HIITCLRQNN AALQDQIDEL QEAFQAQER
1561 QNFLSDWMKV SAIIFAGIAS LSAVCKLVGR LKNLIWPSPV HVELSEGEQA AYAGAKRGAK
1621 QALQVLDLQG GGRIIAQAGN PVMDYEVCSA KNMVAPITFY YADKAQVTQS CLLVKGRLEFV
1681 VNRHVAETDW VSFELRDVRH ERDTVMTSRV NRSMEVDLT FIKVTKGPLF KDNTKKFCSN
1741 KDDFPQKNET VTGIMNTGLP FVFNGKFIIG NHPVNTTTGA TFNHCLHYRA NTRRGWCGSA
1801 VICQVNGKKA VYGMHSAGGG GLAAATIITQ ELVEAAEQNM DRLVPQGAIM EIGTGSVVHV
1861 PRKTKLRRTV AHEIFLPKFE PAVLSRYDPR TEKDVDQVAF SKHTTNMEEL PAVFSMVAKE
1921 YANRVFTKLK KENQLLTQQ AILGLPGMDP MEKDTSPGLP YTQQGLRRTD LVNFETGKMD
1981 HNLDYAHSL MLGHYEDVVY QSFLKDEIRP IEKIHEAKTR IVDVPPFHHC IWGRQLLGRF
2041 ASRFQTNPGL DLGSAIGTDP DVDWTVFAHQ LAEFKYIYDV DYSNFDASHS TAIFEILIQE
2101 FFTPQNGFDP RIGEYLRSLA VSRHAYEDRR VLIRGGLPSG CAATSMINTI INNIVIRAAL
2161 YMTYANFEED DIKVLSYGDD LLIATNYEIN FNLVKERLAP FNYKITPANK TSTFPQTSHL
2221 QDVVFLKRRF VQFNSFLFRP QMETENLKAM VSYCRPGVLK EKLMSIALLA VHSGPDVYDE
2281 IFMPFRRIGV VVPEYSTMLY RWLNLEF (SEQ ID NO:33)

Fig. 81B

1 MACKHGYPDV CPICTAIDVT PGFEYLLLD GEWFPTDLLC VDLDDDVFWP SDSSNQSQTM
61 EWTDIPLICD TVMEPQGNST SSDKSNSQSS GNEGVIINNF YSNQYQNSID LSANGGNAGD
121 GPKTEGQLSN ILGGAANAFA TMAPLLLDEN TEEMENLSDR VDSKAGNSA TNTQSSVGRL
181 HGYGATHRGD HPASCADTAT DKVLAAERY Y TIDLATWTTA QTTFSHIRVP LPHALAGEHG
241 GVFGATLRRH YLAKCGWRVQ VQCNASQFHA GSLLVFLAPE FYTGTGVATS GQEPNKVFLM
301 DTTWQEPQAA PTGFRYDGKN GFFTLNHQNY WQWTVYPHQI LNLRTNTSVD LEVPYVNVAP
361 TSSWTQHAW ALVVAVLTPL QYSTGAATDV AITVSLQPVN PVFNGLRHEA QVPQSPVAVT
421 VREHQGSFYS TNPDTTVPIY GKTIVTPSDY MCGEFTDLLE LCKLPTFLGN LSNDTRVPFF
481 TATNSVPTES LVEYQVTLSC SCMSNSMLAS VARNFNQYRG SLNFLFVFTG SAMTKGKFLI
541 AYTTPGAGKP TTRDQAXQST YAIWDLGLNS SYNFTVPFIS PSHYRQTSYT SPSIAAVDGW
601 LTVWQLTPLT FPANVPPSSD ILTLVSAGND FTLRMPISPT KWIPQGV DNA EKGKVSDDNA
661 SVDFVAEPIK LPENQTRVNF FYDRSSPIGL LRPNQAIEN FSYSADSNGA TNCALLTPLP
721 SYSPDRPGQS PDTSKAPIQW RWISAVTESG TVSNTEPTRT RQDYAFLLFS PFTYYKCDLE
781 VTLSSVGNGV VASLVRWAPT GAPADITTQL TTSTPSIGDT RDPHMWLVGA GNSQTSEVIP
841 YNSPLSVLPA AWFNGWSNFS NTYDFGIAPC SDFGRLWIQG NAPLAIRVRY KKMRFVFCPRP
901 TLFFPWPTPT TTKVNADNPV PILDLENPAA (SEQ ID NO:34)

Fig. 82

10 20 30 40 50 60 70 80 90
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTTGAAATGGGGGGCTGGGCCCTGATGCCAGTCCTTCTCCCTTCCGGGGGTTAACCGGCTGTGTTTGCTAGAGGCACAGAGGGG
< 5' UTR

100 110 120 130 140 150 160 170 180
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CAACATCCAACCTGCTTTTGCGGGGAACGGTGCGGCTCCGATTCTGCGTCGCCAAAGGTGTTAGCGCACCCAAACGGCGCACCTACCAA

190 200 210 220 230 240 250 260 270
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TGTTATTGGTGTGGTCTGCGAGTTCTAGCCTACTCGTTTCTCCCCCGACCATTCACTCACCCACGAAAAGTGTGTTGTAACCATAAGATT

280 290 300 310 320 330 340 350 360
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TAACCCCGGCACGGGATGTGCGATAACCGTAAGACTGGCTCAAGCGCGGAAAGCGCTGTAACCACATGCTGTTAGTCCCTTTATGGCTGC

370 380 390 400 410 420 430 440 450
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAGATGGCTACCCACCTCGGATCACTGAACTGGAGCTCGACCCTCCTTAGTAAGGGAACCGAGAGGCCTTCGTGCAACAAGCTCCGACAC

460 470 480 490 500 510 520 530 540
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AGAGTCCACGTGACTGCTACCACCATGAGTACATGGTTCTCCCTCTCGACCCAGGACTTCTTTTTGAATATCCACGGCTCGATCCAGAG

550 560 570 580 590 600 610 620 630
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GGTGGGGCATGACCCCTAGCATAGCGAGCTACAGCGGGAACGTGTAGCTAGGCCTTAGCGTGCCTTGGATACTGCCTGATAGGGCGACGGC

640 650 660 670 680 690 700 710 720
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CTAGTCGTGTCGGTTCTATAGGTAGCACATACAAATATGCAGAACTCTCATTTTTCTTTCGATACAGCCTCTGGCACCTTTGAAGATGTA
M Q N S H F S F D T A S G T F E D V
5' UTR >< Leader

730 740 750 760 770 780 790 800 810
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ACCGGAACAAAAGTCAAGATCGTTGAATACCCAGATCGGTGAACAATGGTGTGTACGATTCTGCTACTCATTTGGAGATACTGAACCTA
T G T K V K I V E Y P R S V N N G V Y D S S T H L E I L N L

820 830 840 850 860 870 880 890 900
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CAGGGTGAAATTGAAATTTTAAGGTCTTTCAATGAATACCAAATTCGCGCCGCCAAACAACACTCGGACTGGACATCGTGTACGAACTA
Q G E I E I L R S F N E Y Q I R A A K Q Q L G L D I V Y E L

910 920 930 940 950 960 970 980 990
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CAGGGTAATGTTTCAGACAACGTCAAAGAATGATTTTGATTCCCGTGGCAATAATGGTAACATGACCTTCAATTACTACGCAAACACTTAT
Q G N V Q T T S K N D F D S R G N N G N M T F N Y Y A N T Y
L >< VP4

1000 1010 1020 1030 1040 1050 1060 1070 1080
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CAGAATTCAGTAGACTTCTCGACCTCCTCGTCGGCGTCAGGCGCCGGACCCGGGAACCTCCCGGGGCGGATTAGCGGGTCTCCTCACAAAT
Q N S V D F S T S S S A S G A G P G N S R G G L A G L L T N

FIG. 83A

1090 1100 1110 1120 1130 1140 1150 1160 1170
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTCAGTGGAATCTTGAACCCCTCTTGGCTACCTCAAAGATCACAACACCGAAGAAATGGAAAACCTCTGCTGATCGAGTCACAACGCAAACG
F S G I L N P L G Y L K D H N T E E M E N S A D R V T T Q T
VP4 >< VP2

1180 1190 1200 1210 1220 1230 1240 1250 1260
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GCGGGCAACACTGCCATAAACACGCAATCATCATTTGGGTGTGTTGTGTGCCTACGTTGAAGACCCGACCAAATCTGATCCTCCGTCCAGC
A G N T A I N T Q S S L G V L C A Y V E D P T K S D P P S S

1270 1280 1290 1300 1310 1320 1330 1340 1350
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AGCACAGATCAACCCACCACTTTCACTGCCATCGACAGGTGGTACACTGGACGTCTCAATTCTTGGACAAAAGCTGTAAAAACCTTC
S T D Q P T T T F T A I D R W Y T G R L N S W T K A V K T F

1360 1370 1380 1390 1400 1410 1420 1430 1440
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TCTTTTCAGGCCGTCCCGCTTCCCGGGGCCTTTCTGTCTAGGCAGGGAGGCCTCAACGGAGGGGCCTTCACAGCTACCCTACATAGACAC
S F Q A V P L P G A F L S R Q G G L N G G A F T A T L H R H

1450 1460 1470 1480 1490 1500 1510 1520 1530
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTTTTGTATGAAGTGCGGGTGGCAGGTGCAGGTCCAATGTAATTTGACACAATTCCACCAAGGCGCTCTTCTTGTGTCATGGTTCCTGAA
F L M K C G W Q V Q V Q C N L T Q F H Q G A L L V A M V P E

1540 1550 1560 1570 1580 1590 1600 1610 1620
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ACCACCCCTTGATGTCAAGCCCGACGGTAAGGCAAAGAGCTTACAGGAGCTGAATGAAGAACAGTGGGTGGAAATGTCTGACGATTACCGG
T T L D V K P D G K A K S L Q E L N E E Q W V E M S D D Y R

1630 1640 1650 1660 1670 1680 1690 1700 1710
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ACCGGGAAAAACATGCCTTTTTCAGTCTCTTGGCACATACTATCGGCCCCCTAACTGGACTTGGGGTCCCAATTTTCATCAACCCCTATCAA
T G K N M P F Q S L G T Y Y R P P N W T W G P N F I N P Y Q

1720 1730 1740 1750 1760 1770 1780 1790 1800
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GTAACGGTTTTTCCACACCAAATCTGAACGCGAGAACCTCTACCTCGGTAGACATAAACGTCCCATACATCGGGGAGACCCACGCAA
V T V F P H Q I L N A R T S T S V D I N V P Y I G E T P T Q

1810 1820 1830 1840 1850 1860 1870 1880 1890
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TCCTCAGAGACACAGAACTCCTGGACCCCTCCTCGTTATGGTGCTCGTTCCCTAGACTATAAGGAAGGAGCCACAACCTGACCCAGAAATT
S S E T Q N S W T L L V M V L V P L D Y K E G A T T D P E I

1900 1910 1920 1930 1940 1950 1960 1970 1980
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ACATTTTCTGTAAGGCCTACAAGTCCCTACTTCAATGGGCTTCGCAACCGCTACACGGCCGGGACGGACGAAGAACAGGGGCCCCATTCCT
T F S V R P T S P Y F N G L R N R Y T A G T D E E Q G P I P
VP2 >< VP3

1990 2000 2010 2020 2030 2040 2050 2060 2070
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ACGGCACCCAGAGAAAATTCGCTTATGTTTCTCTCAACCCTCCCTGACGACACTGTCCCTGCTTACGGGAATGTGCGTACCCCTCCTGTC
T A P R E N S L M F L S T L P D D T V P A Y G N V R T P P V

FIG. 83B

2080 2090 2100 2110 2120 2130 2140 2150 2160
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AATTACCTCCCTGGTGAAATAACCGACCTTTTGCAACTGGCCCGCATACCCACTCTCATGGCATTGAGCGGGTGCCTGAACCCGTGCCT
N Y L P G E I T D L L Q L A R I P T L M A F E R V P E P V P

2170 2180 2190 2200 2210 2220 2230 2240 2250
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GCCTCAGACACATATGTGCCCTACGTTGCCGTTCCCACCCAGTTCGATGACAGGCCTCTCATCTCCTTCCCGATCACCCCTTCAGATCCC
A S D T Y V P Y V A V P T Q F D D R P L I S F P I T L S D P

2260 2270 2280 2290 2300 2310 2320 2330 2340
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GTCTATCAGAACACCCTGGTTGGCGCCATCAGTTCAAATTTCCGCAATTACCGTGGGTGTATCCAAATCACTCTGACATTTTGTGGACCC
V Y Q N T L V G A I S S N F A N Y R G C I Q I T L T F C G P

2350 2360 2370 2380 2390 2400 2410 2420 2430
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ATGATGGCGAGAGGGAAATTCCTGCTCTCGTATTCTCCCCAAATGGAACGCAACCACAGACTCTTCCGAAGCTATGCAGTGCACATAC
M M A R G K F L L S Y S P P N G T Q P Q T L S E A M Q C T Y

2440 2450 2460 2470 2480 2490 2500 2510 2520
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TCTATTTGGGACATAGGCTTGAACCTCTAGTTGGACCTTCGTCGTCCTTACATCTCGCCAGTGACTACCGTGAAACTCGAGCCATTACC
S I W D I G L N S S W T F V V P Y I S P S D Y R E T R A I T

2530 2540 2550 2560 2570 2580 2590 2600 2610
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AACTCGGTTTACTCCGCTGATGGTTGGTTAGCCTGCACAAAGTTGACCAAAATTACTCTACCACCTGACTGTCCGCAAAGTCCCTGCATT
N S V Y S A D G W F S L H K L T K I T L P P D C P Q S P C I

2620 2630 2640 2650 2660 2670 2680 2690 2700
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CTCTTTTTCGCTTCTGCTGGTGAGGATTACACTCTCCGTCTCCCCGTTGATTGTAATCCTTCCCTATGTGTTCC**CACTCC**ACCGACAACGCC
L F F A S A G E D Y T L R L P V D C N P S Y V F **H S** T D N A
VP3 >< VP1

2710 2720 2730 2740 2750 2760 2770 2780 2790
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GAGACCGGGGTTATTGAGGCGGGTAACACTGACACCGATTTCTCTGGTGAACCTGGCGGCTCCTGGCTCTAACCACACTAATGTCAAGTTC
E T G V I E A G N T D T D F S G E L A A P G S N H T N V K F

2800 2810 2820 2830 2840 2850 2860 2870 2880
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CTGTTTGATCGATCTCGATTATTGAATGTAATCAAGGTACTGGAGAAGGACGCCGTTTCCCCCGCCCTTCCCTACACAAGAAGGTGCG
L F D R S R L L N V I K V L E K D A V F P R P F P T Q E G A

2890 2900 2910 2920 2930 2940 2950 2960 2970
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CAGCAGGATGATGGTTACTTTTGTCTTCTGACCCCCGCCCCAACAGTCGCTTCCCGACCCGCCACTCGTTTCGGCCTGTACGCCAATCCG
Q Q D D G Y F C L L T P R P T V A S R P A T R F G L Y A N P

2980 2990 3000 3010 3020 3030 3040 3050 3060
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TCCGGCAGTGGTGTCTTGCTAACACTTCACTGGACTTCAATTTTATAGCTTGGCCTGTTTCACTTACTTTAGATCGGACCTTGAGGTT
S G S G V L A N T S L D F N F Y S L A C F T Y F R S D L E V

FIG. 83C

3070 3080 3090 3100 3110 3120 3130 3140 3150
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ACGGTGGTCTCACTAGAGCCGGATCTGGAATTTGCTGTAGGGTGGTTTCCTTCTGGCAGTGAATACCAGGCTTCCAGCTTTGTCTACGAC
T V V S L E P D L E F A V G W F P S G S E Y Q A S S F V Y D

3160 3170 3180 3190 3200 3210 3220 3230 3240
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CAGCTGCATGTGCCCTTCCACTTTACTGGGCGCACTCCCCGCGCTTTCGCTAGCAAGGTGGGAAGGTATCTTTCGTGCTCCCTTGGAAC
Q L H V P F H F T G R T P R A F A S K G G K V S F V L P W N

3250 3260 3270 3280 3290 3300 3310 3320 3330
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TCTGTCTCGTCTGTGCTCCCCGTGCGCTGGGGGGGGGCTTCCAAGCTCTCTTCTGCTACGCGGGGTCTACCGGCGCATGCTGATTGGGGG
S V S S V L P V R W G G A S K L S S A T R G L P A H A D W G

3340 3350 3360 3370 3380 3390 3400 3410 3420
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ACTATTTACGCCTTTGTCCCCGTCCTAATGAGAAGAAAAGCACCGCTGTAAACACGTGGCCGTGTACATTCCGGTACAAGAACGCACGT
T I Y A F V P R P N E K K S T A V K H V A V Y I R Y K N A R

3430 3440 3450 3460 3470 3480 3490 3500 3510
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GCCTGGTGGCCCGCATGCTTCCCTTTTCGAGCTACAAGCAGAAAGATGCTGATGCAATCTGGCGATATCGAGACCAATCCTGGTCCTGCT
A W C P S M L P F R S Y K Q K M L M Q S G D I E T N P G P A
VP1^{alt} >< 2A^{alt} VP1 >< 2A 2A >< 2B

3520 3530 3540 3550 3560 3570 3580 3590 3600
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TCTGACAACCCAATTTTGGAGTTTCTTGAAGCAGAAAATGATCTAGTCACTCTGGCCTCTCTCTGGAAGATGGTGCACCTCTGTTCAACAG
S D N P I L E F L E A E N D L V T L A S L W K M V H S V Q Q

3610 3620 3630 3640 3650 3660 3670 3680 3690
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ACCTGGAGAAAGTATGTGAAGAACGATGATTTTTGGCCCAATTTACTCAGCGAGCTAGTGGGGGAAGGCTCTGTGCGCTTGGCCGCCACG
T W R K Y V K N D D F W P N L L S E L V G E G S V A L A A T

3700 3710 3720 3730 3740 3750 3760 3770 3780
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CTATCCAACCAAGCTTCAGTAAAGGCTCTTTTGGGCTGCACTTTCTCTCTCGGGGGCTCAATTACACTGACTTTTACTCTTTACTGATA
L S N Q A S V K A L L G L H F L S R G L N Y T D F Y S L L I

3790 3800 3810 3820 3830 3840 3850 3860 3870
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GAGAAATGCTCTAGTTTCTTTACCGTAGAACCACCTCCTCCACCAGCTGAAAACCTGATGACCAAGCCCTCAGTGAAGTCGAAATTCCGA
E K C S S F F T V E P P P P P A E N L M T K P S V K S K F R

3880 3890 3900 3910 3920 3930 3940 3950 3960
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAACTGTTTAAGATGCAAGGACCCATGGACAAAGTCAAAGACTGGAACCAATAGCTGCCGGCTTGAAGAATTTCAATTTGTTTCGTGAC
K L F K M Q G P M D K V K D W N Q I A A G L K N F Q F V R D
2B >< 2C

3970 3980 3990 4000 4010 4020 4030 4040 4050
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CTAGTCAAAGAGGTGGTCGATTGGCTGCAGGCCTGGATCAACAAAGAGAAAGCCAGCCCTGTCTCCAGTACCAGTTGGAGATGAAGAAG
L V K E V V D W L Q A W I N K E K A S P V L Q Y Q L E M K K

FIG. 83D

4060 4070 4080 4090 4100 4110 4120 4130 4140
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CTCGGGCCTGTGGCCTTGGCTCATGACGCTTTCATGGCTGGTTCCGGGCCCCCTCTTAGCGACGACCAGATTGAATACCTCCAGAACCTC
L G P V A L A H D A F M A G S G P P L S D D Q I E Y L Q N L

4150 4160 4170 4180 4190 4200 4210 4220 4230
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AAATCTCTTGCCCTAACACTGGGGAAGACTAATTTGGCCCAAAGTCTCACCCTATGATCAATGCCAAACAAAGTTCAGCCCAACGAGTT
K S L A L T L G K T N L A Q S L T T M I N A K Q S S A Q R V

4240 4250 4260 4270 4280 4290 4300 4310 4320
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GAACCCGTTGTGGTGGTCCTTAGAGGCAAGCCGGGATGCGGCAAGAGCTTGGCCTCTACGTTGATTGCCAGGCTGTGTCCAAGCGCCTC
E P V V V V L R G K P G C G K S L A S T L I A Q A V S K R L

4330 4340 4350 4360 4370 4380 4390 4400 4410
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TATGGCTCCCAAAGTGTATATTCTCTTCCCCCAGATCCAGATTTCTTCGATGGATACAAAGGACAGTTTCGTGACCTTGATGGATGATTTG
Y G S Q S V Y S L P P D P D F F D G Y K G Q F V T L M D D L

4420 4430 4440 4450 4460 4470 4480 4490 4500
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GGACAAAACCCGGATGGACAAGATTTCTCCACCTTTTGTGATGGTGTGACCGCCCAATTTCTCCCAACATGGCGGACCTTGCAGAG
G Q N P D G Q D F S T F C Q M V S T A Q F L P N M A D L A E

4510 4520 4530 4540 4550 4560 4570 4580 4590
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AAAGGGCGTCCCTTTACCTCCAATCTCATCATTGCAACTACAAATCTCCCCCACTTCAGTCCTGTGACCATTTGCTGATCCTTCTGCAGTC
K G R P F T S N L I I A T T N L P H F S P V T I A D P S A V

4600 4610 4620 4630 4640 4650 4660 4670 4680
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TCTCGCCGTATCAACTACGATCTGACTCTAGAAGTATCTGAGGCCTACAAGAAACACACACGGCTGAATTTTGAAGTTGGCTTTCAGGCGC
S R R I N Y D L T L E V S E A Y K K H T R L N F D L A F R R

4690 4700 4710 4720 4730 4740 4750 4760 4770
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ACAGACGCCCCCCCCATTTATCCTTTTGCTGCCCATGTGCCCTTTGTGGACGTAGCTGTGCGCTTCAAAAATGGTCACCAGAATTTTAAT
T D A P P I Y P F A A H V P F V D V A V R F K N G H Q N F N

4780 4790 4800 4810 4820 4830 4840 4850 4860
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CTCCTAGAGTTGGTCGATTCCATTTGTACAGACATTTCGAGCCAAGCAACAAGGTGCCCCGAAACATGCAGACTCTGGTTCTACAGAGCCCC
L L E L V D S I C T D I R A K Q Q G A R N M Q T L V L Q S P
2C >< 3A

4870 4880 4890 4900 4910 4920 4930 4940 4950
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AACGAGAATGATGACACCCCGTCGACGAGGCGTTGGGTAGAGTTCTCTCCCCCGCTGCGGTCGATGAGGCGCTTGTGACCTCACTCCA
N E N D D T P V D E A L G R V L S P A A V D E A L V D L T P

4960 4970 4980 4990 5000 5010 5020 5030 5040
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GAGGCCGACCCGTTGGCCGTTTGGCTATTCTTGCCAAGCTAGGTCTTGCCCTAGCTGCGGTCACCCCTGGTCTGATAATCTTGGCAGTG
E A D P V G R L A I L A K L G L A L A A V T P G L I I L A V

FIG. 83E

5050 5060 5070 5080 5090 5100 5110 5120 5130
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GGACTCTACAGGTACTTCTCTGGCTCTGATGCAGACCAAGAAGAAACAGAAAGTGAGGGATCTGTCAAGGCACCCAGGAGCCGAAAATGCT
G L Y R Y F S G S D A D Q E E T E S E G S V K A P R S E N A
3A >< 3B^{VEg}

5140 5150 5160 5170 5180 5190 5200 5210 5220
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TATGACGGCCCGAAGAAAACTCTAAGCCCCCTGGAGCACTCTCTCTCATGGAAATGCAACAGCCCAACGTGGACATGGGCTTTGAGGCT
Y D G P K K N S K P P G A L S L M E M Q Q P N V D M G F E A
3B^{VEg} >< 3C^{Pro}

5230 5240 5250 5260 5270 5280 5290 5300 5310
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GCGGTCGCTAAGAAAGTGGTCGTCCCCATTACCTTCATGGTTCCTCAACAGACCTTCTGGGCTTACACAGTCCGCTCTTCTGGTGACCGGC
A V A K K V V V P I T F M V P N R P S G L T Q S A L L V T G

5320 5330 5340 5350 5360 5370 5380 5390 5400
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CGGACCTTCCTAATCAATGAACATACATGGTCCAATCCCTCCTGGACCAGCTTCACAATCCGCGGTGAGGTACACACTCGTGATGAGCCC
R T F L I N E H T W S N P S W T S F T I R G E V H T R D E P

5410 5420 5430 5440 5450 5460 5470 5480 5490
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTCCAAACGGTTCATTTCACTCACCACGGTATTCCCACAGATCTGATGATGGTACGTCTCGGACCGGGCAATTCTTCCCTAACAATCTA
F Q T V H F T H H G I P T D L M M V R L G P G N S F P N N L

5500 5510 5520 5530 5540 5550 5560 5570 5580
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GACAAGTTTGGACTTGACCAGATGCCGGCAGCAACTCCCGTGTGGTTGGCGTTTCGTCCAGTTACGGAACTTCTTCTTCTCTGGAAAT
D K F G L D Q M P A R N S R V V G V S S S Y G N F F F S G N

5590 5600 5610 5620 5630 5640 5650 5660 5670
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTCCTCGGATTTGTTGATTCCATCACCTCTGAACAAGGAACCTTACGCAAGACTCTTTAGGTACAGGGTGACGACCTACAAAGGATGGTGC
F L G F V D S I T S E Q G T Y A R L F R Y R V T T Y K G W C

5680 5690 5700 5710 5720 5730 5740 5750 5760
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GGCTCGGCCCTGGTCTGTGAGGCCGGTGGCGTCCGACGCATCATTTGGCCTGCATTCTGCTGGCGCCCGGTATCGGCGCCGGGACCTAT
G S A L V C E A G G V R R I I G L H S A G A A G I G A G T Y

5770 5780 5790 5800 5810 5820 5830 5840 5850
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ATCTCAAAATTAGGACTAATCAAAGCCCTGAAACACCTCGGTGAACCTTTGGCCACAATGCAAGCACTGATGACTGAATTAGAGCCTGGA
I S K L G L I K A L K H L G E P L A T M Q G L M T E L E P G
3C^{Pro} >< 3D^{pol}

5860 5870 5880 5890 5900 5910 5920 5930 5940
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ATCACCGTACATGTACCCCGGAAATCCAAATTGAGAAAGACGACCGCACACGCGGTGTACAAACCGGAGTTTGAGCCTGCTGTGTTGTCA
I T V H V P R K S K L R K T T A H A V Y K P E F E P A V L S

5950 5960 5970 5980 5990 6000 6010 6020 6030
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAATTTGATCCAGACTGAACAAGGATGTTGACTTGGATGAAGTAATTTGGTCTAAACACACTGCCAATGTCCCTTACCAACCTCCTTTG
K F D P R L N K D V D L D E V I W S K H T A N V P Y Q P P L

FIG. 83F

6040 6050 6060 6070 6080 6090 6100 6110 6120
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTCTACACATACATGTCAGAGTACGCTCATCGAGTCTTCTCCTTCTTGGGGAAAAGACAATGACATTCTGACCGTCAAAGAAGCAATTCCTG
F Y T Y M S E Y A H R V F S F L G K D N D I L T V K E A I L

6130 6140 6150 6160 6170 6180 6190 6200 6210
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GGCATCCCCGGACTAGACCCCATGGATCCCCACACAGCTCCGGGTCTGCCTTACGCCATCAACGGCCTTCGACGTACTGATCTCGTCGAT
G I P G L D P M D P H T A P G L P Y A I N G L R R T D L V D

6220 6230 6240 6250 6260 6270 6280 6290 6300
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTTGTGAACGGTACAGTAGATGCGGCGCTGGCTGTACAAATCCAGAAATTCTTAGACGGTGACTACTCTGACCATGTCTTCCAAACTTTT
F V N G T V D A A L A V Q I Q K F L D G D Y S D H V F Q T F

6310 6320 6330 6340 6350 6360 6370 6380 6390
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CTGAAAGATGAGATCAGACCCTCAGAGAAAGTCCGAGCGGGAAAAACCCGCATTGTTGATGTGCCCTCCCTGGCGCATTGCATTGTGGGC
L K D E I R P S E K V R A G K T R I V D V P S L A H C I V G

6400 6410 6420 6430 6440 6450 6460 6470 6480
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AGAATGTTGCTTGGGCGCTTTGCTGCCAAGTTTCAATCCCATCCTGGCTTTCTCCTCGGCTCTGCTATCGGGTCTGACCCCTGATGTTTTC
R M L L G R F A A K F Q S H P G F L L G S A I G S D P D V F

6490 6500 6510 6520 6530 6540 6550 6560 6570
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TGGACCGTCAATAGGGGCTCAACTCGAGGGGAGAAAGAACACGTATGACGTGGACTACAGTGCCCTTTGACTCTTCACACGGCACTGGCTCC
W T V I G A Q L E G R K N T Y D V D Y S A F D S S H G T G S

6580 6590 6600 6610 6620 6630 6640 6650 6660
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTCGAGGCTCTCATCTCTCACTTTTTCACCGTGGACAATGGTTTTAGCCCTGCGCTGGGACCGTATCTCAGATCCCTGGCTGTCTCGGTG
F E A L I S H F F T V D N G F S P A L G P Y L R S L A V S V

6670 6680 6690 6700 6710 6720 6730 6740 6750
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CACGCTTACGGCGAGCGTCGCATCAAGATTACCGGTGGCCTCCCCTCCGGTTGTGCCGCGACCGCCTGCTGAACACAGTGCTCAACAAT
H A Y G E R R I K I T G G L P S G C A A T S L L N T V L N N

6760 6770 6780 6790 6800 6810 6820 6830 6840
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GTGATCATCAGGACTGCTCTGGCATTGACTTACAAGGAATTTGAATATGACATGGTTGATATCATCGCCTACGGTGACGACCTTCTGGTT
V I I R T A L A L T Y K E F E Y D M V D I I A Y G D D L L V

6850 6860 6870 6880 6890 6900 6910 6920 6930
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GGCACGGATTACGATCTGGACTTCAATGAGGTGGCAGCGCGCTGCCAAGTTGGGGTATAAGATGACTCCTGCCAACAAGGGTTCTGTC
G T D Y D L D F N E V A R R A A K L G Y K M T P A N K G S V

6940 6950 6960 6970 6980 6990 7000 7010 7020
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTCCCTCCGACTTCTCTCTTCCGATGCTGTTTTTCTAAAGCGCAAATTCGTCCAAAACAACGACGGCTTATACAAACCAGTTATGGAT
F P P T S S L S D A V F L K R K F V Q N N D G L Y K P V M D

FIG. 83G

```

      7030      7040      7050      7060      7070      7080      7090      7100      7110
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTAAAGAATTTGGAAGCCATGCTCTCCTACTTCAAACCAGGAACACTACTCGAGAAGCTGCAATCTGTTTCTATGTTGGCTCAACATTCT
L  K  N  L  E  A  M  L  S  Y  F  K  P  G  T  L  L  E  K  L  Q  S  V  S  M  L  A  Q  H  S

      7120      7130      7140      7150      7160      7170      7180      7190      7200
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GGAAAAGAAGAATATGATAGATTGATGCACCCCTTCGCTGACTACGGTGCCGTACCGAGTCACGAGTACCTGCAGGCAAGATGGAGGGCC
G  K  E  E  Y  D  R  L  M  H  P  F  A  D  Y  G  A  V  P  S  H  E  Y  L  Q  A  R  W  R  A

      7210      7220      7230      7240      7250      7260      7270      7280      7290
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTGTTTCGACTGACCCAGATAGCCCAAGGCGCTTCGGTGCTGCCGGCGATTCTGGGAGAACTCAGTCGGAACAGAAAAGGGAAAAAAAAA
L  F  D  *
3Dpol >< 3' UTR

      7300      7310
.....|.....|.....|.....|
AAAAAAAAAAAAAAAAAAAAA
3' UTR >< poly(A)
```

FIG. 83H

1 TTTGAAATGG GGGGCTGGGC CCTGATGCCC AGTCCTTCCT TTCCCCTTCC GGGGGGTAA
 61 CCGGCTGTGT TTGCTAGAGG CACAGAGGGG CAACATCCAA CCTGCTTTTG CGGGGAACGG
 121 TCGGGCTCCG ATTCCTGCGT CGCCAAAGGT GTTAGCGCAC CCAAACGGCG CACCTACCAA
 181 TGTATTGGT GTGGTCTGCG AGTTCTAGCC TACTCGTTTC TCCCCGACC ATTCCTCAC
 241 CCACGAAAAG TGTGTTGTAA CCATAAGATT TAACCCCCGC ACGGGATGTG CGATAACCGT
 301 AAGACTGGCT CAAGCGCGGA AAGCGCTGTA ACCACATGCT GTTAGTCCCT TTATGGCTGC
 361 AAGATGGCTA CCCACCTCGG ATCACTGAAC TGGAGCTCGA CCCTCCTTAG TAAGGGAACC
 421 GAGAGGCCTT CGTGCAACAA GCTCCGACAC AGAGTCCACG TGA CTGCTAC CACCATGAGT
 481 ACATGGTTCT CCCCTCTCGA CCCAGGACTT CTTTTTGAAT ATCCACGGCT CGATCCAGAG
 541 GGTGGGGCAT GACCCCTAGC ATAGCGAGCT ACAGCGGGAA CTGTAGCTAG GCCTTAGCGT
 601 GCCTTGGATA CTGCCTGATA GGGCGACGGC CTAGTCGTGT CGGTTCTATA GGTAGCACAT
 661 ACAAATATGC AGAACTCTCA TTTTCTTTC GATACAGCCT CTGGCACCTT TGAAGATGTA
 721 ACCGGAACAA AAGTCAAGAT CGTTGAATAC CCCAGATCGG TGAACAATGG TGTTTACGAT
 781 TCGTCTACTC ATTTGGAGAT ACTGAACCTA CAGGGTGAAA TTGAAATTTT AAGGTCTTTC
 841 AATGAATACC AAATTCGCGC CGCCAAACAA CAACTCGGAC TGGACATCGT GTACGAACTA
 901 CAGGGTAATG TTCAGACAAC GTCAAAGAAT GATTTTGATT CCCGTGGCAA TAATGGTAAC
 961 ATGACCTTCA ATTACTACGC AAACACTTAT CAGAATTCAG TAGACTTCTC GACCTCCTCG
 1021 TCGGCGTCAG GCGCCGGACC CGGGAACCTC CGGGGCGGAT TAGCGGGTCT CCTCACAAAT
 1081 TTCAGTGGAA TCTTGAACCC TCTTGGCTAC CTCAAAGATC ACAACACCGA AGAAATGGAA
 1141 AACTCTGCTG ATCGAGTCAC AACGCAAACG GCGGGCAACA CTGCCATAAA CACGCAATCA
 1201 TCATTGGGTG TGTGTGTGTC CTACGTTGAA GACCCGACCA AATCTGATCC TCCGTCCAGC
 1261 AGCACAGATC AACCACACAC CACTTTCCTT GCCATCGACA GGTGGTACAC TGGACGTCTC
 1321 AATTCTTGGA CAAAAGCTGT AAAAACCTTC TCTTTTCAGG CCGTCCCGCT TCCCGGGGCC
 1381 TTTCTGTCTA GGCAGGGAGG CCTCAACGGA GGGGCCTTCA CAGCTACCCT ACATAGACAC
 1441 TTTTGTATGA AGTGCGGGTG GCAGGTGCAG GTCCAATGTA ATTTGACACA ATTCCACCAA
 1501 GGCGCTCTTC TTGTTGCCAT GGTTCCTGAA ACCACCCTTG ATGTCAAGCC CGACGGTAAG
 1561 GCAAAGAGCT TACAGGAGCT GAATGAAGAA CAGTGGGTGG AAATGTCTGA CGATTACCGG
 1621 ACCGGGAAAA ACATGCCTTT TCAGTCTCTT GGCACATACT ATCGGCCCCC TAACTGGACT
 1681 TGGGGTCCCA ATTTCAATCA CCCCTATCAA GTAACGGTTT TCCCACACCA AATTCTGAAC
 1741 GCGAGAACCT CTACCTCGGT AGACATAAAC GTCCCATACA TCGGGGAGAC CCCCACGCAA
 1801 TCCTCAGAGA CACAGAACTC CTGGACCCTC CTCGTTATGG TGCTCGTTCC CCTAGACTAT
 1861 AAGGAAGGAG CCACAACCTA CCCAGAAATT ACATTTTCTG TAAGGCCTAC AAGTCCCTAC
 1921 TTCAATGGGC TTCGCAACCG CTACACGGCC GGGACGGACG AAGAACAGGG GCCCATTCCT
 1981 ACGGCACCCA GAGAAAATTC GCTTATGTTT CTCTCAACCC TCCCTGACGA CACTGTCCCT
 2041 GCTTACGGGA ATGTGCGTAC CCCTCCTGTC AATTACCTCC CTGGTGAAAT AACCGACCTT

FIG. 84A

2101 TTGCAACTGG CCCGCATACC CACTCTCATG GCATTTGAGC GGGTGCCTGA ACCCGTGCCT
 2161 GCCTCAGACA CATATGTGCC CTACGTTGCC GTTCCCACCC AGTTCGATGA CAGGCCTCTC
 2221 ATCTCCTTCC CGATCACCCCT TTCAGATCCC GTCTATCAGA ACACCCTGGT TGGCGCCATC

2281 AGTTCAAATT TCGCCAATTA CCGTGGGTGT ATCCAAATCA CTCTGACATT TTGTGGACCC
2341 ATGATGGCGA GAGGGAAATT CCTGCTCTCG TATTCTCCCC CAAATGGAAC GCAACCACAG
2401 ACTCTTCCG AAGCTATGCA GTGCACATAC TCTATTTGGG ACATAGGCTT GAACTCTAGT
2461 TGGACCTTCG TCGTCCCCTA CATCTCGCCC AGTGACTACC GTGAAACTCG AGCCATTACC
2521 AACTCGGTTT ACTCCGCTGA TGGTTGGTTT AGCCTGCACA AGTTGACCAA AATTACTCTA
2581 CCACCTGACT GTCCGCAAAG TCCCTGCATT CTCTTTTTCG CTTCTGCTGG TGAGGATTAC
2641 ACTCTCCGTC TCCCCGTTGA TTGTAATCCT TCCTATGTGT TCCACTCCAC CGACAACGCC
2701 GAGACCGGGG TTATTGAGGC GGGTAACACT GACACCGATT TCTCTGGTGA ACTGGCGGCT
2761 CCTGGCTCTA ACCACACTAA TGTCAAGTTC CTGTTTGATC GATCTCGATT ATTGAATGTA
2821 ATCAAGGTAC TGGAGAAGGA CGCCGTTTTT CCCC GCCCTT TCCCTACACA AGAAGGTGCG
2881 CAGCAGGATG ATGGTACTT TTGTCTTCTG ACCCCCCGCC CAACAGTCGC TTCCCGACCC
2941 GCCACTCGTT TCGGCCTGTA CGCCAATCCG TCCGGCAGTG GTGTTCTTGC TAACACTTCA
3001 CTGGACTTCA ATTTTATAG CTTGGCCTGT TTCACTTACT TTAGATCGGA CCTTGAGGTT
3061 ACGGTGGTCT CACTAGAGCC GGATCTGGAA TTTGCTGTAG GGTGGTTTCC TTCTGGCAGT
3121 GAATACCAGG CTTCCAGCTT TGTCTACGAC CAGCTGCATG TGCCCTTCCA CTTTACTGGG
3181 CGCACTCCCC GCGCTTTCGC TAGCAAGGGT GGGAAGGTAT CTTTCGTGCT CCCTTGGAAC
3241 TCTGTCTCGT CTGTGCTCCC CGTGCCTGG GGGGGGCTT CCAAGCTCTC TTCTGCTACG
3301 CGGGGTCTAC CGGCGCATGC TGATTGGGGG ACTATTTACG CCTTTGTCCC CCGTCCTAAT
3361 GAGAAGAAAA GCACCGCTGT AAAACACGTG GCCGTGTACA TTCGGTACAA GAACGCACGT
3421 GCCTGGTGCC CCAGCATGCT TCCCTTTCGC AGCTACAAGC AGAAGATGCT GATGCAATCT
3481 GGCGATATCG AGACCAATCC TGGTCCTGCT TCTGACAACC CAATTTTGGA GTTCTTGAA
3541 GCAGAAAATG ATCTAGTCAC TCTGGCCTCT CTCTGGAAGA TGGTGCACTC TGTTCAACAG
3601 ACCTGGAGAA AGTATGTGAA GAACGATGAT TTTTGGCCCA ATTTACTCAG CGAGCTAGTG
3661 GGGGAAGGCT CTGTCGCCTT GGCCGCCACG CTATCCAACC AAGCTTCAGT AAAGGCTCTT
3721 TTGGGCCTGC ACTTTCTCTC TCGGGGGCTC AATTACACTG ACTTTTACTC TTTACTGATA
3781 GAGAAATGCT CTAGTTTCTT TACCGTAGAA CCACCTCCTC CACCAGCTGA AAACCTGATG
3841 ACCAAGCCCT CAGTGAAGTC GAAATTCCGA AACTGTTTA AGATGCAAGG ACCCATGGAC
3901 AAAGTCAAAG ACTGGAACCA AATAGCTGCC GGCTTGAAGA ATTTTCAATT TGTTTCGTGAC
3961 CTAGTCAAAG AGGTGGTCGA TTGGCTGCAG GCCTGGATCA ACAAAGAGAA AGCCAGCCCT
4021 GTCCTCCAGT ACCAGTTGGA GATGAAGAAG CTCGGGCCTG TGGCCTTGGC TCATGACGCT

FIG. 84B

4081 TTCATGGCTG GTTCCGGGCC CCCTCTTAGC GACGACCAGA TTGAATACCT CCAGAACCTC
4141 AAATCTCTTG CCCTAACACT GGGGAAGACT AATTTGGCCC AAAGTCTCAC CACTATGATC
4201 AATGCCAAAC AAAGTTCAGC CCAACGAGTT GAACCCGTTG TGGTGGTCCT TAGAGGCAAG
4261 CCGGGATGCG GCAAGAGCTT GGCTCTACG TTGATTGCCC AGGCTGTGTC CAAGCGCCTC
4321 TATGGCTCCC AAAGTGTATA TTCTCTTCCC CCAGATCCAG ATTTCTTCGA TGGATACAAA
4381 GGACAGTTCG TGACCTTGAT GGATGATTTG GGACAAAACC CGGATGGACA AGATTTCTCC
4441 ACCTTTTGTC AGATGGTGTC GACCGCCCAA TTTCTCCCCA ACATGGCGGA CCTTGCAGAG
4501 AAAGGGCGTC CCTTTACCTC CAATCTCATC ATTGCAACTA CAAATCTCCC CCACTTCAGT
4561 CCTGTCACCA TTGCTGATCC TTCTGCAGTC TCTCGCCGTA TCAACTACGA TCTGACTCTA
4621 GAAGTATCTG AGGCCTACAA GAAACACACA CGGCTGAATT TTGACTTGGC TTTCAGGCGC
4681 ACAGACGCCC CCCCCATTTA TCCTTTTGCT GCCCATGTGC CCTTTGTGGA CGTAGCTGTG
4741 CGCTTCAAAA ATGGTCACCA GAATTTTAAT CTCCTAGAGT TGGTCGATTC CATTTGTACA
4801 GACATTCGAG CCAAGCAACA AGGTGCCCGA AACATGCAGA CTCTGGTTCT ACAGAGCCCC
4861 AACGAGAATG ATGACACCCC CGTCGACGAG GCGTTGGGTA GAGTTCTCTC CCCCCTGCG
4921 GTCGATGAGG CGCTTGTCGA CCTCACTCCA GAGGCCGACC CGGTTGGCCG TTTGGCTATT
4981 CTTGCCAAGC TAGGTCTTGC CCTAGCTGCG GTCACCCCTG GTCTGATAAT CTTGGCAGTG
5041 GGACTCTACA GGTACTTCTC TGGCTCTGAT GCAGACCAAG AAGAAACAGA AAGTGAGGGA
5101 TCTGTCAAGG CACCCAGGAG CGAAAATGCT TATGACGGCC CGAAGAAAAA CTCTAAGCCC
5161 CCTGGAGCAC TCTCTCTCAT GGAAATGCAA CAGCCCAACG TGGACATGGG CTTTGAGGCT
5221 GCGGTCGCTA AGAAAGTGGT CGTCCCCATT ACCTTCATGG TTCCCAACAG ACCTTCTGGG
5281 CTTACACAGT CCGCTCTTCT GGTGACCGGC CGGACCTTCC TAATCAATGA ACATACATGG
5341 TCCAATCCCT CCTGGACCAG CTTCACAATC CGCGGTGAGG TACACACTCG TGATGAGCCC
5401 TTCCAAACGG TTCATTTTAC TCACCACGGT ATTCCCACAG ATCTGATGAT GGTACGTCTC
5461 GGACCGGGCA ATTCTTTCCC TAACAATCTA GACAAGTTTG GACTTGACCA GATGCCGGCA
5521 CGCAACTCCC GTGTGGTTGG CGTTTCGTCC AGTTACGGAA ACTTCTTCTT CTCTGGAAAT
5581 TTCCTCGGAT TTGTTGATTC CATCACCTCT GAACAAGGAA CTTACGCAAG ACTCTTTAGG
5641 TACAGGGTGA CGACCTACAA AGGATGGTGC GGCTCGGCC TGGTCTGTGA GGCCGGTGGC
5701 GTCCGACGCA TCATTGGCCT GCATTCTGCT GGCGCCGCCG GTATCGGCGC CGGGACCTAT
5761 ATCTCAAAAT TAGGACTAAT CAAAGCCCTG AAACACCTCG GTGAACCTTT GGCCACAATG
5821 CAAGGACTGA TGA CTGAATT AGAGCCTGGA ATCACC GTAC ATGTACCCCG GAAATCCAAA
5881 TTGAGAAAGA CGACCGCACA CGCGGTGTAC AAACCGGAGT TTGAGCCTGC TGTGTTGTCA
5941 AAATTTGATC CCAGACTGAA CAAGGATGTT GACTTGGATG AAGTAATTTG GTCTAAACAC
6001 ACTGCCAATG TCCCTTACCA ACCTCCTTTG TTCTACACAT ACATGTCAGA GTACGCTCAT

FIG. 84C

6061 CGAGTCTTCT CCTTCTTGGG GAAAGACAAT GACATTCTGA CCGTCAAAGA AGCAATTCTG
6121 GGCATCCCCG GACTAGACCC CATGGATCCC CACACAGCTC CGGGTCTGCC TTACGCCATC
6181 AACGGCCTTC GACGTACTGA TCTCGTCGAT TTTGTGAACG GTACAGTAGA TGCGGCGCTG
6241 GCTGTACAAA TCCAGAAATT CTTAGACGGT GACTACTCTG ACCATGTCTT CCAAACTTTT
6301 CTGAAAGATG AGATCAGACC CTCAGAGAAA GTCCGAGCGG GAAAAACCCG CATTGTTGAT
6361 GTGCCCTCCC TGGCGCATTG CATTGTGGGC AGAATGTTGC TTGGGCGCTT TGCTGCCAAG
6421 TTTCAATCCC ATCCTGGCTT TCTCCTCGGC TCTGCTATCG GGTCTGACCC TGATGTTTTT
6481 TGGACCGTCA TAGGGGCTCA ACTCGAGGGG AGAAAGAACA CGTATGACGT GGACTACAGT
6541 GCCTTTGACT CTTACACCGG CACTGGCTCC TTCGAGGCTC TCATCTCTCA CTTTTTCACC
6601 GTGGACAATG GTTTTAGCCC TGCCTGGGA CCGTATCTCA GATCCCTGGC TGTCTCGGTG
6661 CACGCTTACG GCGAGCGTCG CATCAAGATT ACCGGTGGCC TCCCCTCCGG TTGTGCCGCG
6721 ACCAGCCTGC TGAACACAGT GCTCAACAAT GTGATCATCA GGACTGCTCT GGCATTGACT
6781 TACAAGGAAT TTGAATATGA CATGGTTGAT ATCATCGCCT ACGGTGACGA CCTTCTGGTT
6841 GGCACGGATT ACGATCTGGA CTTCAATGAG GTGGCACGAC GCGCTGCCAA GTTGGGGTAT
6901 AAGATGACTC CTGCCAACAA GGGTTCTGTC TTCCCTCCGA CTTCTCTCTT TTCCGATGCT
6961 GTTTTTCTAA AGCGCAAATT CGTCCAAAAC AACGACGGCT TATACAAACC AGTTATGGAT
7021 TTAAAGAATT TGGAAGCCAT GCTCTCCTAC TTCAAACCAG GAACACTACT CGAGAAGCTG
7081 CAATCTGTTT CTATGTTGGC TCAACATTCT GGAAAAGAAG AATATGATAG ATTGATGCAC
7141 CCCTTCGCTG ACTACGGTGC CGTACCGAGT CACGAGTACC TGCAGGCAAG ATGGAGGGCC
7201 TTGTTGACT GACCCAGATA GCCCAAGGCG CTTCGGTGCT GCCGGCGATT CTGGGAGAAC
7261 TCAGTCGGAA CAGAAAAGGG AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA (SEQ ID
NO:168)

FIG. 84D

1 MQNSHFSFDT ASGTFEDVTG TKVKIVEYPR SVNNGVYDSS THLEILNLQG EIEILRSFNE
61 YQIRAAKQQL GLDIVYELQG NVQTTSKNDF DSRGNNGNMT FNYANTYQN SVDFSTSSSA
121 SGAGPGNSRG GLAGLLTNFS GILNPLGYLK DHNTEEMENS ADRVTTQTAG NTAINTQSSL
181 GVLCAIVEDP TKSDPPSSST DQPTTFTAI DRWYTGR LNS WTKAVKTFSF QAVPLPGAFL
241 SRQGGLNGGA FTATLHRHFL MKCGWQVQVQ CNLTQFHQGA LLVAMVPETT LDVKPDGKAK
301 SLQELNEEQW VEMSDDYRTG KNMPFQSLGT YYRPPNWTWG PNFINPYQVT VFPHQILNAR
361 TSTSVDINVP YIGETPTQSS ETQNSWTLLV MVLVPLDYKE GATTDPEITF SVRPTSPYFN
421 GLRNRYTAGT DEEQGPIPTA PRENSLMFLS TLPDDTVPAY GNVRTPPVNY LPGEITDLLQ
481 LARIPTLMAF ERVPEPVPAS DTYVPYVAVP TQFDDRPLIS FPITLSDPVY QNTLVGAISS
541 NFANYRGCIQ ITLTFCGPM ARGKFLSYS PPNGTQPQTL SEAMQCTYSI WDIGLNSSWT
601 FVVPYISPSD YRETRAITNS VYSADGWFSL HKLTKITLPP DCPQSPCILE FASAGEDYTL
661 RLPVDCNPSY VFHSTDNAET GVIEAGNTDT DFSGELAAPG SNHTNVKFLF DRSRLNVIK
721 VLEKDAVFPR PFPTQEGAQQ DDGYFCLLTP RPTVASRPAT RFGLYANPSG SGVLANTS LD
781 FNFYSLACFT YFRSDLEVTV VSLEPDLEFA VGWFPSGSEY QASSEFVDQL HVPFHFTGRT
841 PRAFASKGGK VSEVLPWNSV SSVLPVRWGG ASKLSSATRG LPAHADWGTI YAFVPRPNEK
901 KSTAVKHVAV YIRYKNARAW CPSMLPFRSY KQKMLMQSGD IETNPGPASD NPILEFLEAE
961 NDLVTLASLW KMVHSVQQTW RKYVKNDDFW PNLLSELVGE GSVALAATLS NQASVKALLG
1021 LHFLSRGLNY TDFYSL LIEK CSSFFTVEPP PPPAENLMTK PSVKS KFRKL FKMQGPMDKV
1081 KDWNQIAAGL KNFQFVRDLV KEVVDWLQAW INKEKAS PVL QYQLEMKKLG PVALAHDAFM
1141 AGSGPPLSDD QIEYLQNLKS LALT LGKTNL AQSLTTMINA KQSSAQ RVEP VVVVLRGKPG
1201 CGKSLASTLI AQAVSKRLYG SQSVYSLPPD PDFFDGYKGQ FVTLMDDLQ NP DGQDFSTF
1261 CQMVSTAQFL PNMADLA EKG RPFTSNLIIA TTNLPHFSPV TIADPSAVSR RINYDLTLEV
1321 SEAYKKHTRL NFDLAFRRTD APPIYPFAAH VPFVDVAVRF KNGHQNFNLL ELVDSICTDI
1381 RAKQOGARNM QTLVLQSPNE NDDTPVDEAL GRVLSPA AVD EALVDLTPEA DPVGR LAILA
1441 KLGLALAAVT PGLIILAVGL YRYFSGSDAD QEETESEGSV KAPRSENAYD GPKKNSKPPG
1501 ALSLMEMQQP NVDMGFEEAV AKKVVPITF MVPNRPSGLT QSALLVTGRT FLINEHTWSN
1561 PSWTSFTIRG EVHTRDEPFQ TVHFTHHGIP TDLMMVRLGP GNSFPNNLDK FGLDQMPARN

FIG. 85A

1621 SRVVGVSSTY GNEFFSGNFL GFVDSITSEQ GTYARLFYR VTTYKGWCGS ALVCEAGGVR
1681 RIIGLHSAGA AGIGAGTYIS KLGLIKALKH LGEPLATMQG LMTELEPGIT VHVPRKSKLR
1741 KTTAHAVYKP EFEPAVLSKF DPRLNKDVDL DEVIWSKHTA NVPYQPPLFY TYMSEYHRV
1801 FSFLGKDNDI LTVKEAILGI PGLDPMDPHT APGLPYAING LRRTDLVDFV NGTVDAALAV
1861 QIQKFLDGDY SDHVFQTFK DEIRPSEKVR AGKTRIVDVP SLAHCIVGRM LLGRFAAKFQ
1921 SHPGFLLGSA IGSDPDVFWT VIGAQLEGRK NTYDVDYSAF DSSHGTGSFE ALISHFFTVD
1981 NGFSPALGPY LRSLAVSVHA YGERRIKITG GLPSGCAATS LLNTVLNNVI IRTALALTYK
2041 EFEYDMVDII AYGDDLLVGT DYDLDFNEVA RRAAKLGYKM TPANKGSVFP PTSSLSDAVF
2101 LKRKFVQNND GLYKPVMDLK NLEAMLSYFK PGTLLLEKLQS VSMLAQHSGK EYDRLMHPF
2161 ADYGAVPSHE YLQARWRALF D* (SEQ ID NO:169)

FIG. 85B

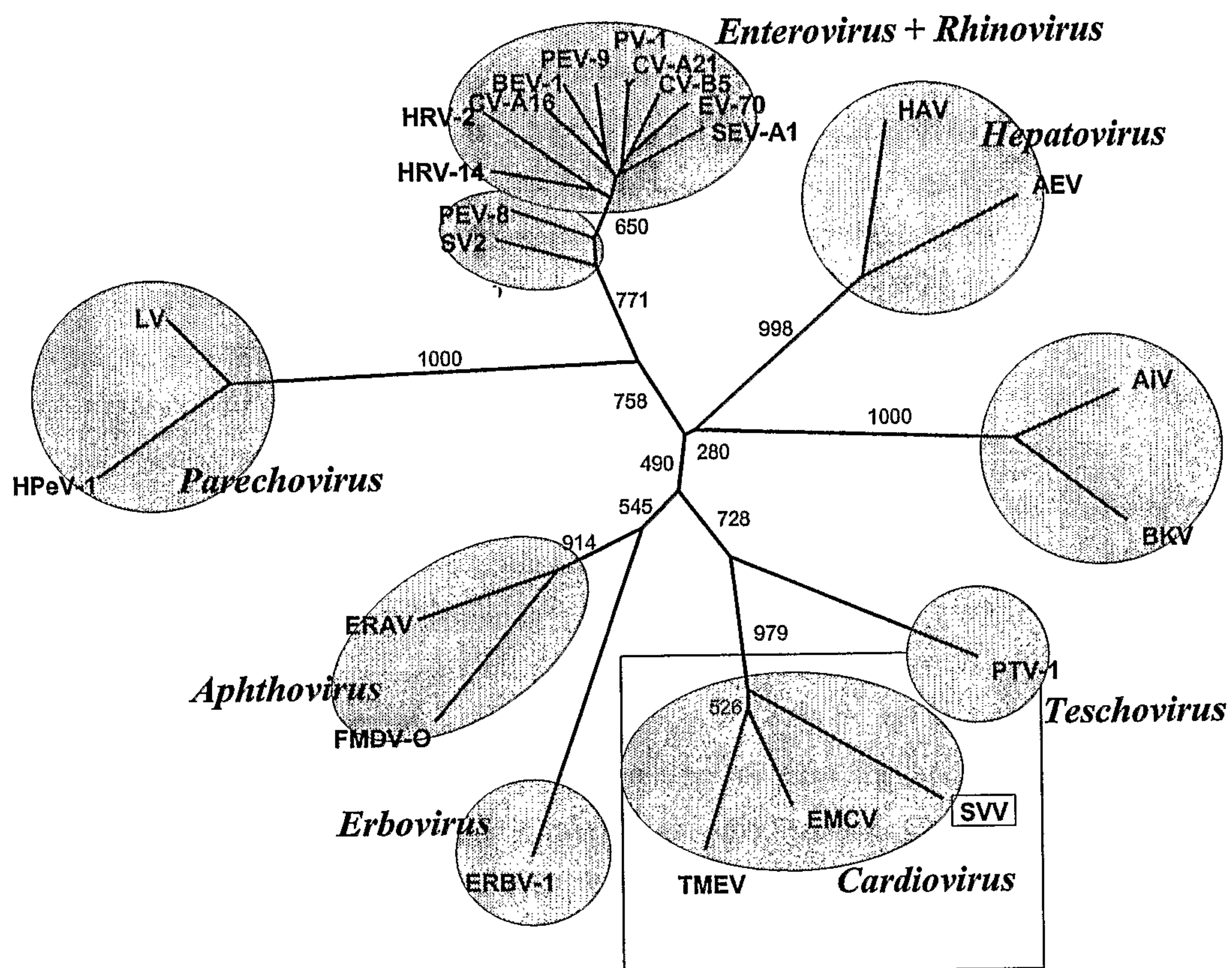


FIG. 86

VP2 (partial) -VP3-VP1-2A (partial)

	VP2 >< VP3															
	10	20	30	40	50	60	70	80								
SVV-001																
89-47752	AATGGGCTTCGCAACCGCTACACGGCCGGGACGGACGAAGAACAGGGGCCATTCCCTACGGCACCAGAGAAAATTCGCT															
131395	AATGGGCTTCGCAACCGCTACACGGCCGGGACGGACGAAGAACAGGGGCCATTCCCTACGGCACCAGAGAACATTCGCT															
Consensus	AATGGGC TCGCAACCGCTACA G CCGGGACGGACGA GAACAGGGGCC ATTCC ACGGCACCAGAGA ATTCGCT															
	90	100	110	120	130	140	150	160								
SVV-001																
89-47752	TATGTTTCTCTCAACCCTCCCTGACGACACTGTCCCTGCTTACGGGAATGTGCGTACCCCTCCTGTCAATTACCTCCCTG															
131395	TATGTTTCTCTCAACCCTCCCTGACGACACTGTCCCTGCTTACGGGAATGTGCGTACCCCTCCTGTCAATTACCTCCCTG															
Consensus	TATGTTTCT TCAAC CT CCTGACGACAC GT CCTGCTTACGGGAATGTGCG ACCCCTCCTGTCAATTACCTCCCTG															
	170	180	190	200	210	220	230	240								
SVV-001																
89-47752	GTGAAATAACCGACCTTTTGCAACTGGCCCGCATACCCACTCTCATGGCATTGTGAGCGGGTGCCCTGAACCCGTGCCTGCC															
131395	GTGAAATAACCGACCTTTTGCAACTGGCCCGCATACCCACTCTCATGGCATTGTGAGCGGGTGCCCTGAACCCGTGCCTGCC															
Consensus	GTGAAATAACCGACCT TTGCAACTGGCCCGCATACCCACTCTCATGGC TTTGAGCGGGTGCC GAACCCGTGCCTGCC															
	250	260	270	280	290	300	310	320								
SVV-001																
89-47752	TCAGACACATATGTGCCCTACGTTGCCGTTCCACCCAGTTTCGATGACAGGCCCTCTCATCTCCTTCCCGATCACCCTTTC															
131395	TCAGATACGATATGTGCCCTACGTTGCCGTTCCACCCAGTTTCGATGACAGGCCCTCTCATCTCCTTCCCGATCACCCTTTC															
Consensus	TCAGA AC TATGTGCCCTACGTTGCCGTTCCACCCCA TT G TGACA GCCTCTCATCTCCTTCCCGATCACCCTTTC															
	330	340	350	360	370	380	390	400								
SVV-001																
89-47752	AGATCCCGTCTATCAGAACACCCCTGGTTGGCGCCATCAGTTCAAATTTGCGCAATTACCGTGGGTGTATCCAAATCACTC															
131395	AGACCCGTCTATCAGAACACCCCTGGTTGGCGCCATCAGTTCAAATTTGCGCAATTACCGTGGGTGTATCCAGATCACTC															
Consensus	AGA CC GTCTATCA AACACCCCTGGT GGCGCCATCAGTTC A TT GCCAA TACCGTGGGTGTATCCA ATCACTC															
	410	420	430	440	450	460	470	480								
SVV-001																
89-47752	TGACATTTTGTGGACCCATGATGGCGAGAGGGAAATTCCTGCTCTCGTATTCTCCCCCAAATGGAACGCAACCACAGACT															
131395	TGACGTTTGTGGACCCATGATGGCAAGAGGGAAATTCCTACTCTCGTATTCTCCCCCAAATGGAACGCAACCACAGACT															
Consensus	TGAC TTTTGTGGACCCATGATGGC AGAGGGAAATTCCT CT T TATTCTCCCCCAAATGGAACGCAACCACAGACT															
	490	500	510	520	530	540	550	560								
SVV-001																
89-47752	CTTTCCGAAGCTATGCAGTGCACATACTCTATTTGGGACATAGGCTTGAACTCTAGTTGGACCTTCGTCGTCCTTACAT															
131395	CTTTCCGAAGCTATGCAGTGCACATACTCTATTTGGGACATAGGCTTGAACTCTAGTTGGACCTTCGTCGTCCTTACAT															
Consensus	CTTTCCGAAGCTATGCAGTGCACATACTCTATTTGGGACATAGGCTTGAACTC AGTTGGACCTTCGTCGTCCTTACAT															

FIG. 87A

	570	580	590	600	610	620	630	640
								
SVV-001	CTCGCCCAGTGACTACCGTGAAACTCGAGCCATTACCAACTCGGTTTACTCCGCTGATGGTTGGTTTAGCCTGCACAAGT							
88-23626	-----TTTAGCCTGCACAAGT							
88-36695	-----TTTAGCCTGCACAAGT							
89-47752	CTCGCCCAGTGACTACCGTGAAACCCGGGCCATTACCAACTCGGTTTACTCCGCTGATGGTTGGTTTAGCCTGCACAAGT							
90-10324	-----TTTAGCCTGCACAAGT							
92-48963	-----TTTAGCCTGCACAAGT							
97-1278	-----TTTAGCCTGCACAAGT							
131395	CTCGCCCAGTGACTACCGTGAAACTCGGGCCATTACCAACTCGGTTTACTCCGCTGATGGTTGGTTTAGTCTGCACAAGT							
Consensus	CTCGCCCAGTGACTACCGTGAAAC CG GCCATTACCAACTCGGTTTACTCCGCTGATGGTTGGTTTAG CTGCACAAGT							
	650	660	670	680	690	700	710	720
								
SVV-001	TGACCAAAATTACTCTACCACCTGACTGTCCGCAAAGTCCCTGCATTCTCTTTTTCGCTTCTGCTGGTGAGGATTACACT							
88-23626	TGACCAAAATTACTCTACCACCTGACTGTCCGCAAAGTCCCTGCATTCTCTTTTTCGCTTCTGCTGGTGAGGATTACACT							
88-36695	TGACCAAAATAACTCTACCACCTGACTGTCCGCAAAGTCCCTGCATTCTCTTTTTCGCTTCTGCTGGTGAGGATTACACT							
89-47752	TGACCAAAATTACTCTCCCACCTGACTGTCCGCAAAGTCCCTGCATTCTCTTTTTCGCTTCTGCTGGTGAGGATTACACT							
90-10324	TGACCAAAATTACTCTACCACCTGACTGTCCGCAAAGTCCCTGCATTCTCTTTTTCGCTTCTGCTGGTGAGGATTACACT							
92-48963	TGACCAAAATTACTCTACCACCTGACTGTCCGCAAAGTCCCTGCATTCTCTTTTTCGCTTCTGCTGGTGAGGATTACACT							
97-1278	TGACCAAAATTACTCTACCACCTGACTGTCCGCAAAGTCCCTGCATTCTCTTTTTCGCTTCTGCTGGTGAGGATTACACT							
131395	TGACCAAAATTACTCTACCACCTGACTGTCCGCAAAGTCCCTGTATTCTCTTTTTCGCTTCTGCTGGTGAGGATTACACC							
Consensus	TGAC AAAAT ACT T CCACCTGACTG CCGCAA TCCCTG TTCTCTTTTTCGCTTCTGCTGGTGAGGATTACAC							
				VP3 >< VP1				
	730	740	750	760	770	780	790	800
								
SVV-001	CTCCGTCTCCCCGTTGATTGTAATCCTTCCTATGTGTTCCACTCCACCGACAACGCCGAGACCGGGTTATTGAGGCGGG							
88-23626	CTCCGTCTCCCCATTGATTGCAATCCTTCCTACGTGTTCCACTCCACCGACAACGCCGAAACTGGGGTTGTGAGGCGGG							
88-36695	CTCCGTCTCCCCATTGATTGCAATCCTTCCTACGTGTTCCACTCCACCGACAACGCCGAAACTGGGGTTGTGAGGCGGG							
89-47752	CTCCGTCTCCCCATTGATTGTAATCCTTCCTACGTGTTCCACTCCACCGACAACGCCGAAACTGGGGTTGTGAGGCGGG							
90-10324	CTCCGTCTCCCCATTGATTGTAATCCTTCCTACGTGTTCCACTCCACCGACAACGCCGAAACTGGGGTTGTGAGGCGGG							
92-48963	CTCCGTCTCCCCGTTGATTGTAATCCTTCCTACGTGTTCCACTCCACCGACAACGCCGAAACTGGGGTTGTGAGGCGGG							
97-1278	CTCCGTCTCCCCGTTGATTGTAATCCTTCCTATGTGTTCCACTCCACCGACAACGCCGAGACTGGGGTTATTGAGGCGGG							
131395	CTCCGTCTCCCCGTTGATTGTAATCCTTCCTATGTGTTCCACTCCACCGACAACGCCGAGACTGGGGTTATTGAGGCGGG							
Consensus	CTCCGTCTCCCC TTGATTG AATCCTTC TA GTGTTCCACTCCACCGACAACGCCGA AC GGGGTT T GAGGCGGG							
	810	820	830	840	850	860	870	880
								
SVV-001	TAACACTGACACCGATTTCTCTGGTGAAGTGGCGGCTCCTGGCTCTAACCACACTAATGTCAAGTTCCTGTTTGATCGAT							
88-23626	TAACACTGACACCGATTTCTCTGGTGAAGTAGCGGCTCCTGGCTCCAACCACACTAATGTCAAGTTCCTGTTTGATCGAT							
88-36695	TAACACTGACACCGATTTCTCTGGTGAAGTAGCGGCTCCTGGCTCCAACCACACTAATGTCAAGTTCCTGTTTGATCGAT							
89-47752	TAACACTGACACCGATTTCTCTGGTGAAGTAGCGGCTCCTGGCTCCAACCACACTAATGTCAAGTTCCTGTTTGATCGAT							
90-10324	TAACACTGACACCGATTTCTCTGGTGAAGTAGCGGCTCCTGGTCCAACCACACTAATGTCAAGTTCCTGTTTGATCGAT							
92-48963	TAACACTGACACCGATTTCTCTGGTGAAGTAGCGGCTCCTGGTTCCAACCACACTAACGTCAAGTTCCTGTTTGATCGAT							
97-1278	TAACACTGACACCGATTTCTCTGGTGAAGTGGCGGCTCCTGGCTCTAACCACACTAATGTCAAGTTCCTGTTTGATCGAT							
131395	TAACACTGACACCGATTTCTCTGGTGAAGTGGCGGCTCCTGGCTCTAACCACACTAATGTCAAGTTCCTGTTTGATCGAT							
Consensus	TAACACTGACACCGATTTCTCTGGTGAAGT GCGGCTCCTGG TC AACCACACTAA GTCAAGTTCCTGTTTGATCGAT							

FIG. 87B

	890	900	910	920	930	940	950	960
SVV-001		CTCGATTATTGAATGTAATCAAGGTACTGGAGAAAGACGCCGTTTCCCCCGCCCTTTCCCTACACAAGAAGGTGCGCAG						
88-23626	CTCGGTTATTGAATGTAATTAAGGTACTGGAGAAAGACGCTGTTTCCCTCACCCCTTTCCCTACGCTAGAAGGTGTGCAA							
88-36695	CTCGGTTATTGAATGTAATTAAGGTACTGGAGAAAGACGCTGTTTCCCTCACCCCTTTCCCTACGCTAGAAGGTGTGCAA							
89-47752	CTCGATTATTGAATGTAATTAAGGTACTGGAGAAAGACGCCGTTTCCCCCGCCCTTTCCCTACGCTAGAAGGTGCGCAA							
90-10324	CTCGATTATTGAATGTAATTAAGGTACTGGAGAAAGACGCCGTTTCCCCCGCCCTTTCCCTACGCTAGAAGGTGCGCAA							
92-48963	CTCGATTATTGAATGTAATTAAGGTACTGGAGAAAGACGCCGTTTCCCCCGCCCTTTCCCTACGCTAGAAGGTGCGCAA							
97-1278	CTCGATTATTGAATGTAATTAAGGTACTGGAGAAAGACGCCGTTTCCCCCGCCCTTTCCCTACACAAGAAGGTGCGCAG							
131395	CTCGATTACTGAATGTAATTAAGGTACTGGAGAAAGACGCCGTTTCCCCCGTCCTTTCCCTACAAAAGAAGGTGCGCAG							
Consensus	CTCG TTA TGAATGTAAT AAGGTACTGGAGAA GACGC GTTTTCCC C CCTTTCCCTAC AGAAGGTG GCA							
	970	980	990	1000	1010	1020	1030	1040
SVV-001		CAGGATGATGGTTACTTTTGTCTTCTGACCCCCCGCCCAACAGTCGCTTCCCGACCCGCCACTCGTTTCGGCCTGTACGC						
88-23626	CAGGATGATGGCTACTTTTGTCTTCTGACCCCCCGCCCAACAGTCGCTTCCCGGCCCTGCCACTCGTTTCGGCCTGTACGC							
88-36695	CAGGATGATGGCTACTTTTGTCTTCTGACCCCCCGCCCAACAGTCGCTTCCCGGCCCTGCCACTCGTTTCGGCCTGTACGC							
89-47752	CAGGATGATGGTTACTTTTGTCTTCTGACCCCCCGCCCAACAGTCGCTTCCCGACCCGCCACTCGTTTCGGCCTGTACGC							
90-10324	CAGGATGATGGTTACTTTTGTCTTCTGACCCCCCGCCCAACAGTCGCTTCCCGACCCGCCACTCGTTTCGGCCTGTACGC							
92-48963	CAGGATGATGGTTACTTTTGTCTTCTGACCCCCCGCCCAACAGTCGCTTCCCGACCCGCCACTCGTTTCGGCCTGTACGC							
97-1278	CAGGACGATGGTTACTTTTGTCTTCTGACCCCCCGCCCAACAGTCGCTTCCCGACCCGCCACTCGTTTCGGCCTGTACGC							
131395	CAGGACGATGGTTACTTTTGTCTTCTGACACCCCGCCCAACAGTCGCTTCCCGACCCGCCACTCGTTTCGGCCTGTACGT							
Consensus	CAGGA GATGG TACTTTTGTCTTCTGAC CCCCGCCCAACAGTCGC TCCCG CC GCCACTCGTTTCGGCCTGTACG							
	1050	1060	1070	1080	1090	1100	1110	1120
SVV-001		CAATCCGTCCGGCAGTGGTGTCTTGCTAACACTTCATTGGACTTTCAATTTTATAGCTTGGCCTGTTTCACCTTACTTTA						
88-23626	CAATCCGTCCGGCAGCGGTGTCTTGCTAATACTTCATTGGACTTTAACTTTTATAGCTTGGCCTGTTTCACCTTACTTTA							
88-36695	CAATCCGTCCGGCAGCGGTGTCTTGCTAATACTTCATTGGACTTTAACTTTTATAGCTTGGCCTGTTTCACCTTACTTTA							
89-47752	CAATCCGTCCGGCAGCGGTGTCTTGCTAATACTTCATTGGACTTTAACTTTTATAGCTTGGCCTGTTTCACCTTACTTTA							
90-10324	CAATCCGTCCGGCAGCGGTGTCTTGCTAATACTTCATTGGACTTTAACTTTTATAGCTTGGCCTGCTTCACCTTACTTTA							
92-48963	CAATCCGTCCGGCAGTGGTGTCTTGCTAATACTTCATTGGACTTTAACTTTTATAGCTTGGCCTGTTTCACCTTACTTTA							
97-1278	CAATCCGTCCGGCAGTGGTGTCTCGCCAACACTTCATTGGACTTTCAATTTTATAGCTTGGCCTGTTTCACCTTACTTTA							
131395	CAATCCGTCCGGCAGTGGTGTCTCGCCAACACTTCATTGGACTTTCAATTTTATAGCTTGGCCTGTTTCACCTTACTTTA							
Consensus	CAATCCGTCCGGCAG GGTGTCT GC AA ACTTCA TGGACTT AA TTTTATAGCTTGGCCTG TTCACCTTACTTTA							
	1130	1140	1150	1160	1170	1180	1190	1200
SVV-001		GATCGGACCTTGAGGTACGGTGGTCTCACTAGAGCCGGATCTGGAATTTGCTGTAGGGTGGTTTCCTTCTGGCAGTGAA						
88-23626	GATCGGACCTCGAGGTACGGTGGTCTCACTAGAGCCGGATCTGGAATTTGCTGTGGGGTGGTTTCCTTCCGGCAGTGAA							
88-36695	GATCGGACCTCGAGGTACGGTGGTCTCACTAGAGCCGGATCTGGAATTTGCTGTGGGGTGGTTTCCTTCCGGCAGTGAA							
89-47752	GATCGGACCTCGAGGTACGGTGGTCTCACTGGAGCCAGATCTGGAATTTGCTGTAGGGTGGTTTCCTTCCGGCAGTGAA							
90-10324	GATCGGACCTCGAGGTACGGTGGTCTCACTGGAGCCAGATCTGGAATTTGCTGTAGGGTGGTTTCCTTCCGGCAGTGAA							
92-48963	GATCGGACCTCGAGGTACGGTGGTCTCACTGGAGCCAGATCTGGAATTTGCTGTAGGATGGTTTCCTTCCGGCAGTGAA							
97-1278	GATCGGACCTTGAGGTACGGTGGTCTCACTGGAGCCAAATCTCGAATTTGCTGTAGGGTGGTTTCCTTCCGGTAGTGAA							
131395	GATCGGACCTTGAAGTTACGGTGGTCTCACTGGAGCCAGATCTGGAATTTGCTGTAGGGTGGTTTCCTTCTGGCAGTGAA							
Consensus	GATCGGACCT GA GTTACGGTGGTCTCACT GAGCC ATCT GAATTTGCTGT GG TGGTTTCCTT GC AGTGAA							

FIG. 87C

	1210	1220	1230	1240	1250	1260	1270	1280
								
SVV-001	TACCAGGCTTCCAGCTTTGTCTACGACCAGCTGCATGTGCCCTTCCACTTTACTGGGCGCACTCCCCGCGCTTTGCTAG							
88-23626	TATCAGGCTTCCAGCTTTGTCTACGACCAGCTGCACGTGCCCTTCCACTTCACTGGACGCACCCCCGCGCTTTTGCTAG							
88-36695	TATCAGGCTTCCAGCTTTGTCTACGACCAGCTGCACGTGCCCTTCCACTTCACTGGACGCACCCCCGCGCTTTTGCTAG							
89-47752	TATCAGGCTTCCAGCTTTGTCTACGACCAGCTGCACGTGCCCTTCCACTTCACTGGACGCACCCCCGCGCTTTTGCTAG							
90-10324	TATCAGGCTTCCAGCTTTGTCTACGACCAGCTGCACGTGCCCTTCCACTTCACTGGACGCACCCCCGCGCTTTTGCTAG							
92-48963	TACCAGGCTTCCAGCTTTGTCTACGACCAGCTGCACGTGCCCTTCCACTTCACTGGGCGCACTCCCCGCGCTTTGCTAG							
97-1278	TACCAGGCTTCCAGCTTTGTCTACGACCAGCTGCACGTGCCCTTCCACTTCACTGGGCGCACTCCCCGCGCTTTGCTAG							
131395	TACCAGGCTTCCAGCTTTGTCTACGACCAGCTGCATGTACCCTTCCACTTTACTGGGCGCACTCCCCGCGCTTTGCTAG							
Consensus	TA CAGGCTTCCAG TTTGTCTACG CCAGCTGCA GT CC TTCCACTT ACTGG CGCAC CCCCCGCGCTT GC AG							
	1290	1300	1310	1320	1330	1340	1350	1360
								
SVV-001	CAAGGGTGGGAAGGTATCTTTTGTGCTCCCTTGGAACTCTGTCTCGTCTGCTCCCCGTGCGCTGGGGGGGGGCTTCCA							
88-23626	CAAGGGTGGGAAGGTATCTTTTGTGCTCCCTTGGAACTCTGTCTCATCTGTGCTCCCCGTGCGCTGGGGGGGAGCTTCCA							
88-36695	CAAGGGTGGGAAGGTATCTTTTGTGCTCCCTTGGAACTCTGTCTCATCTGTGCTCCCCGTGCGCTGGGGGGGAGCTTCCA							
89-47752	CAAGGGTGGGAAGGTATCTTTTGTGCTCCCTTGGAACTCTGTCTCATCTGTGCTCCCCGTGCGCTGGGGGGGGGCTTCCA							
90-10324	CAAGGGTGGGAAGGTATCTTTTGTGCTCCCTTGGAACTCTGTCTCATCTGTGCTCCCCGTGCGCTGGGGGGGGGCTTCCA							
92-48963	CAAGGGTGGGAAGGTATCTTTTGTGCTCCCTTGGAACTCTGTCTCATCTGTGCTCCCCGTGCGCTGGGGGGGGGCTTCCA							
97-1278	CAAGGGTGGGAAGGTATCTTTTGTGCTCCCTTGGAACTCTGTCTCATCTGTGCTCCCCGTGCGCTGGGGGGGGGCTTCCA							
131395	CAAGGGTGGGAAGGTATCTTTTGTGCTCCCTTGGAACTCTGTCTCATCTGTGCTCCCCGTGCGCTGGGGGGGGGCTTCCA							
Consensus	CAAGGGTGGGAAGGT TC TT GTGCTCCCTTGGAACTCTGT TC TCTGTGCTCCCCGTGCGCTGGGGGGG GCTTCCA							
	1370	1380	1390	1400	1410	1420	1430	1440
								
SVV-001	AGCTCTCTTCTGCTACGCGGGGTCTACCGGCGCATGCTGATTGGGGGACTATTTACGCCCTTTGTCCCCCGTCCCAATGAG							
88-23626	AGCTCTCTTCTGCCACGCGGGGTCTACCGGTGCACGCTGACTGGGGGACTATCTACGCCCTTTGTCCCCCGTCCCAATGAA							
88-36695	AGCTCTCTTCTGCCACGCGGGGTCTACCGGTGCACGCTGACTGGGGGACTATCTACGCCCTTTGTCCCCCGTCCCAATGAA							
89-47752	AGCTCTCTTCTGCCACGCGGGGTCTACCGGCGCATGCTGACTGGGGGACTATCTACGCCCTTTGTCCCCCGTCCCAATGAA							
90-10324	AGCTCTCTTCTGCCACGCGGGGTCTACCGGCGCATGCTGACTGGGGGACTATCTACGCCCTTTGTCCCCCGTCCCAATGAA							
92-48963	AGCTCTCTTCTGCCACGCGGGGTCTACCGGCGCATGCTGACTGGGGGACTATCTACGCCCTTTGTCCCCCGTCCCAATGAA							
97-1278	AGCTCTCTTCTGCCACGCGGGGTCTACCGGCGCATGCTGACTGGGGGACTATTTACGCCCTTTGTCCCCCGTCCCAATGAA							
131395	AGCTCTCTTCTGCCACGCGGGGTCTACCGGCGCATGCTGACTGGGGGACTATTTACGCCCTTTGTCCCCCGTCCCAATGAG							
Consensus	AGCTCTCTTCTGC AC CGGGGTCTACC G GCA GCTGA TGGGGGACTAT TACGCCCTTTGTCCCCCGTCC AATGA							
	1450	1460	1470	1480	1490	1500	1510	1520
								
SVV-001	AAGAAAAGCACCCTGTAAAAACACGTGGCCGTGTACATTTCGGTACAAGAACGCACGTGCCTGGTGCCCCAGCATGCTTCC							
88-23626	AAGAAAAGCACCCTGTAAAAACATGTGGCCGTGTACATTTCGGTACAAGAACGCACGCGCCTGGTGTTCCCAACATGCTCCC							
88-36695	AAGAAAAGCACCCTGTAAAAACATGTGGCCGTGTACATTTCGGTACAAGAACGCACGCGCCTGGTGTTCCCAACATGCTCCC							
89-47752	AAGAAAAGCACCCTGTAAAAACACGTGGCCGTGTACATTTCGGTACAAGAACGCACGCGCCTGGTGTTCCCAACATGCTTCC							
90-10324	AAGAAAAGCACCCTGTAAAAACACGTGGCCGTGTACATTTCGGTACAAGAACGCACGCGCCTGGTGTTCCCAACATGCTTCC							
92-48963	AAGAAAAGCACCCTGTAAAAACACGTGGCCGTGTACATTTCGGTACAAGAACGCACGCGCCTGGTGTTCCCAACATGCTTCC							
97-1278	AAGAAAAGCACCCTGTAAAAACACGTGGCCGTGTACATTTCGGTACAAGAACGCACGCGCCTGGTGTTCCCAACATGCTTCC							
131395	AAGAAAAGCACCCTGTAAAAACACGTGGCCGTGTACATTTCGGTACAAGAACGCACGTGCCTGGTGTTCCCAACATGCTTCC							
Consensus	AAGAAAAGCAC GCTG AAA CA GTGGCCGTGTAC TTCGGTACAAGAACGCACG GCCTGGTG CCA CATGCT CC							
	1530	1540	1550	1560				
								
SVV-001	CTTTTCGCAGCTACAAGCAGAAGATGCTGATGCAATCTGGC							
88-23626	CTTTTCGCAGCTATAAGCAGAAGATGCTGATGCAATCTGGC							
88-36695	CTTTTCGCAGCTATAAGCAGAAGATGCTGATGCAATCTGGC							
89-47752	CTTTTCGCAGCTATAAGCAGAAGATGCTGATGCAATCTGGC							
90-10324	CTTTTCGCAGCTATAAGCAGAAGATGCTGATGCAATCTGGC							
92-48963	CTTTTCGCAGCTATAAGCAGAAGATGCTGATGCAATCTGGC							
97-1278	TTTTTCGCAGCTACAAGCAGAAGATGCTGATGCAATCTGGC							
131395	TTTTTCGCAGCTACAAGCAGAAGATGCTGATGCAATCTGGC							
Consensus	TTTTTCGCAGCTA AAGCAGAAGATGCTGATGCAATCTGGC							

FIG. 87D

Partial 2C

	10	20	30	40	50	60	70	80
SVV-001								
89-47752	AAACTGTTTAAGATGCAAGGACCCATGGACAAAGTCAAAGACTGGAACCAATAGCTGCCGGCTTGAAGAATTTCAATT							
131395	AAACTGTTTAAGATGCAAGTACCCATGGACAAAGTCAAAGACTGGAACCAATAGCCGCCGGCTTGAAGAACTTTCAATT							
Consensus	AAACTGTTTAAGATGCAAG ACCCATGGACAAAGTCAAAGACTGGAACCAATAGC GCCGGCTTGAAGAA TTTCAATT							
	90	100	110	120	130	140	150	160
SVV-001								
89-47752	TGTTTCGTGACCTAGTCAAAGAGGTGGTCGATTGGCTGCAGGCCTGGATCAACAAAGAGAAAGCCAGCCCTGTCCTCCAGT							
131395	TGTTTCGTGACCTAGTCAAAGAGGTGGTCGACTGGCTGCAGGCCTGGATCAACAAAGAGAAAGCCAGCCCTGTCCTCCAAT							
Consensus	TGTTTCGTGACCTAGTCAAAGAGGTGGTCGA TGGCTGCAGGCCTGGATCAACAA GAGAAAGCCAGCCCTGTCCTCCA T							
	170	180	190	200	210	220	230	240
SVV-001								
89-47752	ACCAGTTGGAGATGAAGAAGCTCGGGCCTGTGGCCTTGGCTCATGACGCTTTTCATGGCTGGTTCCGGGGCCCCCTCTTAGC							
131395	ACCAGTTGGAGATGAAGAAGCTCGGGCCTGTGGCCTTGGCTCATGACGCTTTTATGGCTGGTTCCGGGGCCCCCTCTTAGC							
Consensus	ACCAGTTGGAGATGAAGAAGCTCGGGCC GTGGC TTGGCTCATGACGCTTT ATGGCTGGTTCCGGGGCCCCCTCTTAGC							
	250	260	270	280	290	300	310	320
SVV-001								
89-47752	GACGACCAGATTGAATACCTCCAGAACCTCAAATCTCTTGCCCTAACACTGGGGAAGACTAATTTGGCCCAAAGTCTCAC							
131395	GACGACCAGATTGAGTATCTCCAGAACCTCAAATCTCTTGCCCTAACACTAGGGAAGACTAATTTGGCCCAAAGTCTCAC							
Consensus	GACGACCAGATTGA TA CTCCAGAACCTCAAATCTCTTGCCCTAACACT GGGAAAGACTAATTTGGCCCAAAGTCTCAC							
	330	340	350	360	370	380	390	400
SVV-001								
89-47752	CACATGATCAATGCCAAACAAAGTTCAGCCCAACGAGTTGAACCCGTTGTGGTGGTCCTTAGAGGCAAGCCGGGATGCG							
131395	CACATGATCAATGCCAAACAAAGTTCAGCCCAACGAGTTGAACCCGTTGTGGTGGTCCTTAGAGGTAAGCCTGGATGTG							
Consensus	CACATGATCAATGCCAAACAAAGTTC GCCCAACGAGTTGAACCCGTTGTGGTGGTCCTTAGAGG AAGCC GGATG G							
	410	420	430	440	450	460	470	480
SVV-001								
89-47752	GCAAGAGCTTGGCCTCTACGTTGATTGCCCAGGCTGTGTCCAAGCGCCTCTATGGCTCCCAAAGTGTATATTCTCTTCCC							
131395	GCAAGAGCTTGGCCTCTACGCTGATTGCTCAGGCTGTGTCCAAGCGCCTCTATGGCTCCCAAAGTGTATATTCCCTCCCC							
Consensus	GCAAGAGCTTGGCCTC ACG TGATTGC CAGGCTGTGTCCAAGCG CTCTATGGCTCCCAAAGTGT TATTC CT CCC							
	490	500	510	520	530	540	550	
SVV-001								
89-47752	CCAGATCCAGATTTCTTCGATGGATACAAAGGACAGTTTCGTGACCTTGATGGATGATTTGGGACAAAACCC							
131395	CCAGATCCAGATTTCTTCGATGGATACAAAGGACAGTTTGTGACCTTGATGGATGATTTGGGACAAAACCC							
Consensus	CC GA CCAGATTTCTT GATGGATACAAAGGACA TT GTGACCTTGATGGATGATTTGGGACAAAACCC							

FIG. 88

Partial 3Dpol and 3' UTR

	10	20	30	40	50	60	70	80
SVV-001		CTTCTGGTTGGCACGGATTACGATCTGGACTTCAATGAGGTGGCACGACGCGCTGCCAAGTTGGGGTATAAGATGACTCC						
NC-88-23626	CTTCTGGTTGGCACGGATNACNATCTGGACTTCAATGAGGTGGCGCGGCGCGCTGCCAAATTGGGGTATAAGATGACGCC							
MN-88-36695	CTTCTGGTTGGCACGGATTACGATCTGGACTTCAATGAGNTGGCGCGGCGCGCTGCCAAATTGGGGTATAAGATGACGCC							
IA-89-47752	CTTCTGGTTGGCACGGATGACGATCTGGACTTCAATGAGGTGGCGCGGCGCGCTGCCAAATTGGGGTATAAGATGACGCC							
NJ-90-10324	CTTCNGGTTGGCACGGATGACGATCTGGACTTCAATGAGGTGGCGCGGCGCGCTGCCAAATTGGGGTATAAGATGACGCC							
IL-92-48963	CTTCTGGTTGNACACGGATNACGATCTGNACTTCAATGAGGTGGCGCGGCGCGCTGCCAAATTGGGGTATAAGATGACGCC							
LA-97-1278	CTTCTGGTTGGCACGGATTACGATCTGGACTTCAATGAGGTGGCGCGACGCGCTGCCAAATTGGGGTATAAGATGACTCC							
CA-131395	CTTCTGGTTGGTACGGATTACGATCTGGACTTCAATGAGGTGGCGCGACGCGCTGCCAAGCTGGGGTATAAGATGACTCC							
Consensus	CTTC GGTG ACGGAT AC ATCTG ACTTCAATGAG TGGC CG CGCGCTGCCAA TGGGGTATAAGATGAC CC							
	90	100	110	120	130	140	150	160
SVV-001		TGCCAACAAAGGGTTCTGTCTTCCCTCCGACTTCCTCTCTTTCCGATGCTGTTTTTCTAAAGCGCAAATTCGTCCAAAACA						
NC-88-23626	TGCCAACAAAGGGTTCCGTCTTCCCTCCGACTTCCTCTCTTTCCAATGCTGTTTTTCTAAACGTAATTCGTCCAAAACA							
MN-88-36695	TGCCAACAAAGGGTTCCGTCTTCCCTCCGACTTCCTCTCTTTCCAATGCTGTTTTTCTAAACGTAATTCGTCCAAAACA							
IA-89-47752	TGCCAACAAAGGGTTCCGTCTTCCCTCCGACTTCCTCTCTTTCCAATGCTGTTTTTCTAAACGTAATTCGTCCAAAACA							
NJ-90-10324	TGCCAACAAAGGGTTCCGTCTTCCCTCCGACTTCCTCTCTTTCCGATGCTGTTTTTCTAAACGCAAATTCGTCCAAAACA							
IL-92-48963	TGCCAACAAAGGGTTCCGTCTTCCCTCCGACTTCCTCTCTTTCCGATGCTGTTTTTCTAAACGCAAATTCGTCCAAAACA							
LA-97-1278	TGCCAACAAAGGGTTCCGTCTTCCCTCCGACTTCCTCTCTTTCCGATGCTGTTTTTCTAAACGCAAATTCGTCCAAAACA							
CA-131395	TGCCAACAAAGGGTTCCGTCTTCCCTCCGACTTCCTCTCTTTCCGACGCTGTTTTTCTAAACGCAAATTCGTCCAAAACA							
Consensus	TGCCAACAAAGGGTTC GTCTTCCCT CGACTTCCTCTCT TCC A GCTGTTTT CTAA CG AAATTCGTCCAAAACA							
	170	180	190	200	210	220	230	240
SVV-001		ACGACGGCTTATACAAACCAGTTATGGATTAAAGAATTTGGAAGCCATGCTCTCCTACTTCAAACCAGGAACACTACTC						
NC-88-23626	ATGACGGCTTGTACAAAGCCAGTTATGGATTCAAAGAATTTGGAAGCCATGCTCTCCTACTTCAAACCAGGAACACTACTC							
MN-88-36695	ATGACGGCTTGTACAAAGCCAGTTATGGATTCAAAGAATTTGGAAGCCATGCTCTCCTACTTCAAACCAGGAACACTACTC							
IA-89-47752	ACGACGGCTTGTACAAACCAGTTATGGATTCAAAGAATTTGGAAGCCATGCTCTCCTACTTCAAACCAGGAACACTACTC							
NJ-90-10324	ACGACGGCTTGTACAAACCAGTTATGGATTCAAAGAATTTGGAAGCCATGCTCTCCTACTTCAAACCAGGAACACTACTC							
IL-92-48963	ACGACGGCTTGTACAAACCAGTTATGGATTCAAAGAATTTGGAAGCCATGCTCTCCTACTTCAAACCAGGAACACTACTC							
LA-97-1278	ACGACGGCTTATACAAACCAGTTATGGATTAAAGAATTTGGAAGCCATGCTCTCCTACTTCAAACCAGGAACACTACTC							
CA-131395	ACGACGGCTTATACAAACCAGTTATGGATTAAAGAATTTGGAAGCCATGCTCTCCTACTTCAAACCAGGAACACTACTC							
Consensus	A GACGGCTT TACAA CCAGTTATGGATT AAAGAATTTGGAAGCCATGCTCTCCTACTTCAAACCAGGAACACTACTC							
	250	260	270	280	290	300	310	320
SVV-001		GAGAAGCTGCAATCTGTTTCTATGTTGGCTCAACATTCTGGAAAAAGAAGAATATGATAGATTGATGCACCCCTTCGCTGA						
NC-88-23626	GAGAAGCTGCAATCTGTTTCTATGTTGGCTCAACATTCTGGAAAAAGAAGAATACGATAGATTGATGCATCCCTTCGCTGA							
MN-88-36695	GAGAAGCTGCAATCTGTTTCTATGTTGGCTCAACATTCTGGAAAAAGAAGAATACGATAGATTGATGCATCCCTTCGCTGA							
IA-89-47752	GAGAAGCTGCAATCTGTTTCTATGTTGGCTCAACATTCTGGAAAAAGAAGAATATGATAGATTGATGCATCCCTTCGCTGA							
NJ-90-10324	GAGAAGCTGCAATCTGTCTCTATGTTGGCTCAACATTCTGGAAAAAGAAGAATATGATAGATTGATGCATCCCTTCGCTGA							
IL-92-48963	GAGAAGCTGCAATCTGTTTCTATGTTGGCTCAACATTCTGGAAAAAGAAGAATATGATAGACTGATGCACCCCTTCGCTGA							
LA-97-1278	GAGAAGCTGCAATCTGTTTCTATGTTGGCTCAACATTCTGGAAAAAGAAGAATATGATAGATTGATGCACCCCTTCGCTGA							
CA-131395	GAGAAGCTGCAATCTGTTTCTATGTTGGCTCAACATTCTGGAAAAAGAAGAATATGATAGATTGATGCACCCCTTCGCTGA							
Consensus	GAGAAGCTGCAATCTGT TCTATGTTGGCTCAACATTCTGGAAAAAGAAGAATA GATAGA TGATGCA CCCTTCGCTGA							

FIG. 89A

SVV-001|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
NC-88-23626 CTACGGTGCCGTACCGAGTCACGAGTACCTGCAGGCAAGATGGAGGGCCTTGTTTCGACTGACCCAGATAGCCCAAGGCGC
MN-88-36695 CTACGGTGCCGTACCGAGTCACGAGTACCTGCAGGCAAGATGGAGGGCCTTGTTTCGATTGACCCAGATAGCCCAAGGCGC
IA-89-47752 CTACGGTGCCGTACCGAGTCACGAGTACCTGCAGGCAAGATGGAGGGCCTTGTTTCGACTGACCCAGATAGCCCAAGGCGC
NJ-90-10324 CTACGGTGCCGTACCGAGTCACGAGTACCTGCAGGCAAGATGGAGGGCCTTGTTTCGACTGACCCAGATAGCCCAAGGCGC
IL-92-48963 CTACGGTGCCGTACCGAGTCACGAGTACCTGCAGGCAAGATGGAGGGCCTTGTTTCGACTGACCCAGATAGCCCAAGGCGC
LA-97-1278 CTACGGTGCCGTACCGAGTCACGAGTACCTGCAGGCAAGATGGAGGGCCTTGTTTCGACTGACCCAGATAGCCCAAGGCGC
CA-131395 CTACGGTGCCGTACCGAGTCACGAGTACCTGCAGGCAAGATGGAGGGCCTTGTTTCGACTGACCCAGATAGCCCAAGGCGC
Consensus CTACGGTGCCGTACCGAGTCACGAGTACCTGCAGGCAAGATGGAGGGCCTTGTTTCGA TGACCCAGA AGCCCAAGGCGC

410 420 430 440 450 460
SVV-001|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
NC-88-23626 TTCGGTGCTGCCGGCGATTCTGGGAGAACTCAGTCGGAACAGAAAAGGGGAAAAAAAAAAAA
MN-88-36695 TTCGGTGCTGACGGTGATTCTGGGAGAACTCAGTCGGAACAGAAAAGGGGAAAAAAAAAAAA
IA-89-47752 TTCGGTGCTGACGGTGATTCTGGGAGAACTCAGTCGGAACAGAAAAGGGGAAAAAAAAAAAA
NJ-90-10324 TTCGGTGCTGACGGTGATTCTGGGAGAACTCAGTCGGAACAGAAAAGGGGAAAAAAAAAAAA
IL-92-48963 CTCGGTGCTGCCGGTGATTNTNGGAGAACTCAGTCGGAACAGAAAAGGGGAAAAAAAAAAAA
LA-97-1278 TTCGGTGCTGCCGGCGATTCTGGGAGAACTCAGTCGGAACAGAAAAGGGGAAAAAAAAAAAA
CA-131395 TTCGGTGCTGCCGGCGATTCTGGGAGAACTCAGTCGGAACAGAAAAGGGGAAAAAAAAAAAA
Consensus T GGTGCTG CGG GATT T GGAGAACTCAGTCGGAACA AAA GGG AAAAAAAAAA

FIG. 89B

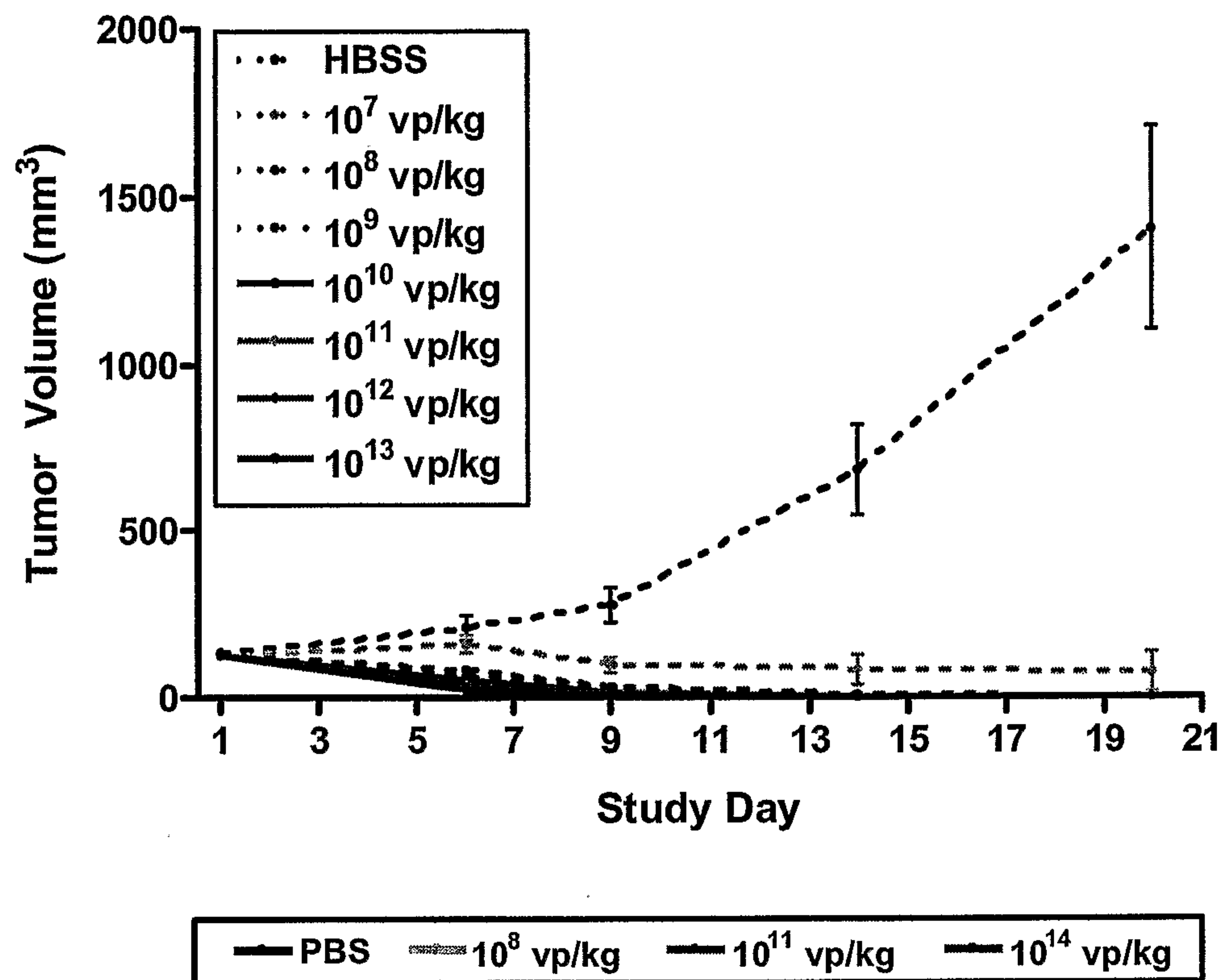


FIG. 90A

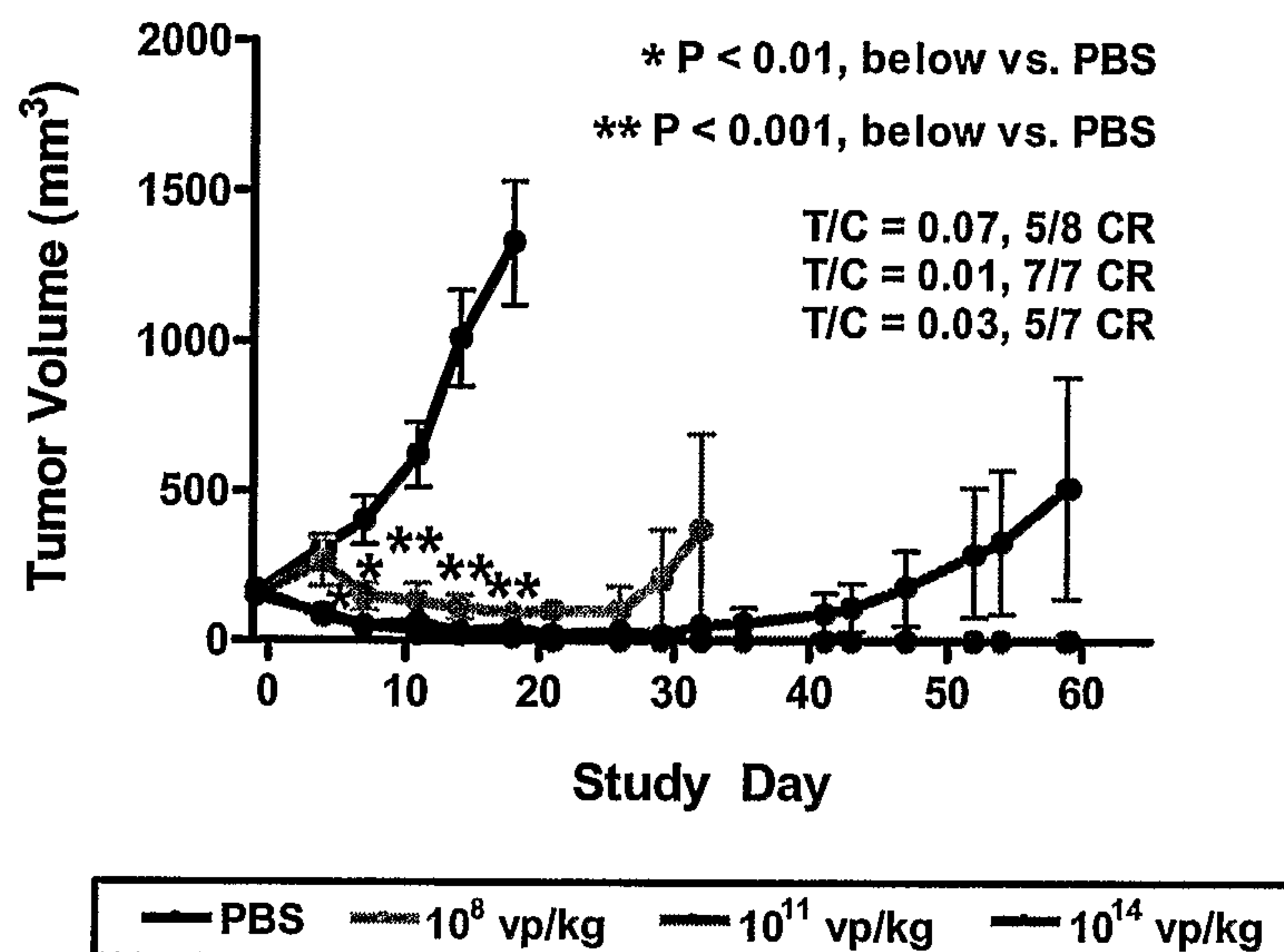


FIG. 90B

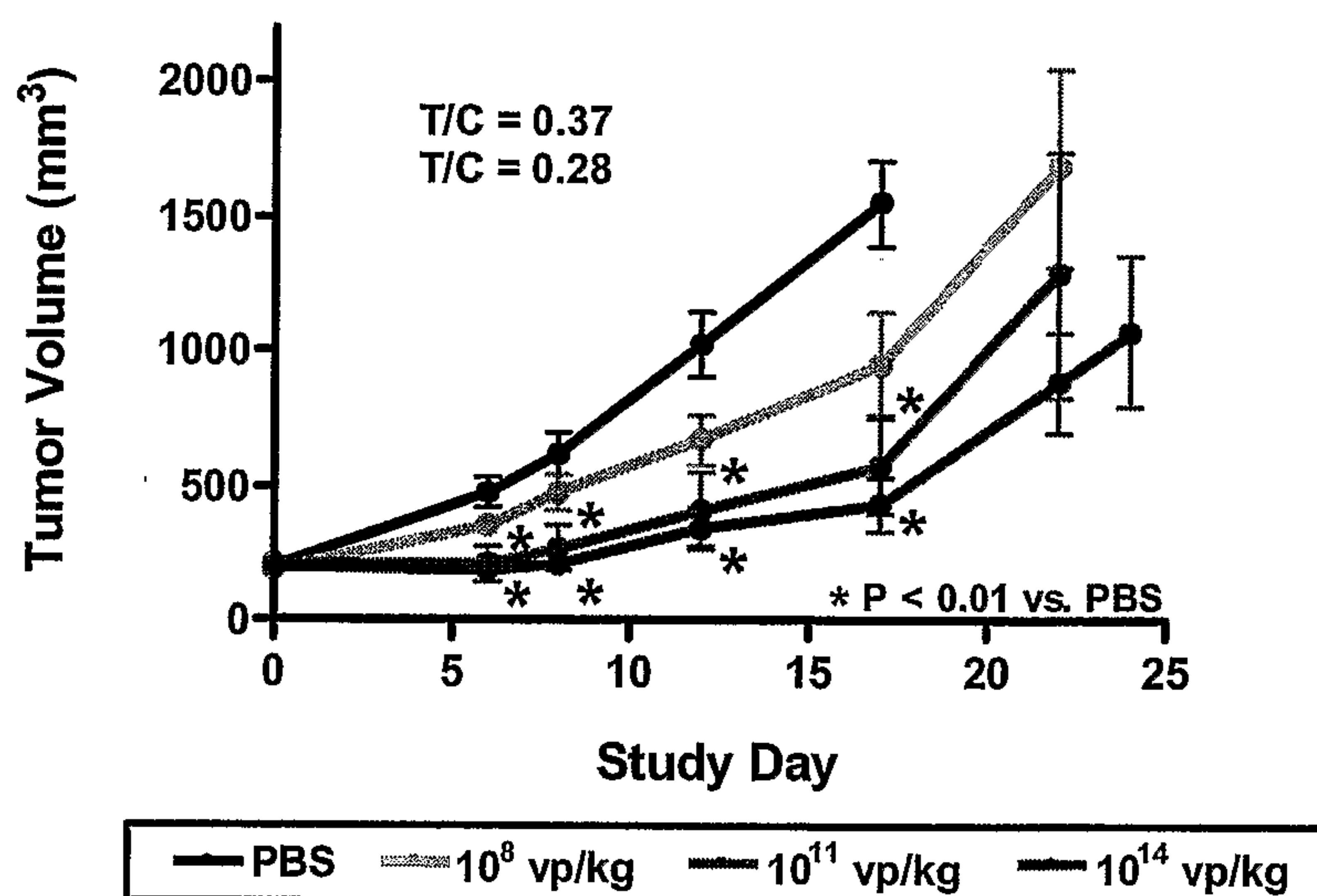


FIG. 90C

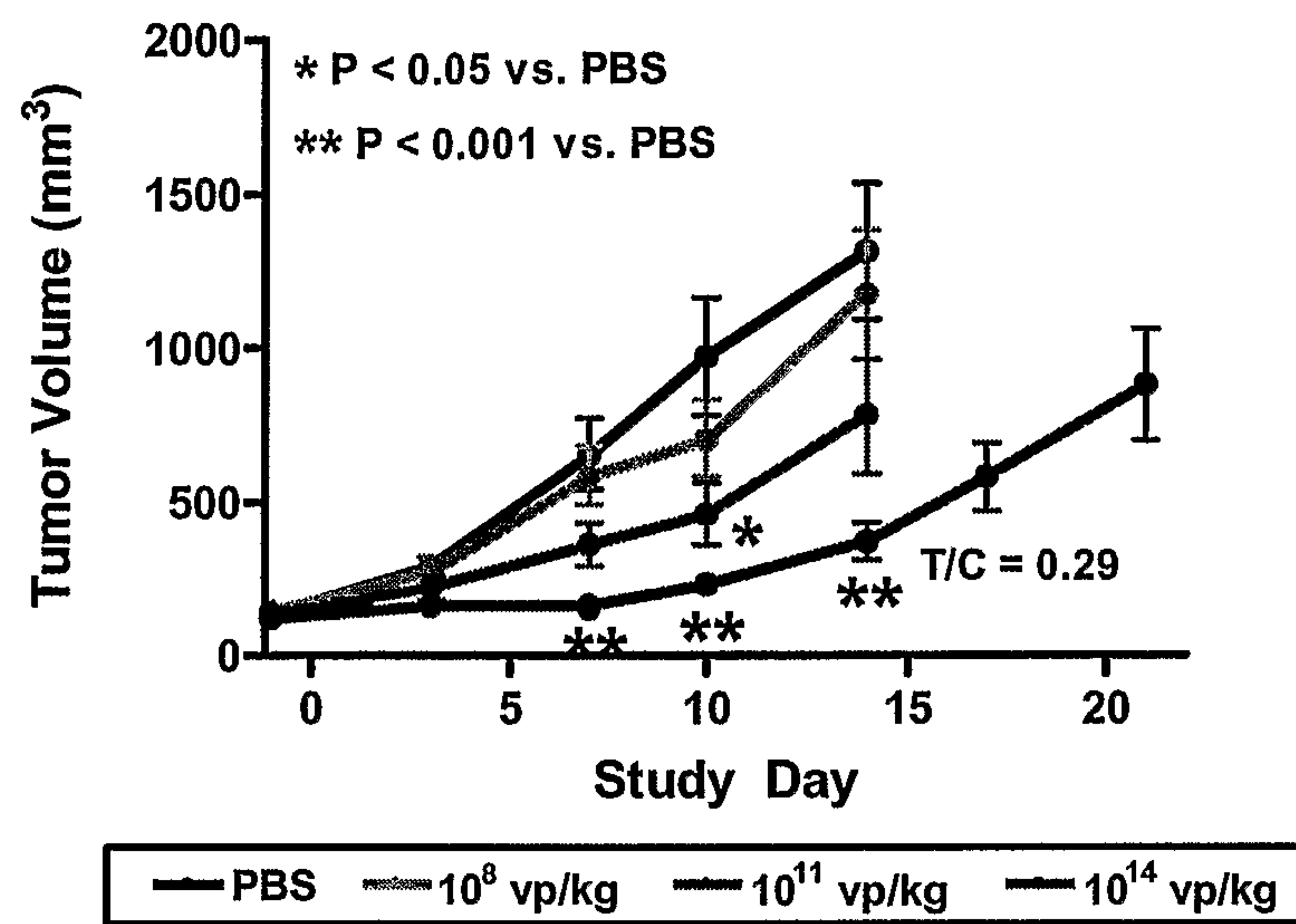


FIG. 90D

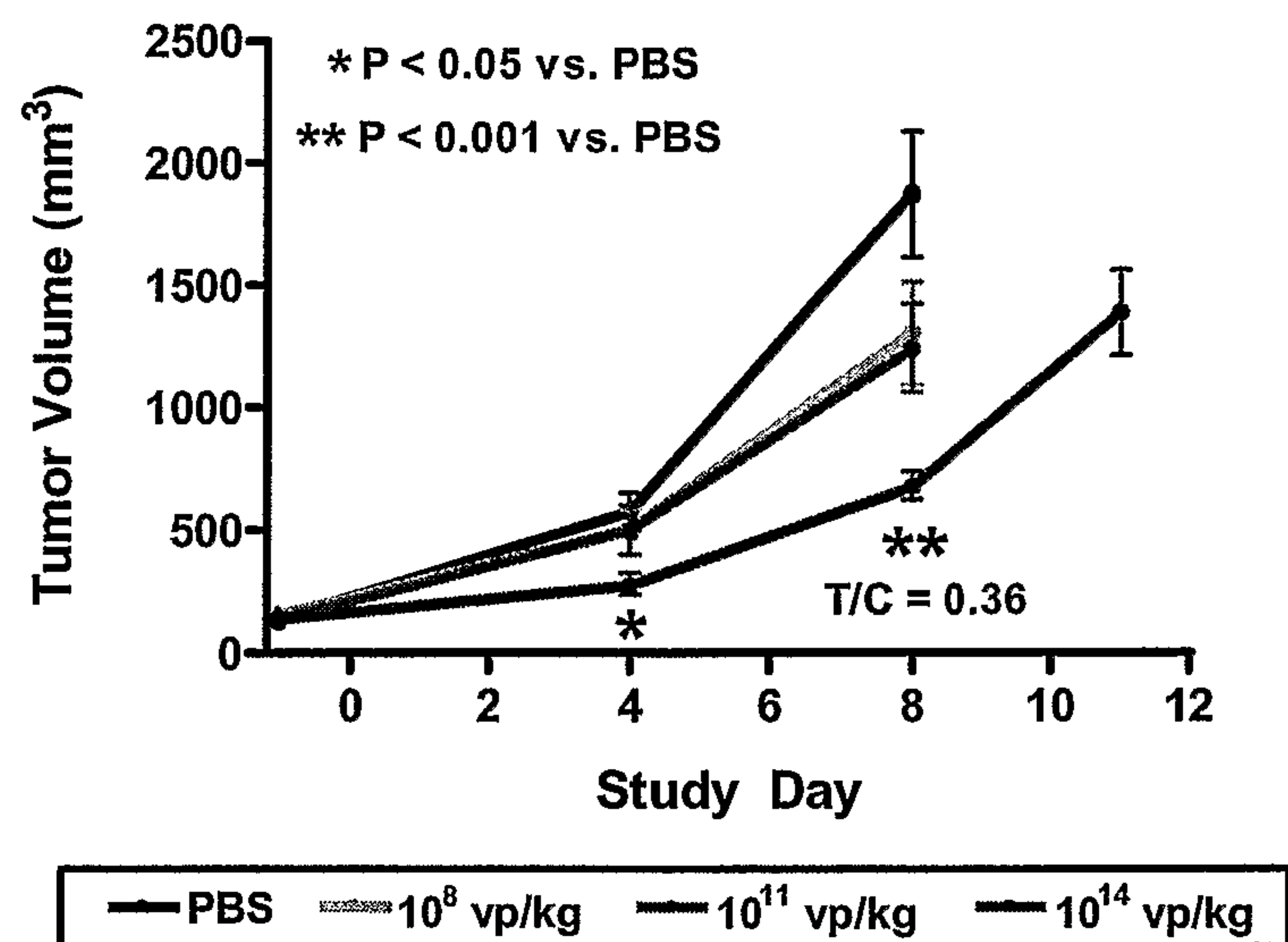


FIG. 90E

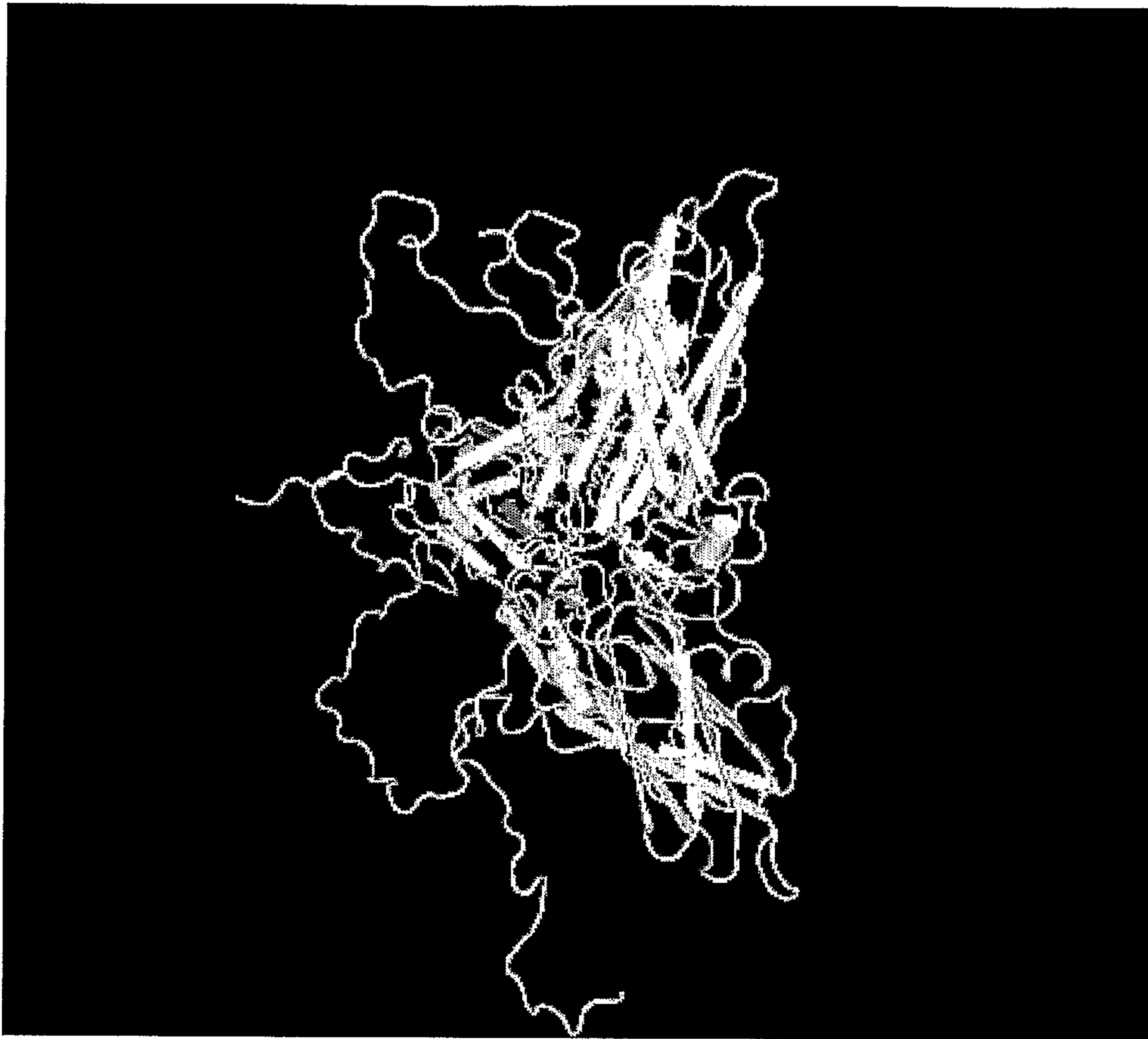


FIG. 91



FIG. 92A



FIG. 92B



FIG. 92C



FIG. 92D

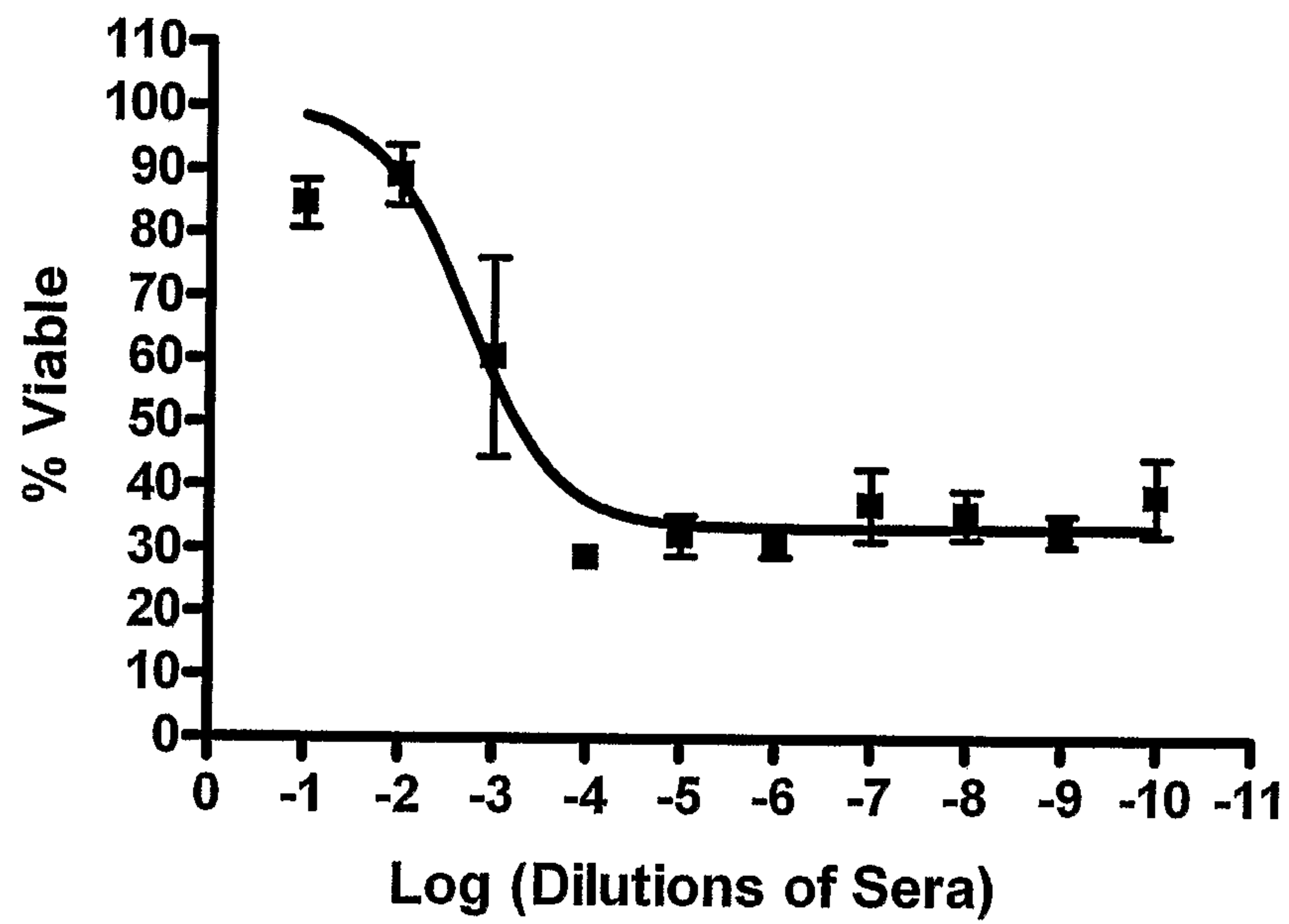


FIG. 93

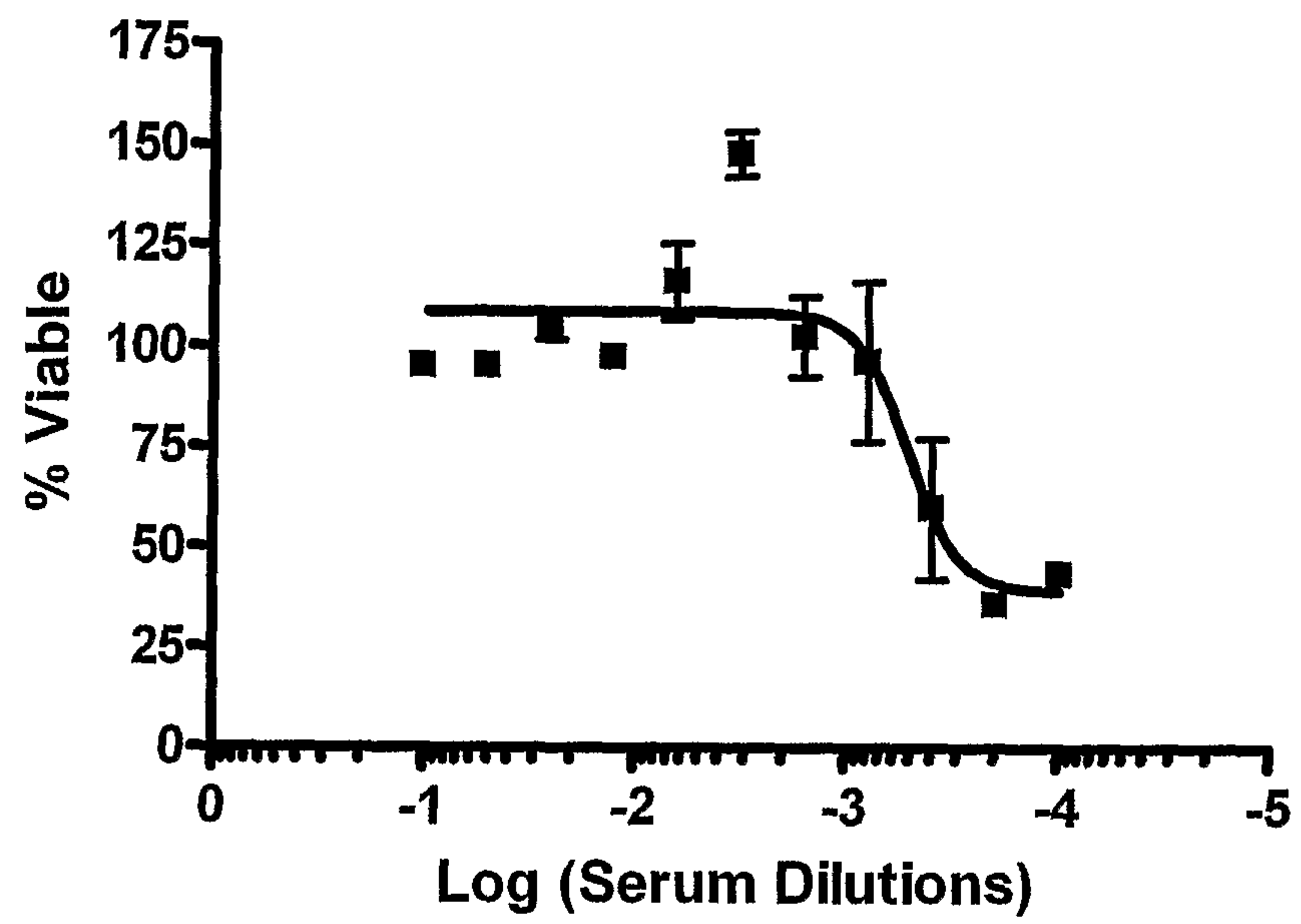


FIG. 94

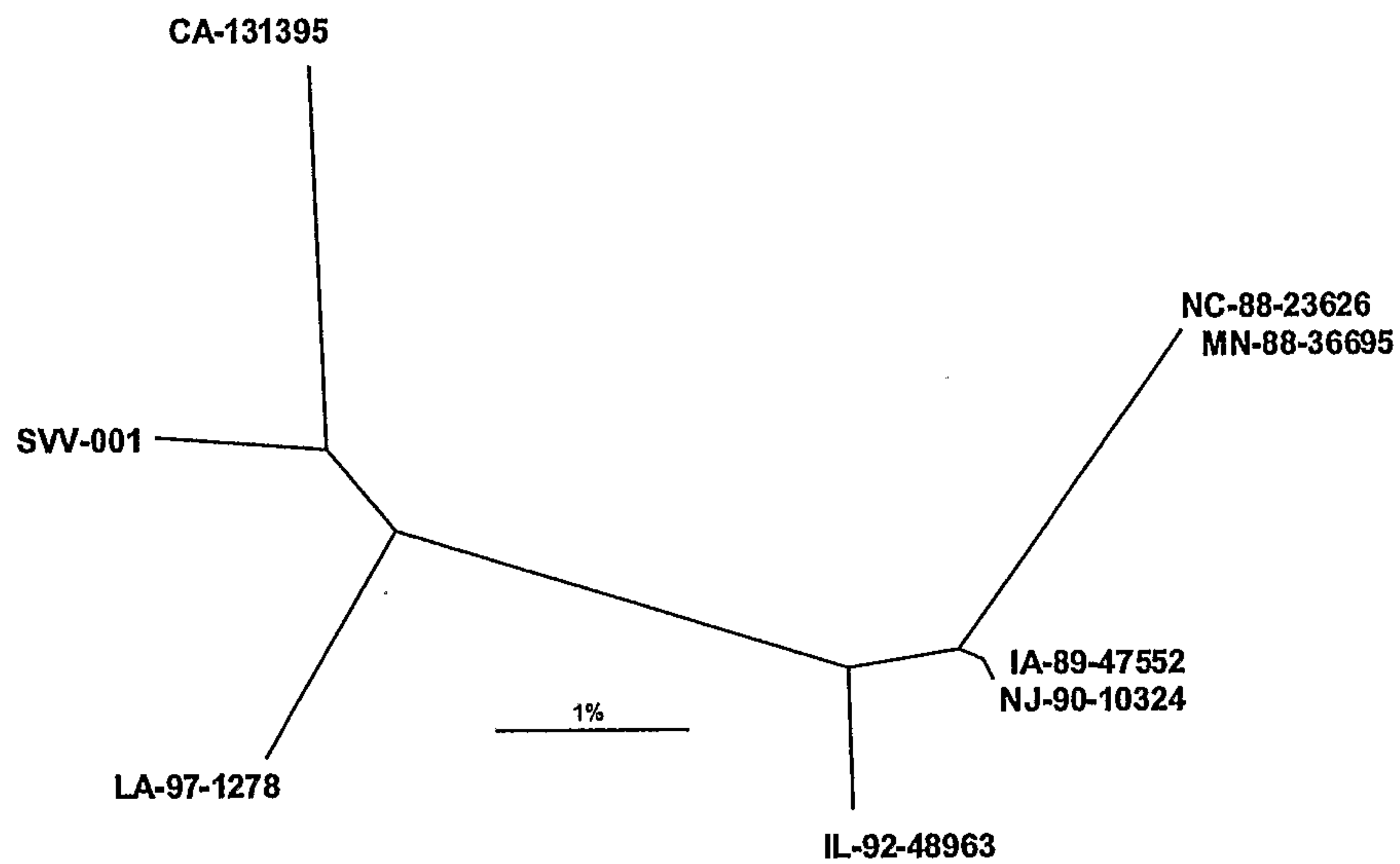


FIG. 95A

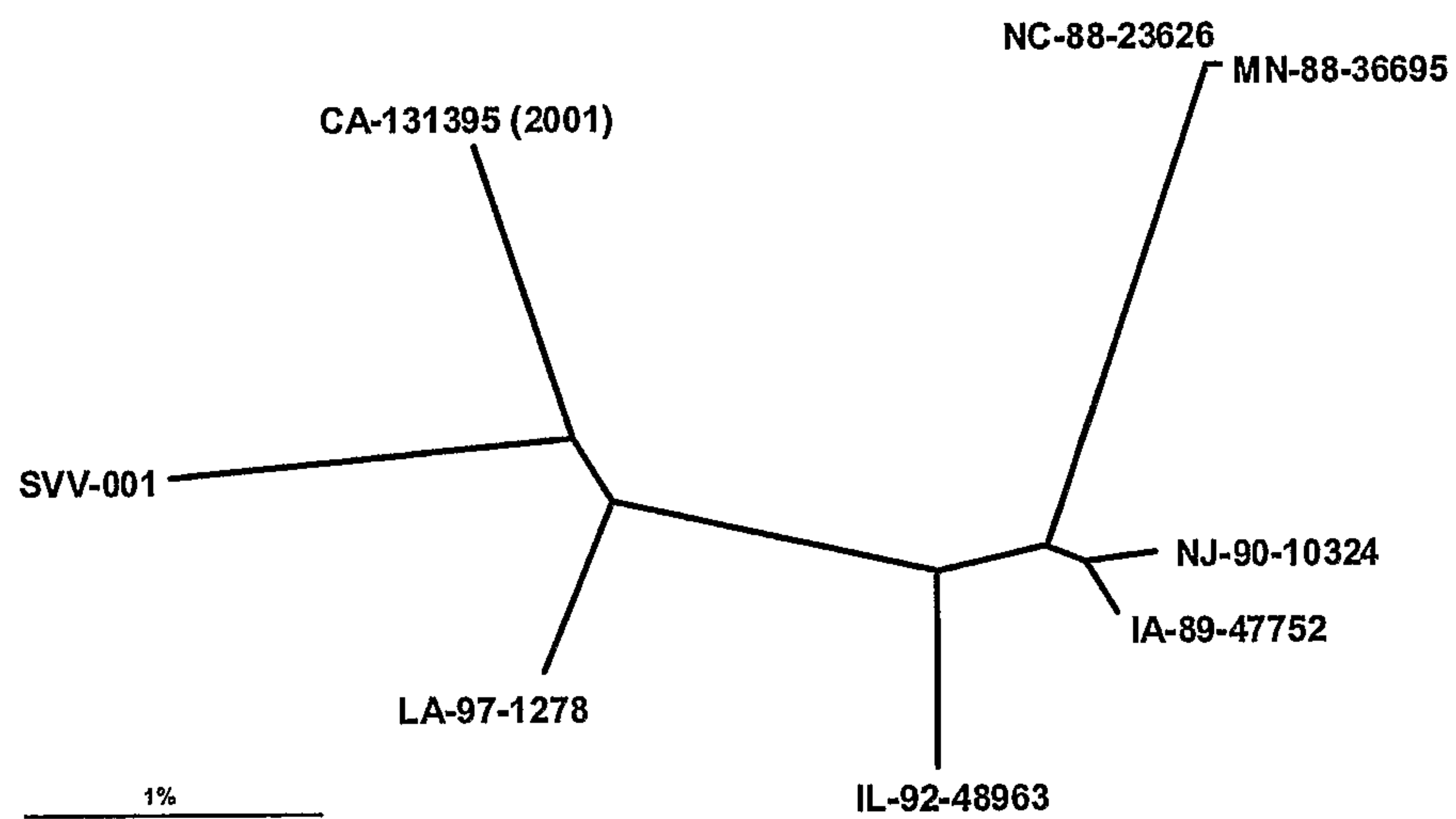


FIG. 95B

