

-1-

## MICROORGANISMS FOR THERAPY

## RELATED APPLICATIONS

Benefit of priority is claimed to each of EP 03 013 826.7, filed 18 June 2003, entitled "Recombinant vaccinia viruses useful as tumor-specific delivery vehicle for cancer gene therapy and vaccination;" EP 03 018 478.2, filed 14 August 2003, entitled "Method for the production of a polypeptide, RNA or other compound in tumor tissue;" and EP 03 024 283.8, filed 22 October 2003, entitled "Use of a Microorganism or Cell to Induce Autoimmunization of an Organism Against a Tumor." Where permitted, the subject matter of each of these applications is incorporated by reference in its entirety.

This application also is related U.S. application Serial No. ~~(Attorney docket number 17248-002wo1 (4802PC))~~, filed the same day herewith. <sup>10/872,156</sup> This application also is related to U.S. Application filed June 10, 2004 ~~(attorney docket number 17248-003002)~~, <sup>Serial No. 10/866,606</sup> entitled "Light emitting microorganisms and cells for diagnosis and therapy of tumors," which is a continuation of U.S. Application Serial No. 10/189,918, filed July 3, 2002; U.S. Application filed May 19, 2004 ~~(attorney docket number 17248-004002)~~, <sup>Serial No. 10/849,664</sup> entitled, "Light emitting microorganisms and cells for diagnosis and therapy of diseases associated with wounded or inflamed tissue" which is a continuation of U.S. Application Serial No. 10/163,763, filed June 5, 2003; International PCT Application WO 03/014380, filed July 31, 2002, entitled "Microorganisms and Cells for Diagnosis and Therapy of Tumors;" PCT Application WO 03/104485, filed June 5, 2003, entitled, "Light Emitting Microorganisms and Cells for Diagnosis and Therapy of Diseases Associated with Wounded or Inflamed tissue;" EP Application No. 01 118 417.3, filed July 31, 2001, entitled "Light-emitting microorganisms and cells for tumour diagnosis/therapy;" EP Application No. 01 125 911.6, filed October 30, 2001, entitled "Light emitting microorganisms and cells for diagnosis and therapy of tumors;" EP Application No. 02 0794 632.6, filed January 28, 2004, entitled "Microorganisms and Cells for Diagnosis and Therapy of Tumors;" and EP Application No. 02 012 552.2, filed June 5, 2002, entitled "Light Emitting

-2-

Microorganisms and Cells for Diagnosis and Therapy of Diseases associated with wounded or inflamed tissue." Where permitted, the subject matter of each of these applications is incorporated by reference in its entirety.

#### FIELD OF THE INVENTION

5 Vaccines that contain attenuated or modified microorganisms, including microbes and cells, and methods for preparing the microorganisms and vaccines are provided. In particular, modified bacteria, eukaryotic cells and viruses are provided and methods of use thereof for treatment of proliferative and inflammatory disorders and for production of products in tumors are provided.

#### 10 BACKGROUND

In the late 19th century, a variety of attempts were made to treat cancer patients with microorganisms. One surgeon, William Coley, administered live Streptococcus <sup>pyogenes</sup> ~~pyogenes~~ to patients with tumors with limited success. In the early 20th century, scientists documented vaccinia viral oncolysis in mice, which <sup>led</sup> ~~lead~~ to administration of several live viruses to patients with tumors from the 1940s through 15 the 1960s. These forays into this avenue of cancer treatment were not successful.

Since that time, a variety of genetically engineered viruses have been tested for treatment of cancers. In one study, for example, nude mice bearing nonmetastatic colon adenocarcinoma cells were systemically injected with a WR strain of vaccinia virus modified by having a vaccinia growth factor deletion and an enhanced green fluorescence protein inserted into the thymidine kinase locus. The virus was observed to have antitumor effect, including one complete response, despite a lack of exogenous therapeutic genes in the modified virus (McCart *et al.* (2001) *Cancer Res* 1:8751-8757). In another study, vaccinia melanoma oncolysate (VMO) was injected into sites near melanoma positive lymph nodes in a Phase III clinical trial of melanoma patients. As a control, New York City Board of Health strain vaccinia virus (VV) was administered to melanoma patients. The melanoma patients treated with VMO had a survival rate better than that for untreated patients, but similar to patients treated with the VV control (Kim *et al.* (2001) *Surgical Oncol* 20 10:53-59). 25 30

-3-

Other studies have demonstrated limited success with this approach. This therapy is not completely effective, particularly for systemically delivered viruses or bacteria. Limitations on the control of microbial vehicle function in vivo result in ineffective therapeutic results as well as raising safety concerns. It would be desirable to improve this type of therapy or to develop more effective approaches for treatments of neoplastic disease. Therefore, among the objects herein, it is an object to provide therapeutic methods and microorganisms for the treatment of neoplastic and other diseases.

### SUMMARY

In a first aspect, there is provided a recombinant vaccinia virus, comprising a modified thymidine kinase (TK) gene and a modified HA gene and a modified F3 gene or interrupted F3 locus, wherein a modified gene is deleted or contains one or more truncations, mutations, insertions or deletions accompanied by at least a partial loss of function.

In a second aspect, there is provided a recombinant pox virus, comprising a modified TK and HA gene and a modified F3 gene or locus that corresponds to the F3 gene in vaccinia, wherein a modified gene or locus is deleted or contains one or more truncations, mutations, insertions or deletions accompanied by at least a partial loss of function.

In a third aspect, there is provided an isolated host cell, comprising a recombinant vaccinia virus of the first aspect.

In a fourth aspect, there is provided a non-human tumor cell, comprising a recombinant virus of the first or second aspect.

In a fifth aspect, there is provided a pharmaceutical composition containing a recombinant virus of the first or second aspect in a pharmaceutically acceptable vehicle.

In a sixth aspect, there is provided a kit, comprising: a composition containing a pharmaceutical composition of the fifth aspect; and a composition comprising a chemotherapeutic compound, wherein: the compositions are packaged as separate compositions or are provided as a single composition.

In a seventh aspect, there is provided a combination, comprising: a composition containing a pharmaceutical composition of the fifth aspect; and a composition comprising a chemotherapeutic compound, wherein: a combination is an association of two products; the compositions are separate compositions or are provided as a single composition.

In an eighth aspect, there is provided a use of the pharmaceutical composition of the fifth aspect for formulation of a medicament for eliminating immunoprivileged cells or tissues.

In a ninth aspect, there is provided a use of a recombinant virus of the first or second aspect for the preparation of a pharmaceutical composition for gene therapy or vaccine therapy.

In a tenth aspect, there is provided use of a recombinant virus of the first or second aspect for formulation of a medicament for eliminating immunoprivileged cells or tissues, in an animal, wherein: the virus accumulates in the immunoprivileged cells; the virus does not accumulate to toxic levels in organs and tissues that do not comprise immunoprivileged cells or tissues.

In an eleventh aspect, there is provided a use of the combination of the seventh aspect for the formulation of a medicament for the treatment of cancer.

In a twelfth aspect, there is provided a method for production of a polypeptide or RNA or a product compound, comprising: isolating the polypeptide or RNA or compound from tumor tissue that has been harvested from a tumor-bearing animal, wherein: the animal is a non-human animal that has been administered a microorganism containing nucleic acid encoding the polypeptide or RNA or a pathway for producing the product compound; the microorganism had accumulated in immunoprivileged cells; and the microorganism did not accumulate to toxic levels in organs and tissues that do not comprise immunoprivileged cells or tissues, wherein the microorganism is a recombinant virus of the first or second aspect.

In a thirteenth aspect, there is provided a method for simultaneously producing a polypeptide, RNA molecule or cellular compound and an antibody that specifically reacts with the polypeptide, RNA molecule or compound, comprising: isolating the antibody from serum that has been obtained from a tumor-bearing animal, wherein the animal is a non-human animal that has been administered a microorganism, wherein the microorganism expressed or produced the compound, polypeptide or RNA molecule, and wherein the microorganism is a recombinant virus of the first or second aspect.

In a fourteenth aspect, there is provided a use of at least two microorganisms for formulation of a medicament for elimination of immunoprivileged cells or tissues in an animal, wherein: the microorganisms accumulate in the immunoprivileged cells, whereby the animal is autoimmunized against the immunoprivileged cells or tissues; and at least one of the microorganisms is a recombinant virus of the first or second aspect.

In a fifteenth aspect, there is provided a combination, comprising at least two microorganisms formulated for administration to an animal for elimination of immunoprivileged cells or tissues, wherein one of the microorganisms is a recombinant virus of the first or second aspect.

In a sixteenth aspect, there is provided a kit comprising the combination of the fifteenth aspect, wherein each microorganism is contained in a composition formulated and packaged for single dosage administration.

In a seventeenth aspect, there is provided a method for the production of antibodies against products encoded by heterologous nucleic acid in immunoprivileged tissues or cells, comprising: isolating antibodies against a protein or RNA from the blood or serum that has been obtained from an animal containing immunoprivileged tissues or cells, wherein the animal is a non-human animal that has been administered a microorganism containing nucleic acid encoding a selected protein or RNA and wherein the microorganism is a recombinant virus of the first or second aspect.

In an eighteenth aspect, there is provided a vaccine, comprising a recombinant virus of the first or second aspect.

In a nineteenth aspect, there is provided a use of the virus of the first or second aspect for formulation of a vaccine for vaccination against smallpox.

Disclosed herein are therapeutic methods and microorganisms, including viruses, bacteria and eukaryotic cells, for uses in the methods for the treatment of neoplastic diseases and other diseases. Diseases for treatment may comprise those in which the targeted tissues and/or cells are immunoprivileged in that they, and often the local environment thereof, somehow escape or are inaccessible to the immune system. Such tissues include tumors and other tissues and cells involved in other proliferative disorders, wounds and other tissues involved in inflammatory responses. The microorganisms, which include bacterial cells, viruses and mammalian cells, are selected or are designed to be non-pathogenic and to preferentially accumulate in the immunoprivileged tissues. The microorganisms, once in the tissues or cells or vicinity thereof, affect the cell membranes of the cells in such tissues so that they become leaky or lyse, but sufficiently slowly so that the targeted cells and tumors leak enough antigen or other proteins for a time sufficient to elicit an immune response.

The microorganisms may be administered by any route, including systemic administration, such as i.v. or using oral or nasal or other delivery systems that direct agents to the lymphatics. In exemplary methods, the microorganisms are used to treat tumors and to prevent recurrence and metastatic spread. Exemplary microorganisms include highly attenuated viruses and bacteria, as well as mammalian cells. The microorganisms may optionally be modified to deliver other products, including other therapeutic products to the targeted tissues.

-4-

When the microorganisms are administered to a host that contains tumors, the tumors in the host essentially become antigen and protein factories. This can be exploited so that the tumors can be used to produce proteins or other cellular products encoded by or produced by the microorganisms. In addition, the host sera  
5 can be harvested to isolate antibodies to products produced by the microorganisms as well as the tumor cells. Hence also disclosed herein are methods for producing gene products by administering the microorganisms to an animal, generally a non-human animal, and harvesting the tumors to isolate the product. Also disclosed herein are methods  
10 for producing antibodies to selected proteins or cell products, such as metabolites or intermediates, by administering a microorganism that expresses or produces the protein or other product to a host, typically a non-human host; and harvesting serum from the host and isolating antibodies that specifically bind to the protein or other product.

Disclosed herein are methods and microorganisms for elimination of  
15 immunoprivileged cells or tissues, particularly tumors. The methods include administration, typically systemic administration, with a microorganism that preferentially accumulates in immunoprivileged cells, such as tumor cells, resulting in leakage proteins and other compounds, such as tumor antigens, resulting in vaccination of the host against non-host proteins and, such as the tumor antigens,  
20 providing for elimination of the immunoprivileged cells, such as tumor cells, by the host's immune system. The microorganisms are selected not for their ability to rapidly lyse cells, but rather for the ability to accumulate in immunoprivileged cells, such as tumors, resulting in a leakage of antigens in a sufficient amount and for a sufficient time to elicit an immune response.

Disclosed herein are uses of microorganisms or cells containing heterologous DNA, polypeptides or RNA to induce autoimmunization of an organism against a tumor. In particular, the microorganisms are selected or designed to accumulate in tumors and to accumulate very little, if at all (to be non-toxic to the host) in non-tumorous cells, tissues or organs, and to in some manner result in the tumor cell  
30 lyses or cell membrane disruption such that tumor antigens leak. Exemplary of such

-5-

microorganisms are the LIVP-derived vaccinia virus and the bacteria described herein and also mammalian cells modified to target the tumors and to disrupt the cells membrane. The microorganisms can be modified to express heterologous products that mediate or increase the leakage of the tumor cell antigens and/or that are therapeutic, such as anti-tumor compounds.

Also disclosed herein are methods for production of antibodies against a tumor by (a) injecting a microorganism or cell containing a DNA sequence encoding a desired polypeptide or RNA into an organism bearing a tumor and (b) isolating antibodies against the tumor.

Disclosed herein are attenuated microorganisms that accumulate in immunoprivileged tissues and cells, such as tumor cells, but do not accumulate to toxic levels in non-targeted organs and tissues, and that upon administration to an animal bearing the immunoprivileged tissues and cells, result in autoimmunity, such as by production of anti-tumor (or anti-tumor antigen) antibodies against the immunoprivileged cells or products thereof. The microorganisms are selected or produced to render the immunoprivileged cells leaky, such as by a slow lysis or apoptotic process. The goal is to achieve such leakiness, but to not lyse the cells so rapidly that the host cannot mount an immune response.

Uses of and methods of use of the microorganisms for eliminating immunoprivileged are disclosed herein. The microorganisms optionally include reporter genes and/or other heterologous nucleic acids that disrupt genes in the microorganism and can also encode and provide therapeutic products or products, such as RNA, including RNAi, that alter gene and/or protein expression in the cells or tissues where the microorganism accumulates. Among the disclosed viruses are attenuated pox viruses that contain a modified TK and HA gene and a modified F3 gene or locus that corresponds to the F3 gene in vaccinia. In particular, disclosed are recombinant vaccinia viruses that contain a modified TK and HA gene and optionally a modified F3 gene or locus, wherein the resulting virus does not accumulate to toxic levels in non-targeted organs. Vaccinia viruses where the TK gene and F3 gene are modified and vaccinia viruses where the HA and F3 gene are modified, and

-6-

viruses where all three genes are modified are disclosed herein. Modification includes inactivation by insertion, deletion or replacement of one or more nucleotide bases whereby an activity or product of the virus is altered. Included among the alterations is insertion of heterologous nucleic acid, such as therapeutic protein-  
5 encoding nucleic acids.

In exemplary embodiments, the vaccinia viruses are Lister strain viruses, particularly LVP strain viruses (LVP refers to the Lister virus from the Institute of Viral Preparations, Moscow, Russia, the original source for this now widely disseminated virus strain). Modifications include modification of the virus at the  
10 unique *NotI* site in the locus designed F3. In particular, the modification is at position 35 of the F3 gene or at position 1475 inside of the *HindIII*-F fragment of vaccinia virus DNA strain LVP.

The heterologous nucleic acid can include regulatory sequence operatively linked to the nucleic acid encoding the protein. Regulatory sequences include  
15 promoters, such as the vaccinia virus early/late promoter p7.5 and an early/late vaccinia pE/L promoter. The heterologous nucleic acid in the microorganism can encode a detectable protein or a product capable of inducing a detectable signal. Inclusion of detectable protein or a product that can generate a detectable signal permits monitoring of the distribution of the administered microorganism as well as  
20 monitoring therapeutic efficacy, since the microorganism will be eliminated when the immunoprivileged cells are eliminated.

Host cells containing the recombinant viruses, such as the triple mutant vaccinia virus exemplified herein are disclosed. Also contemplated are tumor cells that contain any of the microorganisms disclosed herein or used in the disclosed methods.

25 Pharmaceutical compositions containing the microorganisms in a pharmaceutically acceptable vehicle for use in the methods are disclosed herein. The pharmaceutical compositions can be formulated for any mode of administration, including, but not limited to systemic administration such as for intravenous administration or is formulated. The compositions can contain a delivery

-7-

vehicle, such as a lipid-based carrier, including liposomes and micelles associated with the microorganism.

Also disclosed are methods (and uses of the microorganisms) for eliminating immunoprivileged cells, such as tumor cells in an animal, by administering the pharmaceutical compositions to an animal, whereby the virus accumulates in the immunoprivileged cells, thereby mediating autoimmunization resulting in elimination of the cells or a reduction in their number.

Therapeutic methods for eliminating immunoprivileged cells or tissues, in an animal, by administering a microorganism to an animal, where the microorganism accumulates in the immunoprivileged cells; the microorganism does not accumulate in unaffected organs and tissues and has low toxicity in the animal; and the microorganism results in leakage of the cell membranes in the immunoprivileged cells, whereby the animal produces autoantibodies against the cells or products of the cells are disclosed. These methods include tumor treatment, treatment for inflammatory conditions, including wounds, and proliferative disorders, including psoriasis, cancers, diabetic retinopathies, restenosis and other such disorders. It is desirable for the microorganisms to not accumulate in unaffected organs, particularly the ovaries or testes.

The microorganisms include attenuated bacteria, an attenuated viruses and mammalian cells, such as pox viruses and other cytoplasmic viruses, bacteria such as vibrio, E. coli, salmonella, streptococcus and listeria and mammalian cells, such as immune cells, including B cells and lymphocytes, such as T cells, and stem cells.

Disclosed herein are methods for a recombinant vaccinia virus by : (a) generating (i) a vaccinia shuttle plasmid containing the modified F3 gene inserted at restriction site x and (ii) a dephosphorylated wt VV (VGL) DNA digested at a restriction site; (b) transfecting host cells infected with psoralen-UV (PUV)-inactivated helper VV (VGL) with a mixture of constructs (i) and (ii) of step a; and (c) isolating the recombinant vaccinia viruses from the transfectants. Host cells include CV-1 cells.

-8-

Also disclosed are methods for production of a polypeptide or RNA or compound, such as cellular product and uses of the microorganism therefore.

Such methods can include the steps of : (a) administering a

microorganism containing nucleic acid encoding the polypeptide or RNA or  
5 producing the product compound to tumor-bearing animal, where the  
microorganism accumulates in the immunoprivileged cells; and the  
microorganism does not accumulate to toxic levels in organs and tissues that do not  
comprise immunoprivileged cells or tissues; (b) harvesting the tumor tissue from  
the animal; and (c) isolating the polypeptide or RNA or compound from the tumor.

10 As noted, the microorganisms include eukaryotic cells, prokaryotic cells and  
viruses such as a cytoplasmic virus or an attenuated bacterium or a stem cell or an  
immune cell. The bacterium can be selected from among attenuated *vibrio*, *E.*  
*coli*, listeria, salmonella and streptococcus strains. The microorganism can express  
or produce detectable products, such as a fluorescent protein (*i.e.*, green, red and  
15 blue fluorescent proteins and modified variants thereof), and/or luciferase which,  
when contacted with a Lucifer produces light, and also can encode additional  
products, such as therapeutic products. In the methods and uses disclosed herein,  
the animals can be non-human animals or can include humans.

Also disclosed are methods for simultaneously producing a polypeptide,  
20 RNA molecule or cellular compound and an antibody that specifically reacts  
with the polypeptide, RNA molecule or compound, by: a) administering a  
microorganism to a tumor-bearing animal, wherein the microorganism expresses or  
produces the compound, polypeptide or RNA molecule; and b) isolating the  
antibody from serum in the animal. The method optionally includes, after step a)  
25 harvesting the tumor tissue from the animal; and isolating the polypeptide,  
RNA molecule or cellular compound from the tumor tissue.

Also disclosed are methods for eliminating immunoprivileged cells or tissues  
in an animal, such as tumor cells, and uses of the microorganisms therefore by  
administering at least two microorganisms, wherein the microorganisms are  
30 administered simultaneously, sequentially or intermittently, wherein the

-9-

microorganisms accumulate in the immunoprivileged cells, whereby the animal is autoimmunized against the immunoprivileged cells or tissues.

Uses of at least two microorganism for formulation of a medicament for elimination of immunoprivileged cells or tissues, wherein they accumulate in the immunoprivileged cells, whereby the animal is autoimmunized against the immunoprivileged cells or tissues are disclosed. Combinations containing at least two microorganisms formulated for administration to an animal for elimination of immunoprivileged cells or tissues are disclosed. Kits containing packaged combination optionally with instructions for administration and other reagents are disclosed.

Uses of a microorganism encoding heterologous nucleic acid for inducing autoimmunization against products produced in immunoprivileged cells, wherein, when administered, the microorganism accumulates in immunoprivileged tissues and does not accumulate or accumulates at a sufficiently low level in other tissues or organs to be non-toxic to an animal containing the immunoprivileged tissues are disclosed.

Methods for the production of antibodies against products produced in immunoprivileged tissues or cells by: (a) administering a microorganism containing nucleic acid encoding a selected protein or RNA into an animal containing the immunoprivileged tissues or cells; and (b) isolating antibodies against the protein or RNA from the blood or serum of the animal are disclosed.

Also disclosed are methods for inhibiting growth of immunoprivileged cells or tissue in a subject by: (a) administering to a subject a modified microorganism, wherein the modified microorganism encodes a detectable gene product; (b) monitoring the presence of the detectable gene product in the subject until the detectable gene product is substantially present only in immunoprivileged tissue or cells of a subject; and (c) administering to a subject a therapeutic compound that works in conjunction with the microorganism to inhibit growth of immunoprivileged cells or tissue or by: (a) administering to a subject a modified microorganism that encodes a detectable gene product; (b) administering to a subject

-10-

- a therapeutic substance that reduces the pathogenicity of the microorganism; (c) monitoring the presence of the detectable gene product in the subject until the detectable gene product is substantially present only in immunoprivileged tissue or cells of a subject; and (d) terminating or suspending administration of the therapeutic compound, whereby the microorganism increases in pathogenicity and the growth of the immunoprivileged cells or tissue is inhibited.

### DESCRIPTION OF THE FIGURES

Figure 1A: Schematic representation of the recombinant vaccinia virus RVGL8 used. The recombinant vaccinia virus RVGL8 was constructed by using the in vivo recombination method described in Example 1. The complex of wild-type vaccinia virus DNA digested with *NotI* and non-digested plasmid DNA pNZ2 was transfected for in vivo recombination into PUV-VV-infected cells. VGL, wild type vaccinia virus (strain Lister ATCC VR-1549); RVGL8, recombinant vaccinia virus encoding the lacZ gene in the *NotI* site; Not<sub>L</sub> and Not<sub>R</sub>, left and right segments of unique *NotI* restriction site in vaccinia virus genome.; pE/L, synthetic early/late vaccinia virus promoter; p7.5, early/late vaccinia virus promoter; lacZ, lacZ gene of *E. coli*.

Figure 1B: Schematic of the various vaccinia strains described in the Examples.

Results achieved the viruses are described in the Examples.

- Figure 2 sets forth a flow chart for a method for producing products, such as nucleic acid molecules, proteins and metabolic compounds or other cellular products in tumors.

### DETAILED DESCRIPTION

- A. Definitions
- B. Microorganisms for Tumor-Specific Therapy
  - B. Microorganisms for Tumor-Specific Therapy
    - 1. Characteristics
      - a. Attenuated
        - i. Reduced toxicity
        - ii. Accumulate in tumor, not substantially in other organs
        - iii. Ability to Elicit or Enhance Immune Response to Tumor Cell
        - iv. Balance of Pathogenicity and Release of Tumor Antigens

-11-

- b. Immunogenicity
      - c. Replication Competent
      - d. Genetic Variants
        - i. Modified Characteristics
        - ii. Exogenous Gene Expression
        - iii. Detectable gene product
        - iv. Therapeutic gene product
        - v. Expressing a superantigen
        - vi. Expressing a gene product to be harvested
    - 2. Viruses
      - a. Cytoplasmic viruses
        - i. Poxviruses
          - a. Vaccinia Virus
          - b. Modified Vaccinia Viruses
          - c. The F3 Gene
          - d. Multiple Modifications
          - e. The Lister Strain
        - ii. Other cytoplasmic viruses
      - b. Adenovirus, Herpes, Retroviruses
    - 3. Bacteria
      - a. Aerobic bacteria
      - b. Anaerobic bacteria
    - 4. Eukaryotic cells
  - C. Methods for Making an Attenuated Microorganism
    - 1. Genetic Modifications
    - 2. Screening for above characteristics
    - 3. Methods for developing such a microorganism in humans
  - D. Therapeutic Methods
    - 1. Administration
      - a. Steps prior to administering the microorganism
      - b. Mode of administration
      - c. Dosage
      - d. Number of administrations
      - e. Co-administrations
        - i. Administering a plurality of microorganisms
        - ii. Therapeutic compounds
      - f. State of subject
    - 2. Monitoring
      - a. Monitoring microorganismal gene expression
      - b. Monitoring tumor size
      - c. Monitoring antibody titer
      - d. Monitoring general health diagnostics
      - e. Monitoring coordinated with treatment
  - E. Methods of Producing Gene Products and Antibodies
    - 1. Production of Recombinant Proteins and RNA molecules
    - 2. Production of Antibodies
  - F. Pharmaceutical Compositions, combinations and kits
    - 1. Pharmaceutical Compositions
    - 2. Host Cells
    - 3. Combinations
    - 4. Kits
  - G. Examples
- A. Definitions

-12-

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention(s) belong. All patents, patent applications, published applications and publications, websites and other published materials referred to throughout the entire disclosure herein, unless noted otherwise, are incorporated by reference in their entirety. In the event that there are a plurality of definitions for terms herein, those in this section prevail. Where reference is made to a URL or other such identifier or address, it is understood that such identifiers can change and particular information on the internet can come and go, but equivalent information is known and can be readily accessed, such as by searching the internet and/or appropriate databases. Reference thereto evidences the availability and public dissemination of such information.

As used herein, microorganisms refers to isolated cells or viruses, including eukaryotic cells, such as mammalian cells, viruses and bacteria. The microorganisms are modified or selected for their ability to accumulate in tumors and other immunoprivileged cells and tissues, and to minimize accumulation in other tissues or organs. Accumulation occurs by virtue of selection or modification of the microorganisms for particular traits or by proper selection of cells. The microorganism can be further modified to alter a trait thereof and/or to deliver a gene product. The microorganisms provided herein are typically modified relative to wild type to exhibit one or more characteristics such as reduced pathogenicity, reduced toxicity, preferential accumulation in tumor relative to normal organs or tissues, increased immunogenicity, increased ability to elicit or enhance an immune response to tumor cells, increased lytic or tumor cell killing capacity, decreased lytic or tumor cell killing capacity.

As used herein, immunoprivileged cells and tissues refer to cells and tissues, such as solid tumors and wounded tissues, which are sequestered from the immune system. Generally administration of a microorganism elicits an immune response that clears the microorganism; immunoprivileged sites, however, are shielded or sequestered from the immune response, permitting the microorganisms to survive

-13-

and generally to replicate. Immunoprivileged tissues include inflamed tissues, such as wounded tissues, and proliferating tissues, such as tumor tissues.

As used herein, "modified" with reference to a gene refers to a deleted gene, or a gene encoding a gene product having one or more truncations, mutations, insertions or deletions, typically accompanied by at least a change, generally a partial loss of function.

As used herein F3 gene refers to a gene or locus in a virus, such as a vaccinia virus, that corresponds to the F3 gene of vaccinia virus strain LIVP. This includes the F3 gene of any vaccinia virus strain or poxvirus encoding a gene product having substantially the same or at least a related biological function or locus in the genome. F3 genes encompassed herein typically have at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity along the full length of the sequence of nucleotides set forth in SEQ ID No:1. The proteins encoded by F3 genes encompassed herein typically have at least about 50%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 85%, at least about 90%, at least about 93%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the sequence of amino acids set forth SEQ ID No. 2 along the full length thereof. Also included are corresponding loci in other viruses that when modified or eliminated result in reduced toxicity and/or enhanced accumulation in tumors (compared to non-tumorous cells, tissues and organs). The corresponding loci in other viruses equivalent to the F3 gene in LIVP can be determined by the structural location of the gene in the viral genome: the LIVP F3 gene is located on the HindIII-F fragment of vaccinia virus between open reading frames F14L and F15L as defined by Goebel *et al.*, Virology (1990) 179:247-266, and in the opposite orientation of ORFs F14L and F15L; thus corresponding loci in other viruses such as poxviruses including orthopoxviruses are included.

-14-

As used herein, attenuate toxicity of a microorganism means to reduce or eliminate deleterious or toxic effects to a host upon administration of the microorganism compared to the unattenuated microorganism.

As use herein, a microorganism with low toxicity means that upon  
5 administration a microorganism does not accumulate in organs and tissues in the host to an extent that results in damage or harm to organs or that impact on survival of the host to a greater extent than the disease being treated does.

As used herein, subject (or organism) refers to an animal, including a human being.

10 As used herein, animal includes any animal, such as, but are not limited to primates including humans, gorillas and monkeys; rodents, such as mice and rats; fowl, such as chickens; ruminants, such as goats, cows, deer, sheep; ovine, and other animals including pigs, horses, cats, dogs, and rabbits. Non-human animals exclude humans as the contemplated animal.

15 As used herein, accumulation of a microorganism in a targeted tissue refers to the distribution of the microorganism throughout the organism after a time period long enough for the microbes to infect the host's organs or tissues. As one skilled in the art will recognize, the time period for infection of a microbe will vary depending on the microbe, the targeted organ(s) or tissue(s), the immunocompetence of the  
20 host, and dosage. Generally, accumulation can be determined at time point from about 1 day to about 1 week after infection with the microbes. For purposes herein, the microorganisms preferentially accumulate in the target tissue, such as a tumor, but are cleared from other tissues and organs in the host to the extent that toxicity of the microorganism is mild or tolerable and at most not fatal.

25 As used herein, preferential accumulation refers to accumulation of a microorganism at a first location at a higher level than accumulation at a second location. Thus, a microorganism that preferentially accumulates in  
~~immunoprivileged~~  
~~immunoprivileged~~ tissue such as tumor relative to normal tissues or organs refers  
~~immunoprivileged~~  
to a microorganism that accumulates in ~~immunoprivileged~~ tissue such as tumor at  
30 a higher level than the microorganism accumulates in normal tissues or organs.

-15-

As used herein, a "compound" produced in a tumor or other immunoprivileged site refers to any compound that is produced in the tumor by virtue of the presence of an introduced microorganism, generally a recombinant microorganism, expressing one or more genes. For example, a compound produced in a tumor can be, for example, a metabolite, an encoded polypeptide or RNA, or compound that is generated by a recombinant polypeptide (e.g., enzyme) and the cellular machinery of the tumor or immunoprivileged tissue or cells.

As used herein, a delivery vehicle for administration refers to a lipid-based or other polymer based composition, such as liposome, micelle or reverse micelle, that associates with an agent, such as a microorganism provided herein, for delivery into a host animal.

As used herein, the term "viral vector" is used according to its art-recognized meaning. It refers to a nucleic acid vector construct that includes at least one element of viral origin and can be packaged into a viral vector particle. The viral vector particles can be used for the purpose of transferring DNA, RNA or other nucleic acids into cells either in vitro or in vivo. Viral vectors include, but are not limited to, retroviral vectors, vaccinia vectors, lentiviral vectors, herpes virus vectors (e.g., HSV), baculoviral vectors, cytomegalovirus (CMV) vectors, papillomavirus vectors, simian virus (SV40) vectors, ~~vectors~~, semliki forest virus vectors, phage vectors, adenoviral vectors, and adeno-associated viral (AAV) vectors.

As used herein, oncolytic viruses refer to viruses that replicate selectively in tumor cells.

As used herein, "disease or disorder" refers to a pathological condition in an organism resulting from, e.g., infection or genetic defect, and characterized by identifiable symptoms.

As used herein, neoplasm (neoplasia) refers to abnormal new growth, and thus means the same as tumor, which can be benign or malignant. Unlike hyperplasia, neoplastic proliferation persists even in the absence of the original stimulus.

-16-

As used herein, neoplastic disease refers to any disorder involving cancer, including tumor development, growth, metastasis and progression.

As used herein, cancer is a general term for diseases caused by or characterized by any type of malignant tumor.

5 As used herein, malignant, as applies to tumors, refers to primary tumors that have the capacity of metastasis with loss of growth control and positional control.

As used herein, metastasis refers to a growth of abnormal or neoplastic cells distant from the site primarily involved by the morbid process.

As used herein, an anti-cancer agent or compound (used interchangeably  
10 with "anti-tumor or anti-neoplastic agent") refers to any agents or compounds used in anti-cancer treatment. These include any agents, when used alone or in combination with other compounds, that can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of clinical symptoms or diagnostic markers associated with neoplastic disease, tumors and cancer, and can be used in  
15 methods, combinations and compositions provided herein. Exemplary anti-neoplastic agents include the microorganism provided herein used singly or in combination and/or in combination with other agents, such as alkylating agents, antimetabolite, certain natural products, platinum coordination complexes, anthracenediones, substituted ureas, methylhydrazine derivatives, adrenocortical suppressants, certain hormones, <sup>antagonists</sup> ~~antagonists~~ and anti-cancer polysaccharides.  
20

In general, for practice of the methods herein and when using the microorganisms provided herein, the original tumor is not excised, but is employed to accumulate the administered microorganism and as the cells become leaky or lyse to become an antigen or other product factor. The antigens can serve to elicit an  
25 immune response in the host. The antigens and products can be isolated from the tumor.

As used herein, angiogenesis is intended to encompass the totality of processes directly or indirectly involved in the establishment and maintenance of new vasculature (neovascularization), including, but not limited to,

-17-

neovascularization associated with tumors and neovascularization associated with wounds.

As used herein, by homologous means about greater than 25% nucleic acid sequence identity, such as 25%, 40%, 60%, 70%, 80%, 90% or 95%. If necessary the percentage homology will be specified. The terms "homology" and "identity" are often used interchangeably but homology for proteins can include conservative amino acid changes. In general, sequences (protein or nucleic acid) are aligned so that the highest order match is obtained (see, e.g.: Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; Carillo *et al.* (1988) SIAM J Applied Math 48:1073). By sequence identity, the number of identical amino acids is determined by standard alignment algorithm programs, and used with default gap penalties established by each supplier. Substantially homologous nucleic acid molecules would hybridize typically at moderate stringency or at high stringency all along the length of the nucleic acid or along at least about 70%, 80% or 90% of the full length nucleic acid molecule of interest. Also provided are nucleic acid molecules that contain degenerate codons in place of codons in the hybridizing nucleic acid molecule. (For proteins, for determination of homology conservative amino acids can be aligned as well as identical amino acids; in this case percentage of identity and percentage homology vary). Whether any two nucleic acid molecules have nucleotide sequences that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% "identical" can be determined using known computer algorithms such as the "FASTA" program, using for example, the default parameters as in Pearson *et al.* (1988) Proc. Natl. Acad. Sci. USA 85:2444 (other programs include the GCG program package (Devereux, J., *et al.*, Nucleic Acids Research 12(I):387 (1984)), BLASTP, BLASTN, FASTA Atschul, S.F., *et al.*, J Molec Biol

-18-

215:403 (1990); Guide to Huge Computers, Mrtin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo *et al.* (1988) SIAM J Applied Math 48:1073). For example, the BLAST function of the National Center for Biotechnology Information database can be used to determine identity. Other commercially or publicly  
5 available programs include, DNASTar "MegAlign" program (Madison, WI) and the University of Wisconsin Genetics Computer Group (UWG) "Gap" program (Madison WI). Percent homology or identity of proteins and/or nucleic acid molecules can be determined, for example, by comparing sequence information using a GAP computer program (*e.g.*, Needleman *et al.* (1970) J. Mol. Biol. 48:443,  
10 as revised by Smith and Waterman ((1981) Adv. Appl. Math. 2:482).

Briefly, a GAP program defines similarity as the number of aligned symbols (*i.e.*, nucleotides or amino acids) that are similar, divided by the total number of symbols in the shorter of the two sequences. Default parameters for the GAP program can include: (1) a unary comparison matrix (containing a value of 1 for  
15 identities and 0 for non-identities) and the weighted comparison matrix of Gribskov *et al.* (1986) Nucl. Acids Res. 14:6745, as described by Schwartz and Dayhoff, eds., ATLAS OF PROTEIN SEQUENCE AND STRUCTURE, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.  
20 Therefore, as used herein, the term "identity" represents a comparison between a test and a reference polypeptide or polynucleotide.

As used herein, recitation that amino acids of a polypeptide correspond to amino acids in a disclosed sequence, such as amino acids set forth in the Sequence listing, refers to amino acids identified upon alignment of the polypeptide with the  
25 disclosed sequence to maximize identity or homology (where conserved amino acids are aligned) using a standard alignment algorithm, such as the GAP algorithm.

As used herein, the term "at least 90% identical to" refers to percent identities from 90 to 100% relative to the reference polypeptides. Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes  
30 a test and reference polynucleotide length of 100 amino acids are compared, no

-19-

more than 10% (*i.e.*, 10 out of 100) of amino acids in the test polypeptide differs from that of the reference polypeptides. Similar comparisons can be made between a test and reference polynucleotides. 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.*, 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or amino acid substitutions, insertions or deletions. At the level of homologies or 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.

As used herein, primer refers to an oligonucleotide containing two or more deoxyribonucleotides or ribonucleotides, typically more than three, from which synthesis of a primer extension product can be initiated. Experimental conditions conducive to synthesis include the presence of nucleoside triphosphates and an agent for polymerization and extension, such as DNA polymerase, and a suitable buffer, temperature and pH.

As used herein, chemiluminescence refers to a chemical reaction in which energy is specifically channeled to a molecule causing it to become electronically excited and subsequently to release a photon thereby emitting visible light. Temperature does not contribute to this channeled energy. Thus, chemiluminescence involves the direct conversion of chemical energy to light energy.

As used herein, luminescence refers to the detectable EM radiation, generally, UV, IR or visible EM radiation that is produced when the excited product of an exergic chemical process reverts to its ground state with the emission of light. Chemiluminescence is luminescence that results from a chemical reaction. Bioluminescence is chemiluminescence that results from a chemical reaction using biological molecules (or synthetic versions or analogs thereof) as substrates and/or enzymes.

-20-

As used herein, bioluminescence, which is a type of chemiluminescence, refers to the emission of light by biological molecules, particularly proteins. The essential condition for bioluminescence is molecular oxygen, either bound or free in the presence of an oxygenase, a luciferase, which acts on a substrate, a luciferin.

5 Bioluminescence is generated by an enzyme or other protein (luciferase) that is an oxygenase that acts on a substrate luciferin (a bioluminescence substrate) in the presence of molecular oxygen, and transforms the substrate to an excited state, which, upon return to a lower energy level releases the energy in the form of light.

As used herein, the substrates and enzymes for producing bioluminescence  
10 are generically referred to as luciferin and luciferase, respectively. When reference is made to a particular species thereof, for clarity, each generic term is used with the name of the organism from which it derives, for example, bacterial luciferin or firefly luciferase.

As used herein, luciferase refers to oxygenases that catalyze a light emitting  
15 reaction. For instance, bacterial luciferases catalyze the oxidation of flavin mononucleotide (FMN) and aliphatic aldehydes, which reaction produces light. Another class of luciferases, found among marine arthropods, catalyzes the oxidation of Cypridina (Vargula) luciferin, and another class of luciferases catalyzes the oxidation of Coleoptera luciferin.

20 Thus, luciferase refers to an enzyme or photoprotein that catalyzes a bioluminescent reaction (a reaction that produces bioluminescence). The luciferases, such as firefly and Gaussia and Renilla luciferases, are enzymes which act catalytically and are unchanged during the bioluminescence generating reaction. The luciferase photoproteins, such as the aequorin photoprotein to which luciferin is  
25 non-covalently bound, are changed, such as by release of the luciferin, during bioluminescence generating reaction. The luciferase is a protein that occurs naturally in an organism or a variant or mutant thereof, such as a variant produced by mutagenesis that has one or more properties, such as thermal stability, that differ from the naturally-occurring protein. Luciferases and modified mutant or variant

-21-

forms thereof are well known. For purposes herein, reference to luciferase refers to either the photoproteins or luciferases.

Thus, reference, for example, to "Renilla luciferase" means an enzyme isolated from member of the genus *Renilla* or an equivalent molecule obtained from any other source, such as from another related copepod, or that has been prepared synthetically. It is intended to encompass *Renilla* luciferases with conservative amino acid substitutions that do not substantially alter activity. Suitable conservative substitutions of amino acids are known to those of skill in this art and can be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, *e.g.*, Watson *et al.* *Molecular Biology of the Gene*, 4th Edition, 1987, The Benjamin/Cummings Pub. co., p.224).

As used herein, "Aequora GFP" refers to GFPs from the genus *Aequora* and to mutants or variants thereof. Such variants and GFPs from other species are well known and are <sup>available</sup> ~~available~~ and known to those of skill in the art. This nomenclature encompass GFPs with conservative amino acid substitutions that do not substantially alter activity and physical properties, such as the emission spectra and ability to shift the spectral output of bioluminescence generating systems. The luciferases and luciferin and activators thereof are referred to as bioluminescence generating reagents or components. Typically, a subset of these reagents will be provided or combined with an article of manufacture. Bioluminescence will be produced upon contacting the combination with the remaining reagents. Thus, as used herein, the component luciferases, luciferins, and other factors, such as O<sub>2</sub>, Mg<sup>2+</sup>, Ca<sup>2+</sup> are also referred to as bioluminescence generating reagents (or agents or components).

As used herein, bioluminescence substrate refers to the compound that is oxidized in the presence of a luciferase, and any necessary activators, and generates light. These substrates are referred to as luciferins herein, are substrates that undergo oxidation in a bioluminescence reaction. These bioluminescence substrates include any luciferin or analog thereof or any synthetic compound with which a

-22-

luciferase interacts to generate light. Typical substrates include those that are oxidized in the presence of a luciferase or protein in a light-generating reaction. Bioluminescence substrates, thus, include those compounds that those of skill in the art recognize as luciferins. Luciferins, for example, include firefly luciferin, Cypridina (also known as Vargula) luciferin (coelenterazine), bacterial luciferin, as well as synthetic analogs of these substrates or other compounds that are oxidized in the presence of a luciferase in a reaction the produces bioluminescence.

As used herein, capable of conversion into a bioluminescence substrate means susceptible to chemical reaction, such as oxidation or reduction, that yields a bioluminescence substrate. For example, the luminescence producing reaction of bioluminescent bacteria involves the reduction of a flavin mononucleotide group (FMN) to reduced flavin mononucleotide (FMNH<sub>2</sub>) by a flavin reductase enzyme. The reduced flavin mononucleotide (substrate) then reacts with oxygen (an activator) and bacterial luciferase to form an intermediate peroxy flavin that undergoes further reaction, in the presence of a long-chain aldehyde, to generate light. With respect to this reaction, the reduced flavin and the long chain aldehyde are substrates.

As used herein, a bioluminescence generating system refers to the set of reagents required to conduct a bioluminescent reaction. Thus, the specific luciferase, luciferin and other substrates, solvents and other reagents that can be required to complete a bioluminescent reaction form a bioluminescence system. Thus a bioluminescence generating system refers to any set of reagents that, under appropriate reaction conditions, yield bioluminescence. Appropriate reaction conditions refers to the conditions necessary for a bioluminescence reaction to occur, such as pH, salt concentrations and temperature. In general, bioluminescence systems include a bioluminescence substrate, luciferin, a luciferase, which includes enzymes luciferases and photoproteins, and one or more activators. A specific bioluminescence system may be identified by reference to the specific organism from which the luciferase derives; for example, the Renilla bioluminescence system includes a Renilla luciferase, such as a luciferase isolated from the Renilla or

-23-

produced using recombinant means or modifications of these luciferases. This system also includes the particular activators necessary to complete the bioluminescence reaction, such as oxygen and a substrate with which the luciferase reacts in the presence of the oxygen to produce light.

5           As used herein, a fluorescent protein refers to a protein that possesses the ability to fluoresce (*i.e.*, to absorb energy at one wavelength and emit it at another wavelength). For example, a green fluorescent protein refers to a polypeptide that has a peak in the emission spectrum at about 510 nm.

          As used herein, genetic therapy or gene therapy involves the transfer of  
10   heterologous nucleic acid, such as DNA, into certain cells, target cells, of a mammal, particularly a human, with a disorder or conditions for which such therapy is sought. The nucleic acid, such as DNA, is introduced into the selected target cells, such as directly or in a vector or other delivery vehicle, in a manner such that the heterologous nucleic acid, such as DNA, is expressed and a therapeutic product  
15   encoded thereby is produced. Alternatively, the heterologous nucleic acid, such as DNA, can in some manner mediate expression of DNA that encodes the therapeutic product, or it can encode a product, such as a peptide or RNA that in some manner mediates, directly or indirectly, expression of a therapeutic product. Genetic therapy also can be used to deliver nucleic acid encoding a gene product that replaces a  
20   defective gene or supplements a gene product produced by the mammal or the cell in which it is introduced. The introduced nucleic acid can encode a therapeutic compound, such as a growth factor inhibitor thereof, or a tumor necrosis factor or inhibitor thereof, such as a receptor therefor, that is not normally produced in the mammalian host or that is not produced in therapeutically effective amounts or at a  
25   therapeutically useful time. The heterologous nucleic acid, such as DNA, encoding the therapeutic product can be modified prior to introduction into the cells of the afflicted host in order to enhance or otherwise alter the product or expression thereof. Genetic therapy also can involve delivery of an inhibitor or repressor or other modulator of gene expression.

-24-

As used herein, heterologous nucleic acid is nucleic acid that is not normally produced *in vivo* by the microorganism from which it is expressed or that is produced by a microorganism but is at a different locus or expressed differently or that mediates or encodes mediators that alter expression of endogenous nucleic acid, such as DNA, by affecting transcription, translation, or other regulatable biochemical processes. Heterologous nucleic acid is often not endogenous to the cell into which it is introduced, but has been obtained from another cell or prepared synthetically. Heterologous nucleic acid, however, can be endogenous, but is nucleic acid that is expressed from a different locus or altered in its expression or sequence. Generally, although not necessarily, such nucleic acid encodes RNA and proteins that are not normally produced by the cell or in the same way in the cell in which it is expressed. Heterologous nucleic acid, such as DNA, also can be referred to as foreign nucleic acid, such as DNA. Thus, heterologous nucleic acid or foreign nucleic acid includes a nucleic acid molecule not present in the exact orientation or position as the counterpart nucleic acid molecule, such as DNA, is found in a genome. It also can refer to a nucleic acid molecule from another organism or species (*i.e.*, exogenous). Any nucleic acid, such as DNA, that one of skill in the art would recognize or consider as heterologous or foreign to the cell in which the nucleic acid is expressed is herein encompassed by heterologous nucleic acid; heterologous nucleic acid includes exogenously added nucleic acid that also is expressed endogenously. Examples of heterologous nucleic acid include, but are not limited to, nucleic acid that encodes traceable marker proteins, such as a protein that confers drug resistance, nucleic acid that encodes therapeutically effective substances, such as anti-cancer agents, enzymes and hormones, and nucleic acid, such as DNA, that encodes other types of proteins, such as antibodies. Antibodies that are encoded by heterologous nucleic acid can be secreted or expressed on the surface of the cell in which the heterologous nucleic acid has been introduced.

As used herein, a therapeutically effective product for gene therapy is a product that is encoded by heterologous nucleic acid, typically DNA, (or an RNA product such as dsRNA, RNAi, including siRNA, that, upon introduction of the

-25-

nucleic acid into a host, a product is expressed that ameliorates or eliminates the symptoms, manifestations of an inherited or acquired disease or that cures the disease. Also included are biologically active nucleic acid molecules, such as RNAi and antisense.

5           As used herein, cancer or tumor treatment or agent refers to any therapeutic regimen and/or compound that, when used alone or in combination with other treatments or compounds, can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of clinical symptoms or diagnostic markers associated with deficient angiogenesis.

10           As used herein, nucleic acids include DNA, RNA and analogs thereof, including peptide nucleic acids (PNA) and mixtures thereof. Nucleic acids can be single or double-stranded. When referring to probes or primers, which are optionally labeled, such as with a detectable label, such as a fluorescent or radiolabel, single-stranded molecules are provided. Such molecules are typically of a length such that  
15           their target is statistically unique or of low copy number (typically less than 5, generally less than 3) for probing or priming a library. Generally a probe or primer contains at least 14, 16 or 30 contiguous nucleotides of sequence complementary to or identical to a gene of interest. Probes and primers can be 10, 20, 30, 50, 100 or more nucleic acids long.

20           As used herein, operative linkage of heterologous nucleic to regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences refers to the relationship between such nucleic acid, such as DNA, and such sequences of nucleotides. For example, operative linkage of heterologous DNA to a promoter refers to the physical  
25           relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA. Thus, operatively linked or operationally associated refers to the functional relationship of nucleic acid, such as DNA, with regulatory and effector sequences of nucleotides, such as promoters,  
30           enhancers, transcriptional and translational stop sites, and other signal sequences.

-26-

For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it can be necessary to remove, add or alter 5' untranslated portions of the clones to eliminate extra, potentially inappropriate alternative translation initiation (*i.e.*, start) codons or other sequences that can interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, *e.g.*, Kozak J. Biol. Chem. 266:19867-19870 (1991)) can be inserted immediately 5' of the start codon and can enhance expression. The desirability of (or need for) such modification can be empirically determined.

As used herein, a sequence complementary to at least a portion of an RNA, with reference to antisense oligonucleotides, means a sequence of nucleotides having sufficient complementarity to be able to hybridize with the RNA, generally under moderate or high stringency conditions, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA (or dsRNA) can thus be tested, or triplex formation can be assayed. The ability to hybridize depends on the degree of <sup>complementarity</sup> ~~complementarity~~ and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an encoding RNA it can contain and still form a stable duplex (or triplex, as the case can be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

As used herein, amelioration of the symptoms of a particular disorder such as by administration of a particular pharmaceutical composition, refers to any lessening, whether permanent or temporary, lasting or transient that can be attributed to or associated with administration of the composition.

As used herein, antisense polynucleotides refer to synthetic sequences of nucleotide bases complementary to mRNA or the sense strand of double-stranded

-27-

DNA. Admixture of sense and antisense polynucleotides under appropriate conditions leads to the binding of the two molecules, or hybridization. When these polynucleotides bind to (hybridize with) mRNA, inhibition of protein synthesis (translation) occurs. When these polynucleotides bind to double-stranded DNA, inhibition of RNA synthesis (transcription) occurs. The resulting inhibition of translation and/or transcription leads to an inhibition of the synthesis of the protein encoded by the sense strand. Antisense nucleic acid molecules typically contain a sufficient number of nucleotides to specifically bind to a target nucleic acid, generally at least 5 contiguous nucleotides, often at least 14 or 16 or 30 contiguous nucleotides or modified nucleotides complementary to the coding portion of a nucleic acid molecule that encodes a gene of interest.

As used herein, antibody refers to an immunoglobulin, whether natural or partially or wholly synthetically produced, including any derivative thereof that retains the specific binding ability of the antibody. Hence antibody includes any protein having a binding domain that is homologous or substantially homologous to an immunoglobulin binding domain. Antibodies include members of any immunoglobulin class, including IgG, IgM, IgA, IgD and IgE.

As used herein, antibody fragment refers to any derivative of an antibody that is less than full length, retaining at least a portion of the full-length antibody's specific binding ability. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab)2, single-chain Fvs (scFV), FV, dsFV diabody and Fd fragments. The fragment can include multiple chains linked together, such as by disulfide bridges. An antibody fragment generally contains at least about 50 amino acids and typically at least 200 amino acids.

As used herein, a Fv antibody fragment is composed of one variable heavy chain domain (VH) and one variable light chain domain linked by noncovalent interactions.

As used herein, a dsFV refers to an Fv with an engineered intermolecular disulfide bond, which stabilizes the VH-VL pair.

-28-

As used herein, a F(ab)<sub>2</sub> fragment is an antibody fragment that results from digestion of an immunoglobulin with pepsin at pH 4.0-4.5; it can be recombinantly produced to produce the equivalent fragment.

As used herein, Fab fragments are antibody fragments that result from  
5 digestion of an immunoglobulin with papain; it can be recombinantly produced to produce the equivalent fragment.

As used herein, scFVs refer to antibody fragments that contain a variable light chain (VL) and variable heavy chain (VH) covalently connected by a polypeptide linker in any order. The linker is of a length such that the two variable  
10 domains are bridged without substantial interference. Included linkers are (Gly-Ser)<sub>n</sub> residues with some Glu or Lys residues dispersed throughout to increase solubility.

As used herein, humanized antibodies refer to antibodies that are modified to include human sequences of amino acids so that administration to a human does not  
15 provoke an immune response. Methods for preparation of such antibodies are known. For example, to produce such antibodies, the encoding nucleic acid in the hybridoma or other prokaryotic or eukaryotic cell, such as an E. coli or a CHO cell, that expresses the monoclonal antibody is altered by recombinant nucleic acid techniques to express an antibody in which the amino acid composition of the non-  
20 variable region is based on human antibodies. Computer programs have been designed to identify such non-variable regions.

As used herein, diabodies are dimeric scFV; diabodies typically have shorter peptide linkers than scFVs, and they generally dimerize.

As used herein, production by recombinant means by using recombinant  
25 DNA methods means the use of the well known methods of molecular biology for expressing proteins encoded by cloned DNA.

As used herein the term assessing or determining is intended to include quantitative and qualitative determination in the sense of obtaining an absolute value for the activity of a product, and also of obtaining an index, ratio, percentage, visual

-29-

or other value indicative of the level of the activity. Assessment can be direct or indirect.

As used herein, biological activity refers to the *in vivo* activities of a compound or ~~microorganisms~~<sup>microorganisms</sup> or physiological responses that result upon *in vivo* administration ~~of~~ thereof or of composition or other mixture. Biological activity, thus, encompasses therapeutic effects and pharmaceutical activity of such compounds, compositions and mixtures. Biological activities can be observed in *in vitro* systems designed to test or use such activities.

As used herein, an effective amount of a microorganism or compound for treating a particular disease is an amount that is sufficient to ameliorate, or in some manner reduce the symptoms associated with the disease. Such an amount can be administered as a single dosage or can be administered according to a regimen, whereby it is effective. The amount can cure the disease but, typically, is administered in order to ameliorate the symptoms of the disease. Repeated administration can be required to achieve the desired amelioration of symptoms.

As used herein equivalent, when referring to two sequences of nucleic acids, means that the two sequences in question encode the same sequence of amino acids or equivalent proteins. When equivalent is used in referring to two proteins or peptides or other molecules, it means that the two proteins or peptides have substantially the same amino acid sequence with only amino acid substitutions (such as, but not limited to, conservative changes) or structure and the ~~any~~ changes do not substantially alter the activity or function of the protein or peptide. When equivalent refers to a property, the property does not need to be present to the same extent (e.g., two peptides can exhibit different rates of the same type of enzymatic activity), but the activities are usually substantially the same. Complementary, when referring to two nucleotide sequences, means that the two sequences of nucleotides are capable of hybridizing, typically with less than 25%, 15% or 5% mismatches between opposed nucleotides. If necessary, the percentage of complementarity will be specified. Typically the two molecules are selected such that they will hybridize under conditions of high stringency.

-30-

As used herein, an agent or compound that modulates the activity of a protein or expression of a gene or nucleic acid either decreases or increases or otherwise alters the activity of the protein or, in some manner, up- or down-regulates or otherwise alters expression of the nucleic acid in a cell.

5 As used herein, a method for treating or preventing neoplastic disease means that any of the symptoms, such as the tumor, metastasis thereof, the vascularization of the tumors or other parameters by which the disease is characterized are reduced, ameliorated, prevented, placed in a state of remission, or maintained in a state of remission. It also means that the hallmarks of neoplastic disease and metastasis can  
10 be eliminated, reduced or prevented by the treatment. Non-limiting examples of the hallmarks include uncontrolled degradation of the basement membrane and proximal extracellular matrix, migration, division, and organization of the endothelial cells into new functioning capillaries, and the persistence of such functioning capillaries.

As used herein, a prodrug is a compound that, upon in vivo administration, is  
15 metabolized or otherwise converted to the biologically, pharmaceutically or therapeutically active form of the compound. To produce a prodrug, the pharmaceutically active compound is modified such that the active compound is regenerated by metabolic processes. The prodrug can be designed to alter the metabolic stability or the transport characteristics of a drug, to mask side effects or  
20 toxicity, to improve the flavor of a drug or to alter other characteristics or properties of a drug. By virtue of knowledge of pharmacodynamic processes and drug metabolism in vivo, those of skill in this art, once a pharmaceutically active compound is known, can design prodrugs of the compound (see, e.g., Nogrady (1985) Medicinal Chemistry A Biochemical Approach, Oxford University Press,  
25 New York, pages 388-392).

As used herein, a promoter region or promoter element or regulatory region refers to a segment of DNA or RNA that controls transcription of the DNA or RNA to which it is operatively linked. The promoter region includes specific sequences that are sufficient for RNA polymerase recognition, binding and transcription  
30 initiation. This portion of the promoter region is referred to as the promoter. In

-31-

addition, the promoter region includes sequences that modulate this recognition, binding and transcription initiation activity of RNA polymerase. These sequences can be cis acting or can be responsive to trans acting factors. Promoters, depending upon the nature of the regulation, can be constitutive or regulated. Exemplary  
5 promoters contemplated for use in prokaryotes include the bacteriophage T7 and T3 promoters.

As used herein, a receptor refers to a molecule that has an affinity for a ligand. Receptors can be naturally-occurring or synthetic molecules. Receptors also can be referred to in the art as anti-ligands. As used herein, the receptor and anti-  
10 ligand are interchangeable. Receptors can be used in their unaltered state or bound to other polypeptides, including as homodimers. Receptors can be attached to, covalently or noncovalently, or in physical contact with, a binding member, either directly or indirectly via a specific binding substance or linker. Examples of receptors, include, but are not limited to: antibodies, cell membrane receptors  
15 surface receptors and internalizing receptors, monoclonal antibodies and antisera reactive with specific antigenic determinants (such as on viruses, cells, or other materials), drugs, polynucleotides, nucleic acids, peptides, cofactors, lectins, sugars, polysaccharides, cells, cellular membranes, and organelles.

As used herein, sample refers to anything that can contain an analyte for  
20 which an analyte assay is desired. The sample can be a biological sample, such as a biological fluid or a biological tissue. Examples of biological fluids include urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal fluid, tears, mucus, amniotic fluid or the like. Biological tissues are aggregates of cells, usually of a particular kind together with their intercellular substance that form one of the  
25 structural materials of a human, animal, plant, bacterial, fungal or viral structure, including connective, epithelium, muscle and nerve tissues. Examples of biological tissues also include organs, tumors, lymph nodes, arteries and individual cell(s).

As used herein: stringency of hybridization in determining percentage mismatch is as follows:

30 1) high stringency: 0.1 x SSPE, 0.1% SDS, 65°C

-32-

2) medium stringency: 0.2 x SSPE, 0.1% SDS, 50°C

3) low stringency: 1.0 x SSPE, 0.1% SDS, 50°C

Those of skill in this art know that the washing step selects for stable hybrids and also know the ingredients of SSPE (see, e.g., Sambrook, E.F. Fritsch, T. Maniatis, in: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989), vol. 3, p. B.13, see, also, numerous catalogs that describe commonly used laboratory solutions). SSPE is pH 7.4 phosphate-buffered 0.18 M NaCl. Further, those of skill in the art recognize that the stability of hybrids is determined by  $T_m$ , which is a function of the sodium ion concentration and temperature: ( $T_m = 81.50^\circ\text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G+C) - 600/l$ ), so that the only parameters in the wash conditions critical to hybrid stability are sodium ion concentration in the SSPE (or SSC) and temperature. Any nucleic acid ~~molecules~~ <sup>molecules</sup> provided herein can also include those that hybridize under conditions of at least low stringency, generally moderate or high stringency, along at least 70, 80, 90% of the full length of the disclosed molecule. It is understood that equivalent stringencies can be achieved using alternative buffers, salts and temperatures. By way of example and not limitation, procedures using conditions of low stringency are as follows (see also Shilo and Weinberg, Proc. Natl. Acad. Sci. USA 78:6789-6792 (1981)):

Filters containing DNA are pretreated for 6 hours at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 ~~µg~~ <sup>µg</sup>/ml denatured salmon sperm DNA (10X SSC is 1.5 M sodium chloride, and 0.15 M sodium citrate, adjusted to a pH of 7). Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10<sup>6</sup> cpm <sup>32</sup>P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 40°C, and then washed for 1.5 hours at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 hours at 60°C. Filters are blotted dry and

-33-

exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which can be used are well known in the art (e.g., as employed for cross-species hybridizations).

By way of example and not way of limitation, procedures using conditions of moderate stringency include, for example, but are not limited to, procedures using such conditions of moderate stringency are as follows: Filters containing DNA are pretreated for 6 hours at 55°C in a solution containing 6X SSC, 5X Denhart's solution, 0.5% SDS and 100 <sup>μg</sup>/~~μg~~ ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution and 5-20 X 10<sup>6</sup> cpm <sup>32</sup>P-labeled probe is used. Filters are incubated in hybridization mixture for 18- 20 hours at 55°C, and then washed twice for 30 minutes at 60°C in a solution containing 1X SSC and 0.1% SDS. Filters are blotted dry and exposed for autoradiography. Other conditions of moderate stringency which can be used are well-known in the art. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.1% SDS. By way of example and not way of limitation, procedures using conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 hours to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 <sup>μg</sup>/ml denatured salmon sperm DNA. Filters are hybridized for 48 hours at 65°C in prehybridization mixture containing 100 <sup>μg</sup>/~~μg~~ ml denatured salmon sperm DNA and 5-20 X 10<sup>6</sup> cpm <sup>32</sup>P- of <sup>32</sup>P-labeled probe. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 minutes before autoradiography. Other conditions of high stringency which can be used are well known in the art.

The term substantially identical or homologous or similar varies with the context as understood by those skilled in the relevant art and generally means at least 60% or 70%, preferably means at least 80%, more preferably at least 90%, and most preferably at least 95% , 96%, 97%, 98%, 99% or greater identity.

-34-

As used herein, substantially identical to a product means sufficiently similar so that the property of interest is sufficiently unchanged so that the substantially identical product can be used in place of the product.

As used herein, substantially pure means sufficiently homogeneous to appear  
5 free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of  
10 the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound can, however, be a mixture of stereoisomers or isomers. In such instances, further purification might increase the specific activity of the compound.

As used herein, a molecule, such as an antibody, that specifically binds to a polypeptide typically has a binding affinity ( $K_a$ ) of at least about  $10^6$  l/mol,  $10^7$  l/mol,  $10^8$  l/mol,  $10^9$  l/mol,  $10^{10}$  l/mol or greater and binds to a protein of interest generally with at least 2-fold, 5-fold, generally 10-fold or even 100-fold or greater, affinity than to other proteins. For example, an antibody that specifically binds to  
20 the protease domain compared to the full-length molecule, such as the zymogen form, binds with at least about 2-fold, typically 5-fold or 10-fold higher affinity, to a polypeptide that contains only the protease domain than to the zymogen form of the full-length. Such specific binding also is referred to as selective binding. Thus, specific or selective binding refers to greater binding affinity (generally at least 2-  
25 fold, 5-fold, 10-fold or more) to a targeted site or locus compared to a non-targeted site or locus.

As used herein, the terms a therapeutic agent, therapeutic compound, therapeutic regimen, or chemotherapeutic include conventional drugs and drug therapies, including vaccines, which are known to those skilled in the art.

-35-

As used herein, treatment means any manner in which the symptoms of a condition, disorder or disease are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the microorganisms described and provided herein.

5       As used herein, proliferative disorders include any disorders involving abnormal proliferation of cells. Such disorders include, but are not limited to, neoplastic diseases, psoriasis, restenosis, macular degeneration, diabetic retinopathies, inflammatory <sup>responses</sup> ~~repenses~~ and disorders, including wound healing responses.

10       As used herein, vector (or plasmid) refers to discrete elements that are used to introduce heterologous nucleic acid into cells for either expression or replication thereof. The vectors typically remain episomal, but can be designed to effect integration of a gene or portion thereof into a chromosome of the genome. Also contemplated are vectors that are artificial chromosomes, such as yeast artificial  
15       chromosomes and mammalian artificial chromosomes. Selection and use of such vectors are well known to those of skill in the art. An expression vector includes vectors capable of expressing DNA that is operatively linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Thus, an expression vector refers to a recombinant DNA or RNA  
20       construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

25       As used herein, a combination refers to any association between two or among more items.

As used herein, a composition refers to any mixture. It can be a solution, a suspension, an emulsion, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

-36-

As used herein, fluid refers to any composition that can flow. Fluids thus encompass compositions that are in the form of semi-solids, pastes, solutions, aqueous mixtures, gels, lotions, creams and other such compositions.

As used herein, a kit is a packaged combination optionally including  
5 instructions for use of the combination and/or other reactions and components for such use.

For clarity of disclosure, and not by way of limitation, the detailed description is divided into the subsections that follow.

**B. Microorganisms for Tumor-Specific Therapy**

10 Provided herein are microorganisms, and methods for making and using such microorganisms for therapy of neoplastic disease and other proliferative disorders and inflammatory disorders. The microbe (or microorganism)-mediated treatment methods provided herein involve administration of ~~microorganisms~~<sup>microorganisms</sup> to hosts, accumulation of the microorganism in the targeted cell or tissue, such as in a tumor,  
15 resulting in leaking or lysing of the cells, whereby an immune response against leaked or released antigens is mounted, thereby resulting in an inhibition of the tissues or cells in which the microorganism accumulates.

In addition to the gene therapeutic methods of cancer treatment, live attenuated microorganisms can be used for vaccination, such as in cancer  
20 vaccination or antitumor immunity. Immunization, for example, against a tumor can include a tumor-specific T-cell-mediated response through microbe-delivered antigens or cytokines. To do so, the microbes can be specifically targeted to the tumor tissues, with minimal infection to any other key organs and also can be modified or provided to produce the antigens and/or cytokines.

25 The microorganisms provided herein and the use of such microorganisms herein can accumulate in immunoprivileged cells or ~~immunoprivileged~~<sup>immunoprivileged</sup> tissues, including tumors and/or metastases, and also including wounded tissues and cells. While the microorganisms provided herein can typically be cleared from the subject to whom the microorganisms are administered by activity of the subject's immune  
30 system, microorganisms can nevertheless accumulate, survive and proliferate in

-37-

immunoprivileged cells and tissues such as tumors because such <sup>immunoprivileged</sup> ~~immunoprivileged~~ areas are sequestered from the host's immune system. Accordingly, the methods provided herein, as applied to tumors and/or metastases, and therapeutic methods relating thereto, can readily be applied to other immunoprivileged cells and tissues, including wounded cells and tissues.

#### 1. Characteristics

The microorganisms provided herein and used in the methods herein are attenuated, immunogenic, and replication competent.

##### a. Attenuated

The microbes used in the methods provided herein are typically attenuated. Attenuated microbes have a decreased capacity to cause disease in a host. The decreased capacity can result from any of a variety of different modifications to the ability of a microbe to be pathogenic. For example, a microbe can have reduced toxicity, reduced ability to accumulate in non-tumorous organs or tissue, reduced ability to cause cell lysis or cell death, or reduced ability to replicate compared to the non-attenuated form thereof. The attenuated microbes provided herein, however, retain at least some capacity to replicate and to cause immunoprivileged cells and tissues, such as tumor cells to leak or lyse, undergo cell death, or otherwise cause or enhance an immune response to immunoprivileged cells and tissues, such as tumor cells.

##### i. Reduced toxicity

Microbes can be toxic to their hosts by manufacturing one or more compounds that worsen the health condition of the host. Toxicity to the host can be manifested in any of a variety of manners, including septic shock, neurological effects, or muscular effects. The microbes provided herein can have a reduced toxicity to the host. The reduced toxicity of a microbe of the present methods and compositions can range from a toxicity in which the host experiences no toxic effects, to a toxicity in which the host does not typically die from the toxic <sup>effects</sup> ~~affects~~ of the microbes. In some embodiments, the microbes are of a reduced toxicity such that a host typically has no significant long-term effect from the presence of the

-38-

- microbes in the host, beyond any affect on tumorous, metastatic or necrotic organs or tissues. For example, the reduced toxicity can be a minor fever or minor infection, which lasts for less than about a month, and following the fever or infection, the host experiences no adverse <sup>effects</sup> ~~affects~~ resultant from the fever or
- 5 infection. In another example, the reduced toxicity can be measured as an unintentional decline in body weight of about 5% or less for the host after administration of the microbes. In other examples, the microbe has no toxicity to the host.
- <sup>Exemplary</sup> ~~Exemplary~~ vaccinia viruses of the LIVP strain (a widely available attenuated
- 10 Lister strain) that have reduced toxicity compared to other vaccinia viruses employed and are further modified. Modified LIVP were prepared. These LIVP include insertions in the TK and HA genes and optionally in the locus designed F3. As an example of reduced toxicity, these recombinant vaccinia viruses were tested for their toxicity to mice with impaired immune systems (nude mice) relative to the
- 15 corresponding wild type vaccinia virus. <sup>Intravenous</sup> ~~Intervenous~~ (i.v.) injection of wild type vaccinia virus VGL (strain LIVP) at  $1 \times 10^7$  PFU/mouse causes toxicity in nude mice: three mice out of seven lost the weight and died (one mouse died in one week after virus injection, one mouse died ten days after virus injection). <sup>Recombinant</sup> ~~Recombinant~~
- 20 vaccinia virus designated RVGL8 (LacZ inserted into F3 locus) did not show toxic effects in nude mice after i.v. injection of  $1 \times 10^7$  PFU/mouse. There were no readily detectable signs of RVGL8 virus-related toxicity.  $\phi$  Therefore, insertion into NotI site (located in F3 gene) of vaccinia virus genome strain LIVP reduces toxicity of the vaccinia virus to the host. Similar modifications can be made to other pox viruses and other viruses to reduce toxicity thereof. Such modifications can be
- 25 empirically identified, if necessary.

**ii. Accumulate in immunoprivileged cells and tissues, such as tumor, not substantially in other organs**

- Microbes can accumulate in any of a variety of tissues and organs of the host. Accumulation can be evenly distributed over the entire host organism, or can
- 30 be concentrated in one or a few organs or tissues. The microbes provided herein can accumulate in targeted tissues, such as immunoprivileged cells and tissues, such as

-39-

tumors and also metastases. In some embodiments, the microbes provided herein exhibit accumulation in immunoprivileged cells and tissues, such as tumor cells relative to normal organs or tissues that is equal to or greater than the accumulation that occurs with wild type microbes. In other embodiments the microbes provided  
5 herein exhibit accumulation in immunoprivileged cells and tissues, such as tumor cells that is equal to or greater than the accumulation in any other particular organ or tissue. For example, the microbes provided herein can demonstrate an accumulation in immunoprivileged cells and tissues, such as tumor cells that is at least about 2-fold greater, at least about 5-fold greater, at least about 10-fold greater, at least about  
10 100-fold greater, at least about 1,000-fold greater, at least about 10,000-fold greater, at least about 100,000-fold greater, or at least about 1,000,000-fold greater, than the accumulation in any other particular organ or tissue.

In some embodiments, a microbe can accumulate in targeted tissues and cells, such as immunoprivileged cells and tissues, such as tumor cells, without  
15 accumulating in one or more selected tissues or organs. For example, a microbe can accumulate in tumor cells without accumulating in the brain. In another example, a microbe can accumulate in tumor cells without accumulating in neural cells. In another example, a microbe can accumulate in tumor cells without accumulating in ovaries. In another example, a microbe can accumulate in tumor cells without  
20 accumulating in the blood. In another example, a microbe can accumulate in tumor cells without accumulating in the heart. In another example, a microbe can accumulate in tumor cells without accumulating in the bladder. In another example, a microbe can accumulate in tumor cells without accumulating in testes. In another example, a microbe can accumulate in tumor cells without accumulating in the  
25 spleen. In another example, a microbe can accumulate in tumor cells without accumulating in the lungs.

One skilled in the art can determine the desired capability for the microbes to selectively accumulate in targeted tissue or cells, such as in a immunoprivileged cells and tissues, such as tumor rather than non-target organs or tissues, according to  
30 a variety of factors known in the art, including, but not limited to, toxicity of the

-40-

microbes, dosage, tumor to be treated, immunocompetence of host, and disease state of the host.

Provided herein as an example of selective accumulation in immunoprivileged cells and tissues, such as tumors relative to normal organs or tissues, presence of various vaccinia viruses was assayed in tumor samples and different organs. Wild type VGL virus was recovered from tumor, testes, bladder, and liver and as well as from brain. Recombinant virus RVGL8 was found mostly in tumors (in mouse #24, virus was found in testes, bladder and liver; in mouse #22 in testes), and no virus was recovered from brain tissue in six tested animals. Therefore, this finding demonstrates the tumor accumulation properties of a recombinant vaccinia virus of the LVP strain with an insertion in the F3 gene for tumor therapy purposes.

**iii. Ability to Elicit or Enhance Immune Response to Tumor Cells**

The microorganisms herein cause or enhance an immune response to antigens in the targeted tissues or cells, such as immunoprivileged cells and tissues, such as tumor cells. The immune response can be triggered by any of a variety of mechanisms, including the presence of ~~immunostimulatory~~ <sup>immunostimulatory</sup> cytokines and the release antigenic compounds that can cause an immune response.

Cells, in response to an infection such as a microorganismal infection, can send out signals to stimulate an immune response against the cells. Exemplary signals sent from such cells include antigens, cytokines and chemokines such as interferon-gamma and interleukin-15. The microorganism provided herein can cause targeted cells to send out such signals in response to infection by the microbes, resulting in a stimulation of the host's immune system against the targeted cells or tissues, such as tumor cells.

In another embodiment, targeted cells or tissues, such as tumor cells, can contain one or more compounds that can be recognized by the host's immune system in mounting an immune response against a tumor. Such antigenic compounds can be compounds on the cell surface or the tumor cell, and can be protein, carbohydrate, lipid, nucleic acid, or combinations thereof. Microbe-mediated

-41-

release of antigenic compounds can result in triggering the host's immune system to mount an immune response against the tumor. The amount of antigenic compound released by the tumor cells is any amount sufficient to trigger an immune response in a subject ; for example, the antigenic compounds released from one or more  
5 tumor cells can trigger a host immune response<sup>actions</sup> in the organism that is known to be accessible to leukocytes.

The time duration of antigen release is an amount of time sufficient for the host to establish an immune response to one or more tumor antigens. In some embodiments, the <sup>duration</sup> ~~duration~~ is an amount of time sufficient for the host to establish a  
10 sustained <sup>immune</sup> ~~immune~~ response to one or more tumor antigens. One skilled in the art can determine such a time duration based on a variety of factors affecting the time duration for a subject to develop an immune response, including the level of the tumor antigen in the subject, the number of different tumor antigens, the antigenicity of the antigen, the immunocompetence of the host, and the access of the antigenic  
15 material to the vasculature of the host. Typically, the duration of antigen release can be at least about a week, at least about 10 days, at least about two weeks, or at least about a month.

The microorganism provided herein can have any of a variety of properties that can cause target cells and tissues, such as tumor cells, to release antigenic  
20 compounds. Exemplary properties are the ability to lyse cells and the ability to elicit apoptosis in tumor cells. Microbes that are unable to lyse tumor cells or cause tumor cell death can nevertheless be used in the methods provided herein when such microbes can cause some release or display of antigenic compounds from tumor cells. A variety of mechanisms for antigen release or display without lysis or cell  
25 death are known in the art, and any such mechanism can be used by the microbes provided herein, including, but not limited to, secretion of antigenic compounds, enhanced cell membrane permeability, or altered cell surface expression or altered MHC presentation in tumor cells when the tumor cells can be accessed by the host's immune system. Regardless of the mechanism by which the host's immune system  
30 is activated, the net result of the presence of the microbes in the tumor is a

-42-

stimulation of the host's immune system, at least in part, against the tumor cells. In one example, the microbes can cause an immune response against tumor cells not infected by the microbes.

In one embodiment, the microbes provided herein can cause tumor cells to release an antigen that is not present on the tumor cell surface. Tumor cells can produce compounds such as proteins that can cause an immune response; however, in circumstances in which the antigenic compound is not on the tumor cell surface, the tumor can proliferate, and even metastasize, without the antigenic compound causing an immune response. Within the scope of the present methods, the microbes provided herein can cause antigenic compounds within the cell to release away from the cell and away from the tumor, which can result in triggering an immune response to such an antigen. Even if not all cells of a tumor are releasing antigens, the immune response can initially be targeted toward the "leaky" tumor cells, and the bystander effect of the immune response can result in further tumor cell death around the "leaky" tumor cells.

#### iv. Balance of Pathogenicity and Release of Tumor Antigens

Typical methods of involving treatment of targeted cells and tissues, such as immunoprivileged cells and tissues, such as tumors, are designed to cause rapid and complete removal thereof. For example, many viruses, bacterial or eukaryotic cells can cause lysis and/or apoptosis in a variety of cells, including tumor cells. Microorganisms that can vigorously lyse or cause cell death can be highly pathogenic, and can even kill the host. Furthermore, therapeutic methods based upon such rapid and complete lysis are typically therapeutically ineffective.

In contrast, the microorganisms provided herein are not aggressive in causing cell death or lysis. They can have only a limited or no ability to cause cell death as long as they accumulate in the target cells or tissues and result in alteration of cell membranes to cause leakage of antigens <sup>against</sup> ~~again~~ which an immune response is mounted. It is desirable that their apoptotic or lytic effect is sufficiently slow or ineffective to permit sufficient antigenic leakage for a sufficient time for the host to mount an effective immune response against the target tissues. Such immune

-43-

response <sup>alone</sup> ~~alone~~ or in combination with the lytic/apoptotic effect of the microorganism results in elimination of the target tissue and also elimination of future development, such as metastases and reoccurrence, of such tissues or cells. ~~Ø~~

While the microbes provided herein can have a limited ability to cause cell death, the microbes provided herein can nevertheless <sup>stimulate</sup> ~~stimulate~~ the host's immune system to attack tumor cells. As a result, such microorganisms also are typically unlikely to have substantial toxicity to the host.

In one embodiment, the microbes have a limited, or no, ability to cause tumor cell death, while still causing or enhancing an immune response against tumor cells. In one example, the rate of <sup>microorganism</sup> ~~microorganism~~-mediated tumor cell death is less than the rate of tumor cell growth or replication. In another example, the rate of microorganism-mediated tumor cell death is slow enough for the host to establish a sustained immune response to one or more tumor antigens. Typically, the time for of cell death is sufficient to establish an anti-tumor immune response and can be at least about a week, at least about 10 days, at least about two weeks, or at least about a month, depending upon the host and the targeted cells or tissues.

In another embodiment, the microbes provided herein can cause cell death in tumor cells, without causing substantial cell death in non-tumor tissues. In such an embodiment, the microbes can aggressively kill tumor cells, as long as no substantial cell death occurs in non-tumor cells, and optionally, so long as the host has sufficient capability to mount an immune response against the tumor cells.

In one embodiment, the ability of the microbes to cause cell death is slower than the host's immune response against the microbes. The ability for the host to control infection by the microbes can be determined by the immune response (e.g., antibody titer) against microorganismal antigens. Typically, after the host has mounted immune response against the microbes, the microbes can have reduced pathogenicity in the host. Thus, when the ability of the microbes to cause cell death is slower than the host's immune response against the microbes, microbe-mediated cell death can occur without risk of serious disease or death to the host. In one

-44-

example, the ability of the microbes to cause tumor cell death is slower than the host's immune response against the microbes.

**b. Immunogenicity**

5 The microorganisms provided herein also can be immunogenic. An immunogenic microorganism can create a host immune response against the microorganism. In one embodiment, the microorganisms can be sufficiently immunogenic to result in a large anti-(microorganism) antibody titer. The microorganisms provided herein can have the ability to elicit an immune response. The immune response can be activated in response to viral antigens or can be  
10 activated as a result of microorganismal-infection induced cytokine or chemokine production. Immune response against the microorganism can decrease the likelihood of pathogenicity toward the host organism.

Immune response against the microorganism also can result in target tissue or cell, such as tumor cell, killing. In one embodiment, the immune response  
15 against microorganismal infection can result in an immune response against tumor cells, including developing antibodies against tumor antigens. In one example, an immune response mounted against the microorganism can result in tumor cell killing by the "bystander effect," where uninfected tumor cells nearby infected tumor cells are killed at the same time as infected cells, or alternatively, where uninfected tumor  
20 cells nearby extracellular microorganisms are killed at the same time as the microorganisms. As a result of bystander effect tumor cell death, tumor cell antigens can be released from cells, and the host organism's immune system can mount an immune response against tumor cell antigens, resulting in an immune response against the tumor itself.

25 In one embodiment, the microorganism can be selected or modified to express one or more antigenic compounds, including superantigenic compounds. The antigenic compounds such as superantigens can be endogenous gene products or can be exogenous gene products. Superantigens, including toxoids, are known in the art and described elsewhere herein.

30

-45-

**c. Replication Competent**

The microorganisms provided herein can be replication competent. In a variety of viral or bacterial systems, the administered microorganism is rendered replication incompetent to limit pathogenicity risk to the host. While replication incompetence can protect the host from the microorganism, <sup>it also</sup> ~~that also~~ limits the ability of the microorganism to infect and kill tumor cells, and typically results in only a short-lived <sup>affect</sup> ~~affect~~. In contrast, the microorganisms provided herein can be attenuated but replication competent, resulting in low toxicity to the host and accumulation mainly or solely in tumors. Thus, the microorganisms provided herein can be replication competent without creating a pathogenicity risk to the host.

Attenuation of the microorganisms provided herein can include, but is not limited to, reducing the replication competence of the microorganism. For example, a microorganism can be modified to decrease or eliminate an activity related to replication, such as a transcriptional activator that regulates replication in the microorganism. In an example, a microorganism, such as a virus, can have the viral thymidine kinase gene modified.

**d. Genetic Variants**

The microorganisms provided herein can be modified from their wild type form. Modifications can include any of a variety of changes, and typically include changes to the genome or nucleic acid molecules of the microorganisms. Exemplary nucleic acid molecular modifications include truncations, insertions, deletions and mutations. In an exemplary modification, a microorganismal gene can be modified by truncation, insertion, deletion or mutation. In an exemplary insertion, an exogenous gene can be inserted into the genome of the microorganism.

**i. <sup>Modified</sup> Modified Characteristics**

Modifications of the microorganisms provided herein can result in a modification of microorganismal characteristics, including those provided herein such as pathogenicity, toxicity, ability to preferentially accumulate in tumor, ability to lyse cells or cause cell death, ability to elicit an immune response against tumor cells, immunogenicity, replication competence. Variants can be obtained by general

-46-

methods such as mutagenesis and passage in cell or tissue culture and selection of desired properties, as is known in the art, as exemplified for respiratory syncytial virus in Murphy *et al.*, Virus Res. 1994, 32:13-26.

5 Variants also can be obtained by mutagenic methods in which nucleic acid residues of the microorganism are added, removed or modified relative to the wild type. Any of a variety of known mutagenic methods can be used, including recombination-based methods, restriction endonuclease-based methods, and PCR-based methods. Mutagenic methods can be directed against particular nucleotide sequences such as genes, or can be random, where selection methods based on  
10 desired characteristics can be used to select mutated microorganisms. Any of a variety of microorganismal modifications can be made, according to the selected microorganism and the particular known modifications of the selected microorganism.

## ii. Exogenous Gene Expression

15 The microorganisms provided herein also can have the ability to express one or more exogenous genes. Gene expression can include expression of a protein encoded by a gene and/or expression of an RNA molecule encoded by a gene. In some embodiments, the microorganisms can express exogenous genes at levels high enough that permit harvesting products of the exogenous genes from the tumor.  
20 Expression of endogenous genes can be controlled by a constitutive <sup>promoter</sup> promoter, or by an inducible <sup>promoter</sup> promoter. Expression can also be influenced by one or more proteins or RNA molecules expressed by the microorganism. An exemplary inducible <sup>promoter</sup> promoter system can include a chimeric transcription factor containing a progesterone receptor fused to the yeast GAL4 DNA-binding domain and to the  
25 activation domain of the herpes simplex virus protein VP16, and a synthetic promoter containing a series of GAL4 recognition sequences upstream of the adenovirus major late E1B TATA box, linked to one or more exogenous genes; in this exemplary system, administration of RU486 to a subject can result in induction of the exogenous genes. Exogenous genes expressed can include genes encoding a  
30 therapeutic gene product, genes encoding a detectable gene product such as a gene

-47-

product that can be used for imaging, genes encoding a gene product to be harvested, genes encoding an antigen of an antibody to be harvested. The microorganisms provided herein can be used for expressing genes in vivo and in vitro. Exemplary proteins include reporter proteins (E. coli  $\beta$ -galactosidase,  $\beta$ -glucuronidase, xanthineguanine phosphoribosyltransferase), proteins facilitating detection, *i.e.*, a detectable protein or a protein capable of inducing a detectable signal, (*e.g.*, luciferase, green and red fluorescent proteins, transferrin receptor), proteins useful for tumor therapy (pseudomonas A endotoxin, diphtheria toxin, p53, Arf, Bax, tumor necrosis factor-<sup>alpha</sup>, HSV TK, E. coli purine nucleoside phosphorylase, angiostatin, endostatin, different cytokines) and many other proteins.

### iii. Detectable gene product

The microorganisms provided herein can express one or more genes whose products are detectable or whose products can provide a detectable signal. A variety of detectable gene products, such as detectable proteins are known in the art, and can be used with the microorganisms provided herein. Detectable proteins include receptors or other proteins that can specifically bind a detectable compound, proteins that can emit a detectable signal such as a fluorescence signal, enzymes that can catalyze a detectable reaction or catalyze formation of a detectable product.

In some embodiments, the microorganism expresses a gene encoding a protein that can emit a detectable signal or <sup>that</sup> ~~the~~ can catalyze a detectable reaction. A variety of DNA sequences encoding proteins that can emit a detectable signal or <sup>that</sup> ~~the~~ can catalyze a detectable reaction, such as luminescent or fluorescent proteins, are known and can be used in the microorganisms and methods provided herein.

Exemplary genes encoding light-emitting proteins include genes from bacterial luciferase from *Vibrio harveyi* (Belas *et al.*, Science 218 (1982), 791-793), bacterial luciferase from *Vibrio fischerii* (Foran and Brown, Nucleic acids Res. 16 (1988), 177), firefly luciferase (de Wet *et al.*, Mol. Cell. Biol. 7(1987), 725-737), aequorin from *Aequorea victoria* (Prasher *et al.*, Biochem. 26 (1987), 1326-1332), Renilla luciferase from *Renilla reniformis* (Lorenz *et al.*, PNAS USA 88 (1991), 4438-4442) and green fluorescent protein from *Aequorea victoria* (Prasher *et al.*, Gene 111

-48-

(1987), 229-233). Transformation and expression of these genes in microorganisms can permit detection of microorganismal colonies, for example, using a low light imaging camera. Fusion of the lux A and lux B genes can result in a fully functional luciferase protein (Escher *et al.*, PNAS 86 (1989), 6528-6532). This fusion gene (Fab2) has introduced into a variety of microorganisms followed by microorganismal infection and imaging based on luciferase expression. In some embodiments, luciferases expressed in bacteria can require exogenously added substrates such as decanal or coelenterazine for light emission. In other embodiments, microorganisms can express a complete lux operon, which can include proteins that can provide luciferase substrates such as decanal. For example, bacteria containing the complete lux operon sequence, when injected intraperitoneally, intramuscularly, or intravenously, allowed the visualization and localization of bacteria in live mice indicating that the luciferase light emission can penetrate the tissues and can be detected externally (Contag *et al.*, Mol. Microbiol. 18 (1995), 593-603).

In other embodiments, the microorganism can express a gene that can bind a detectable compound or that can form a product that can bind a detectable compound. A variety of gene products, such as proteins, that can specifically bind a detectable compound are known in the art, including receptors, metal binding proteins, ligand binding proteins, and antibodies. Any of a variety of detectable compounds can be used, and can be imaged by any of a variety of known imaging methods. Exemplary compounds include receptor ligands and antigens for antibodies. The ligand can be labeled according to the imaging method to be used. Exemplary imaging methods include any of a variety of magnetic resonance methods such as magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS), and also include any of a variety of tomographic methods including computed tomography (CT), computed axial tomography (CAT), electron beam computed tomography (EBCT), high resolution computed tomography (HRCT), hypocycloidal tomography, positron emission tomography (PET), single-photon

-49-

emission computed tomography (SPECT), spiral computed tomography and ultrasonic tomography.

Labels appropriate for magnetic resonance imaging are known in the art, and include, for example, gadolinium chelates and iron oxides. Use of chelates in contrast agents is known in the art. Labels appropriate for tomographic imaging methods are known in the art, and include, for example,  $\beta$ -emitters such as  $^{11}\text{C}$ ,  $^{13}\text{N}$ ,  $^{15}\text{O}$  or  $^{64}\text{Cu}$  or (b)  $\gamma$ -emitters such as  $^{123}\text{I}$ . Other exemplary radionuclides that can, be used, for example, as tracers for PET include  $^{55}\text{Co}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{60}\text{Cu(II)}$ ,  $^{67}\text{Cu(II)}$ ,  $^{57}\text{Ni}$ ,  $^{52}\text{Fe}$  and  $^{18}\text{F}$ . Examples of useful radionuclide-labeled agents are  $^{64}\text{Cu}$ -labeled engineered antibody fragment (Wu *et al.*, PNAS USA 97 (2002), 8495-8500),  $^{64}\text{Cu}$ -labeled somatostatin (Lewis *et al.*, J. Med. Chem. 42(1999), 1341-1347),  $^{64}\text{Cu}$ -pyruvaldehyde-bis (N4methylthiosemicarbazone)( $^{64}\text{Cu}$ -PTSM) (Adonai *et al.*, PNAS USA 99 (2002), 3030-3035),  $^{52}\text{Fe}$ -citrate (Leenders *et al.*, J. Neural. Transm. Suppl. 43 (1994), 123-132),  $^{52}\text{Fe}/^{52\text{m}}\text{Mn}$ -citrate (Calonder *et al.*, J. Neurochem. 73 (1999), 2047-2055) and  $^{52}\text{Fe}$ -labeled iron (III) hydroxide-sucrose complex (Beshara *et al.*, Br. J. Haematol. 104 (1999), 288-295, 296-302).

#### iv. Therapeutic gene product

The microorganisms provided herein can express one or more genes whose products cause cell death or whose products cause an anti-tumor immune response, such genes can be considered therapeutic genes. A variety of therapeutic gene products, such as toxic or apoptotic proteins, or siRNA, are known in the art, and can be used with the microorganisms provided herein. The therapeutic genes can act by directly killing the host cell, for example, as a channel-forming or other lytic protein, or by triggering apoptosis, or by inhibiting essential cellular processes, or by triggering an immune response against the cell, or by interacting with a compound that has a similar effect, for example, by converting an less active compound to a cytotoxic compound.

In some embodiments, the microorganism can express a therapeutic protein. A large number of therapeutic proteins that can be expressed for tumor treatment are known in the art, including, but not limited to tumor suppressors, toxins, cytostatic

-50-

proteins, and cytokines. An exemplary, non-limiting list of such proteins includes WT1, p53, p16, Rb, BRCA1, cystic fibrosis transmembrane regulator (CFTR), Factor VIII, low density lipoprotein receptor, beta-galactosidase, alpha-galactosidase, beta-glucocerebrosidase, insulin, parathyroid hormone, alpha-1-antitrypsin, rsCD40L, Fas-ligand, TRAIL, TNF, antibodies, microcin E492, diphtheria toxin, Pseudomonas <sup>exotoxin</sup> ~~exotoxin~~, <sup>Escherichia</sup> ~~Escherichia~~ coli Shig toxin, Escherichia coli Verotoxin 1, and hyperforin.

In other embodiments, the microorganism can express a protein that converts a less active compound into a compound that causes tumor cell death. Exemplary methods of conversion of such a prodrug compound include enzymatic conversion and photolytic conversion. A large variety of protein/compound pairs are known in the art, and include, but are not limited to Herpes simplex virus thymidine kinase/gancyclovir, varicella zoster thymidine kinase/gancyclovir, cytosine deaminase/5-fluorouracil, purine nucleoside phosphorylase/6-methylpurine deoxyriboside, beta lactamase/cephalosporin-doxorubicin, carboxypeptidase G2/4-[(2-chloroethyl)(2-mesuloxylethyl)amino]benzoyl-L-glutamic acid, cytochrome P450/acetaminophen, horseradish peroxidase/indole-3-acetic acid, nitroreductase/CB1954, rabbit carboxylesterase/7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, mushroom tyrosinase/bis-(2-chloroethyl)amino-4-hydroxyphenylaminomethanone 28, beta galactosidase/1-chloromethyl-5-hydroxy-1,2-dihydro-3H-benz[e]indole, beta glucuronidase/epirubicin-glucuronide, thymidine phosphorylase/5'-deoxy-5-fluorouridine, deoxycytidine kinase/cytosine arabinoside, and linamerase/linamarin.

In another embodiment, the therapeutic gene product can be an siRNA molecule. The siRNA molecule can be directed against expression of a tumor-promoting gene, such as, but not limited to, an oncogene, growth factor, angiogenesis promoting gene, or a receptor. The siRNA molecule also can be directed against expression of any gene essential for cell growth, cell replication or cell survival. The siRNA molecule also can be directed against expression of any gene that stabilizes the cell membrane or otherwise limits the number of tumor cell

-51-

antigens released from the tumor cell. Design of an siRNA can be readily determined according to the selected target of the siRNA; methods of siRNA design and downregulation of genes are known in the art, as exemplified in U.S. Pat. Pub. No. 20030198627.

5 In one embodiment, the therapeutic compound can be controlled by a regulatory sequence. Suitable regulatory sequences which, for example, are functional in a mammalian host cell are well known in the art. In one example, the regulatory sequence can contain a natural or synthetic vaccinia virus promoter. In another embodiment, the regulatory sequence contains a poxvirus promoter. When  
10 viral microorganisms are used, strong late promoters ~~are~~ can be used to achieve high levels of expression of the foreign genes. Early and intermediate-stage promoters, however, can also be used. In one embodiment, the promoters contain early and late promoter elements, for example, the vaccinia virus early/late promoter p7.5, vaccinia late promoter p11, a synthetic early/late vaccinia pE/L promoter (Patel *et al.*, (1988), Proc. Natl. Acad. Sci. USA 85, 9431-9435; Davison and Moss, (1989), J  
15 Mol Biol 210, 749-769; Davison *et al.*, (1990), Nucleic Acids Res. 18, 4285-4286; Chakrabarti *et al.*, (1997), BioTechniques 23, 1094-1097).

#### v. Expressing a superantigen

The microorganisms provided herein can be modified to express one or more  
20 superantigens. Superantigens are antigens that can activate a large immune response, often brought about by a large response of T cells. A variety of superantigens are known in the art including, but not limited to, <sup>diphtheria</sup> ~~diphtheria~~ toxin, staphylococcal enterotoxins (SEA, SEB, SEC1, SEC2, SED, SEE and SEH), Toxic Shock Syndrome Toxin 1, Exfoliating Toxins (EXf), Streptococcal Pyrogenic  
25 Exotoxin A, B and C (SPE A, B and C), Mouse Mammary Tumor Virus proteins (MMTV), Streptococcal M proteins, Clostridial Perfringens Enterotoxin (CPET), mycoplasma arthritis superantigens.

Since many superantigens also are toxins, if expression of a microorganism of reduced toxicity is desired, the superantigen can be modified to retain at least  
30 some of its superantigenicity while reducing its toxicity, resulting in a compound

-52-

such as a toxoid. A variety of recombinant superantigens and toxoids of superantigens are known in the art, and can readily be expressed in the microorganisms provided herein. Exemplary toxoids include toxoids of ~~diphtheria~~ <sup>diphtheria</sup> toxin, as exemplified in U.S. Pat. No. 6,455,673 and toxoids of Staphylococcal enterotoxins, as exemplified in U.S. Pat. Pub. No. 20030009015.

**vi. Expressing a gene product to be harvested**

Exemplary genes expressible by a microorganism for the purpose of harvesting include human genes. An exemplary list of genes includes the list of human genes and genetic disorders authored and edited by Dr. Victor A. McKusick and his colleagues at Johns Hopkins University and elsewhere, and developed for the World Wide Web by NCBI, the National Center for Biotechnology Information. Online Mendelian Inheritance in Man, OMIM™. Center for Medical Genetics, Johns Hopkins University (Baltimore, Md.) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, Md.), 1999. and those available in public databases, such as pubmed and genbank (see, e.g., (ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) These genes include, but are not limited to: 239f2h9, 3pk, 4ebp1, 4ebp2, al1, al2m1, al2m2, al2m3, al2m4, al5, alb, albg, alst, a2m, a2mr, a2mrap, aa, aaa, aab, aabt, aac1, aac2, aact, aadac, aanat, aars, aas, aat, aavs1, abc1, abc2, abc3, abc7, abc8, abcr, abil, abli1, abli2, abli1, abo, abp, abp1, abpa, abpx, abr, acaa, acac, acaca, acacb, acad1, acadm, acads, acadsb, acadv1, acat, acat1, acat2, acc, accb, accn1, accn2, accpn, ace1, ach, ache, achm1, achm2, achrb, achrd, achrg, acs, acly, aco1, aco2, acox, acox1, acox2, acox3, acp1, acp2, acp5, acpp, acr, acrv1, acs3, acs3, acs4, act2, act35, acta1, acta2, acta3, actb, actc, actg1, actg2, actl1, actn1, actn2, actn3, actsa, acug, acvr1, acvr2b, acvrl1, acvrlk1, acvrlk2, acvrlk3, acyl1, ad1, ad2, ad3, ad4, ad5, ada, adam10, adam11, adam12, adam3, adam3a, adam3b, adam8, adar, adarb1, adarb2, adcp1, adcp2, adcy1, adcy2, adcy3, adcy3, adcy4, adcy5, adcy6, adcy7, adcy8, adcy9, adcyap1, adcyap1r1, add1, add2, add3, add1, adfn, adh1, adh2, adh3, adh4, adh5, adh7, adhps, adhcl@, adhr, adhr, adk, ad1, adm, admlx, adora1, adora2a, adora2b, adora21, adora21, adora3, adprt, adrala, adralb, adralc, adrald, adra2a, adra2b,

-53-

adra2c, adra211, adra212, adra2r, adrb1, adrb1r, adrb2, adrb2r11, adrb3, adrbk1,  
 adrbk2, adsl, adss, adtb1, adx, adxr, ae1, ae2, ae3, aegl1, aemk, aes, afl0, afl7, af4,  
 af6, af8t, af9, afd1, afdn, afg3, afg311, afm, afp, afx1, aga, agcl, ager, agl, agmx1,  
 agmx2, agp1, agp7, agps, agrn, agrp, agrt, ags, agt, agt1, agtr1, agtr1a, agtr2, agtr11,  
 5 agxt, ahc, ahcy, ahd, ahds, ahnak, aho2, ahr, ahsg, ahx, aib1, aic, aic1, aied, aih1,  
 aih2, aih3, aim1, air, airc, aire, ak1, ak2, ak3, akap149, akt1, akt2, aku, alad, alas1,  
 alas2, alb, alb2, alba, alcam, ald, aldh1, aldh10, aldh2, aldh3, aldh4, aldh5, aldh6,  
 aldh9, ald11, aldoa, aldob, aldod, aldr1, alds, alk, alk1, alk2, alk3, alk6, alms1,  
 alox12, alox15, alox5, alp, alpi, alp1, alpp, alpp12, alr, alr, als1, als2, als4, als5, alss,  
 10 ambn, ambp, amcd1, amcd2b, amcn, amcn1, amcx1, amd1, amdm, amelx, amely,  
 amfr, amg, amg1, amgx, amh, amhr, amhr2, aml1, aml1t1, aml2, aml3, amog,  
 ampd1, ampd2, ampd3, amph, amph1, ampk, amt, amy1a, amy1b, amy1c, amy2a,  
 amy2b, an2, anc, ancr, ang, angl, anh1, ank1, ank2, ank3, anop1, anova, anp, anpep,  
 anpra, anprb, anprc, ans, ant1, ant2, ant3, ant3y, anx1, anx11, anx13, anx2, anx214,  
 15 anx3, anx4, anx5, anx6, anx7, anx8, aoah, aoc2, aox1, ap2tf, apah1, apba1, apba2,  
 apbb1, apbb2, apc, apcs, ape, apeced, apch, apex, api1, api2, api3, apj, aplp, aplp1,  
 aplp2, apnh, apo31, apoal, apoa2, apoa4, apob, apobec1, apoc1, apoc2, apoc3,  
 apoc4, apod, apoe, apoer2, apoh, apolmt, apolp1@, apolp2@, app, appbp1, appl1,  
 aprf, aprt, aps, apt1, apt1lg1, apx1, apy, aqdq, aqp0, aqp1, aqp2, aqp21, aqp3, aqp4,  
 20 aqp5, aqp6, aqp7, ar, arl, ara, araf1, araf2, arcn1, ard1, ard1, areg, arf1, arf2, arf3,  
 arf41, arf5, arg, arg1, args, arh12, arh6, arh9, arha, arhb, arhc, arhg, arhgap2,  
 arhgap3, arhgap6, arhgdia, arhgdib, arhh, arix, arl2, armd1, arnt, arnt1, aro, arp,  
 arp1, arpkd, arr3, arrb1, arrb2, arsa, arsacs, arsb, arsc1, arsc2, arsd, arse, arsf, art,  
 art1, art3, art4, arts, arvd1, arvd2, arvd3, arvd4, as, asat, asb, ascl1, ascl2, asclt1,  
 25 asd1, asd2, asgr1, asgr2, ash1, asip, asl, asln, asm1, asma, asmd, asmt, asmtlx,  
 asnty, asnrs, asns, aspa, ass, astm1, astn, asv, at, at1, at2r1, at3, ata, atbfl, atcay,  
 atfl, ath1, aths, atm, atoh1, atox1, atpla1, atpla2, atpla3, atpla11, atp1b1, atp1b2,  
 atp1b3, atp1b11, atp1g1, atp2a1, atp2a2, atp2a3, atp2b, atp2b1, atp2b2, atp2b2,  
 atp2b3, atp2b4, atp4a, atp4b, atp5, atp5a, atp5b, atp5g1, atp5g2, atp5g3, atp5o,  
 30 atp6a, atp6b1, atp6c, atp6e, atp6n1, atp7a, atp7b, atpm, atpsb, atpsk1, atpsk2, atq1,

atr, atr, atr1, atr1, atr2, atrc1, atrc2, atrx, ats, atsv, atx1, atx2, au, aufl, aufla, aut,  
avcd, aved, avp, avpr1a, avpr1b, avpr2, avpr3, avrp, avsd, awa1, ax1, ax1lg, axsf,  
azfl, azf2, azgp1, azu1, b120, b144, blg1, b29, b2m, b2mr, b3galt4, b4galt1, ba2r,  
bab1, bag1, bai1, bai2, bai3, bak1, bam22, bap1, bap135, bapx1, bard1, bark2, bas,  
5 bat1, bat2, bat3, bat4, bat5, bax, bb1, bbbg1, bbbg2, bbs1, bbs2, bbs3, bbs4, bbs5,  
bcas1, bcat1, bcat2, bcate2, bcd1, bcei, bche, bckdha, bckdha, bcl1, bcl10, bcl2,  
bcl2a1, bcl212, bcl3, bcl5, bcl6, bcl7, bcl7a, bcl8, bcl9, bclw, bcm, bcm1, bcma,  
bcns, bcns, bcp, bcpm, bcpr, bcr, bcr12, bcr13, bcr14, bcs1, bct1, bct2, bdb, bdb1,  
bdc, bde, bdkrb1, bdkrb2, bdmf, bdmr, bdnf, bed, bedp, bek, bene, bevi, bf, bfl, bf2,  
10 bfhd, bfic, bfls, bfnc2, bfp, bfsp1, bft, bglap, bgmr, bgn, bgp, bhd, bhpcdh, bhr1,  
bicd1, bid, bigh3, bin1, bir, bjs, bkma1, blast1, blau, blk, blm, blmh, bltr, blvra,  
blvrb, blym, bma1, bmd, bmh, bml1, bmp1, bmp2, bmp2a, bmp2b1, bmp3, bmp4,  
bmp5, bmp6, bmp7, bmp8, bmp1a, bmp1b, bmx, bmyb, bn51t, bnc, bnc1, bnp,  
bor, bpad, bpag1, bpag2, bpes, bpes1, bpes2, bpgm, bph1, bpi, br, br140, braf, brca1,  
15 brca2, brca3, brcaox, bred1, bred2, brdt, brf1, brhc, bric, brks, brn3a, brn3b, brn3c,  
brn1, brw1c, bs, bsap, bsep, bsf2, bsg, bsnd, bss1, bst1, bst2, btak, btc, btd, bteb,  
bteb1, btg1, btg2, bths, btk, btk1, btn, bts, bub1b, bubr1, bw1a, bw1b, bws,  
bwscr1a, bwscr1b, bzrp, bzx, c11orf13, c1nh, c1qa, c1qb, c1qbp, c1qg, c1r, c1s, c2,  
c21orf1, c21orf2, c21orf3, c2ta, c3, c3br, c3dr, c3g, c4a, c4b, c4bpa, c4bpb, c4f, c4s,  
20 c5, c5ar, c5r1, c6, c7, c8a, c8b, c8g, c9, ca1, ca12, ca125, ca2, ca21h, ca3, ca4, ca5,  
ca6, ca7, ca8, ca9, caaf1, cabp9k, cac, cac@, caca, cacd, cacna1a, cacna1b, cacna1c,  
cacna1d, cacna1e, cacna1f, cacna1s, cacna2, cacnb1, cacnb2, cacnb3, cacnb4, cacng,  
cacn1a1, cacn1a2, cacn1a3, cacn1a4, cacn1a5, cacn1a6, cacn1a2a, cacn1b1,  
cacnlg, cacc, cact, cacy, cad, cad11, cadas1, cae1, cae3, caf, cafla, caga, cagb, cain,  
25 cak, cak1, cal11, calb1, calb2, calb3, calc1, calc2, calca, calcb, calcr, cald1, calla,  
calm1, calm2, calm3, calm11, calm13, calna, calna3, calnb, calnb1, calr, cals, calt,  
calu, cam, camk4, camkg, caml1, camlg, camp, can, canp3, canx, cap2, cap3, cap37,  
capb, capg, cap1, capn1, capn2, capn3, capn4, cappa2, cappb, capr, caps, capza2,  
capzb, car, carp, cars, cart1, cas, cas2, cas1, casp1, casp10, casp2, casp3, casp3,  
30 casp4, casp5, casp6, casp7, casp8, casq1, casq2, casr, cast, cat, cat1, cat4, catfl,

-55-

catm, cav1, cav2, cav3, cbbm, cbd, cbfa1, cbfa2, cbfa2t1, cbfa3, cbfb, cbg, cbl,  
 cbl2, cbln2, cbp, cbp, cbp2, cbp68, cbr1, cbs, cbt, cbt1, cc10, cca, cca1, cca11,  
 cca12, ccb11, ccckr5, ccg1, ccg2, cch11a1, cch11a2, cch11a3, cch1b1, cck, cckar,  
 cckbr, cc1, ccm1, ccm2, ccm3, ccn1, ccna, ccnb1, ccnc, ccnd1, ccnd2, ccnd3, ccne,  
 5 ccnf, ccng1, ccnh, ccnt, cent1, cco, ccr10, ccr2, ccr3, ccr9, cesp, cct, ccv, cczs, cd,  
 cd10, cd11a, cd11b, cd11c, cd13, cd137, cd14, cd15, cd151, cd156, cd16, cd164,  
 cd18, cd19, cd1a, cd1b, cd1c, cd1d, cd1e, cd2, cd20, cd22, cd23, cd24, cd26, cd27,  
 cd271, cd28, cd281g, cd281g2, cd30, cd32, cd33, cd34, cd36, cd3611, cd3612,  
 cd37, cd38, cd39, cd3911, cd3d, cd3e, cd3g, cd3z, cd4, cd40, cd401g, cd41b, cd43,  
 10 cd44, cd45, cd46, cd47, cd48, cd49b, cd49d, cd5, cd53, cd57, cd58, cd59, cd51, cd6,  
 cd63, cd64, cd68, cd69, cd7, cd70, cd71, cd72, cd74, cd79a, cd79b, cd80, cd81,  
 cd82, cd82, cd86, cd8a, cd8b, cd8b1, cd9, cd94, cd95, cd97, cd99, cda, cda1, cda3,  
 cdan1, cdan2, cdan3, cdb2, cdc2, cdc20, cdc25a, cdc25b, cdc25c, cdc27, cdc211,  
 cdc212, cdc214, cdc34, cdc42, cdc51, cdc7, cdc711, cded1, cded2, cded3, cde11,  
 15 cdcre1, cdgl1, cdgd1, cdgg1, cdgs2, cdh1, cdh11, cdh12, cdh13, cdh14, cdh15,  
 cdh16, cdh16, cdh17, cd2, cdh3, cdh3, cdh5, cdh7, cdh8, cdhb, cdhh, cdhp, cdhs,  
 cdk2, cdk3, cdk4, cdk5, cdk7, cdk8, cdk9, cdkn1, cdkn1a, cdkn1b, cdkn1c, cdkn2a,  
 cdkn2b, cdkn2d, cdkn3, cdkn4, cd11, cdm, cdmp1, cdmt, cdp1, cdp2, cdp3, cdp4, cdp5,  
 cdr2, cdr3, cdr62a, cdsn, cdsp, cdtb, cdw50, cdx1, cdx2, cdx3, cdx4, cea, cebp,  
 20 cebpa, cebpb, cebpd, cebpe, cecr, cel, cel1, cen1, cenpa, cenpb, cenpc, cenpc1,  
 cenpe, cenpf, cerd4, ces, ces1, cetn1, cets, cf, cf2r, cfag, cfag, cfc, cfd1, cfeom1,  
 cfeom2, cfh, cfl1, cfl2, cfnd, cfns, cfr, cgl, cga, cgat, cgb, cgd, cgf1, cgh, cgrp,  
 cgs23, cgt, cgthba, chac, chat, chl1, chd1, chd2, chd3, chd4, chd5, chdr, chl1, chl2,  
 ched, chek1, chga, chgb, chgc, chh, chi311, chip28, chit, chk1, chl1, chl2, chm,  
 25 chm1, chn, chn1, chn2, chop10, chr, chr39a, chr39b, chr39c, chrml, chrml2, chrml3,  
 chrml4, chrml5, chrna1, chrna2, chrna3, chrna4, chrna5, chrna7, chrnb1, chrnb2,  
 chrnb3, chrnb4, chrnd, chrne, chrng, chrs, chs1, chx10, ciipx, cip1, cirbp, cish,  
 ck2a1, ckap1, ckb, ckbb, ckbe, ckm, ckmm, ckmt1, ckmt2, ckn1, ckn2, ckr3, ckr11,  
 ckr13, cl, cl100, clal, clal, clac, clapb1, clapm1, claps3, clc, clc7, clck2, clcn1,  
 30 clcn2, clcn3, clcn4, clcn5, clcn6, clcn7, clcnka, clcnkb, cld, cldn3, cldn5, clg, clg1,

-56-

clg3, clg4a, clg4b, cli, clim1, clim2, clk2, clk3, cln1, cln2, cln3, cln5, cln6, cln80,  
 clns1a, clns1b, clp, clpp, clps, clta, cltb, cltc, cltc11, cltd, clth, clu, cma1, cmah,  
 cmar, cmd1, cmd1a, cmd1b, cmd1c, cmd1d, cmd1e, cmd1f, cmd3a, cmdj, cmh1,  
 cmh2, cmh3, cmh4, cmh6, cmkbr1, cmkbr2, cmkbr3, cmkbr5, cmkbr6, cmkbr7,  
 5 cmkbr8, cmkbr9, cmkbr12, cmklr1, cmkr11, cmkr12, cml, cmm, cmm2, cmoat, cmp,  
 cmpd1, cmpd2, cmpd2, cmpd3, cmpx1, cmt1a, cmt1b, cmt2a, cmt2b, cmt2d, cmt2d,  
 cmt4a, cmt4b, cmtnd, cmtx1, cmtx2, cna1, ena2, cnbp1, enc, cncg1, cncg2, cncg31,  
 cnd, cng3, cnga1, cnga3, cngb1, cnn1, cnn2, cnn3, cnp, cnr1, cnstn, cntf, cntfr, cntnl,  
 co, coca1, coca2, coch, cod1, cod2, coh1, coil, col10a1, col11a1, col11a2, col12a11,  
 10 col13a1, col15a1, col16a1, col17a1, col18a1, col19a1, colla1, colla2, collar,  
 col2a1, col3a1, col4a1, col4a2, col4a3, col4a4, col4a5, col4a6, col5a1, col5a2,  
 col6a1, col6a2, col6a3, col7a1, col8a1, col8a2, col9a1, col9a1, col9a2, col9a3, colq,  
 comp, comt, copeb, copt1, copt2, cord1, cord2, cord5, cord6, cort, cot, cox10, cox4,  
 cox5b, cox6a1, cox6b, cox7a1, cox7a2, cox7a3, cox7am, cox8, cp, cp107, cp115,  
 15 cp20, cp47, cp49, cpa1, cpa3, cpb2, cpb2, cpd, cpe, cpetr2, cpm, cpi, cpi1, cpi2,  
 cpo, cpp, cpp32, cpp32, cppi, cps1, cpsb, cpsd, cpt1a, cpt1b, cpt2, cpu, cpx, cpx,  
 cpxd, cr1, cr2, cr3a, crabp1, crabp2, crapb, crarf, crat, crbp1, crbp2, crd, crd1, creb1,  
 creb2, crebbp, creb11, crem, crfb4, crfr2, crh, crhbp, crhr, crhr1, crhr2, crip, crk,  
 crk1, crm1, crmp1, crmp2, crp, crp1, crs, crs1c, crs2, crs3, crsa, crt, crt11, crtm, crx,  
 20 cry1, cry2, crya1, crya2, cryaa, cryab, cryb1, cryb2, cryb3, cryba1, cryba2, cryba4,  
 crybb1, crybb2, crybb3, cryg1, cryg2, cryg3, cryg4, cryg8, cryg@, cryga, crygb,  
 crygc, crygd, crygs, crym, cryz, cs, csa, csb, csbp1, csci, csd, csd2, csda, cse, csell1,  
 csf1, csf1r, csf2, csf2ra, csf2rb, csf2ry, csf3, csf3r, csh1, csh2, csk, csmf, csn1,  
 csn10, csn2, csn3, csnb1, csnb2, csnb3, csnk1a1, csnk1d, csnk1e, csnk1g2, csnk2a1,  
 25 csnk2a2, csnk2b, csnu3, cso, cspb, cspg1, cspg2, cspg3, csr, csrb, csrp, csrp1, csrp2,  
 cst1, cst1, cst2, cst3, cst4, cst4, cst5, cst6, csta, cstb, csx, ct2, ctaa1, ctaa2, ctag, ctb,  
 ctbp1, ctbp2, ctgf, cth, cthm, ctk, ctla1, ctla3, ctla4, ctla8, ctm, cttna1, cttna2,  
 cttnb1, cttnnd, cttnnd1, cttnr, cttns, ctp, ctpct, ctps, ctr1, ctr2, ctrb1, ctr1, ctsa, ctsb,  
 ctsc, ctsd, ctse, ctsg, ctsg12, ctsh, ctsk, cts1, ctss, ctsw, ctsz, ctx, cubn, cul3, cul4b,  
 30 cul5, cutl1, cvap, cvd1, cvl, cx26, cx31, cx32, cx37, cx40, cx43, cx46, cx50, cxb3s,

-57-

excr4, exorf4, cyb5, cyb561, cyba, cybb, cyc1, cyk4, cyld1, cyp, cyp1, cyp11a,  
 cyp11b1, cyp11b2, cyp17, cyp19, cyp1a1, cyp1a2, cyp1b1, cyp21, cyp24, cyp27,  
 cyp27a1, cyp27b1, cyp2a, cyp2a3, cyp2a6, cyp2b, cyp2c, cyp2c19, cyp2c9, cyp2d,  
 cyp2d@, cyp2e, cyp2e1, cyp2f1, cyp2j2, cyp3a4, cyp4a11, cyp4b1, cyp51, cyp7,  
 5 cyp7a1, cyr61, cyrn1, cyrn2, czp3, d10s105e, d10s170, d10s170, d11s302e,  
 d11s636, d11s813e, d11s833e, d12s2489e, d12s53e, d13s1056e, d13s25, d14s46e,  
 d15s12, d15s226e, d15s227e, d16s2531e, d16s469e, d17s136e, d17s811e, d18s892e,  
 d19s204, d19s381e, d1s111, d1s155e, d1s166e, d1s1733e, d1s2223e, d1s61, d2h,  
 d2s201e, d2s448, d2s488e, d2s69e, d3s1231e, d3s1319e, d3s48e, d4, d4s90,  
 10 d5s1708, d5s346, d6, d6s1101, d6s207e, d6s2245e, d6s228e, d6s229e, d6s230e,  
 d6s231e, d6s51e, d6s52e, d6s54e, d6s81e, d6s82e, d7s437, d8s2298e, d9s46e, da1,  
 da2b, dab2, dac, dad1, daf, dag, dag1, dag2, dagk1, dagk4, dam10, dam6, damox,  
 dan, dao, dap, dap3, dap5, dapk1, dar, dat1, dax1, daxx, daz, dazh, daz1, dba,  
 dbccr1, dbcn, dbh, dbi, dbi, db1, dbm, dbn1, dbp, dbp, dbp1, dbp2, dbpa, dbt, dbx,  
 15 dby, dcc, dce, dci, dck, den, dcoh, dcpl, dcr, dcr3, det, dctn1, dcx, ddb1, ddb2, ddc,  
 ddh1, ddh2, ddit1, ddit3, ddost, ddp, ddpac, ddr, ddx1, ddx10, ddx11, ddx12, ddx15,  
 ddx16, ddx2a, ddx3, ddx5, ddx6, ddx9, dec, decr, def1, def4, def5, def6, defa1,  
 defa4, defa5, defa6, defb1, defb2, dek, denn, dents, dep1, der12, des, dff1, dffa,  
 dffrx, dffry, dfn1, dfn2, dfn3, dfn4, dfn6, dfna1, dfna10, dfna11, dfna12, dfna13,  
 20 dfna2, dfna2, dfna4, dfna5, dfna6, dfna7, dfna8, dfna9, dfnb1, dfnb12, dfnb13,  
 dfnb14, dfnb16, dfnb17, dfnb18, dfnb2, dfnb3, dfnb4, dfnb5, dfnb6, dfnb7, dfnb8,  
 dfnb9, dgcr, dgcr2, dgcr2, dgcr6, dgil, dgka, dgkq, dgpt, dgpt, dgs, dgs2, dgsi, dgu,  
 dhc2, dhcr7, dhfr, dhlag, dhp, dhpr, dhps, dhrrd, dhtr, di, di1, dia, dia1, dia2, dia4,  
 diaph1, diaph2, dif2, diff6, dipi, dir, dkc, dkc1, dlc1, dld, dlgl, dlgl2, dlgl3, dlgl4, dlst,  
 25 dlx1, dlx2, dlx2, dl3, dlx4, dlx5, dlx6, dlx7, dlx8, dm, dm2, dmahp, dmbt1, dmd,  
 dmda1, dmd1, dmh, dmh, dmp1, dmpk, dmsfh, dmt, dmt1, dmtn, dna21, dnah,  
 dnah1, dnah11, dnah12, dnah2, dnahc1, dnahc11, dnahc2, dnahc3, dnase1,  
 dnase111, dnase113, dnase2, dnch2, dnc1, dncm, dnecl, dne11, dn1, dn11, dn111,  
 dnml, dnmt1, dnmt2, dnpk1, dns, dntr, do, doc1, doc2, dock1, dock180, dod, dok1,  
 30 dom, dp1, dp1, dp2, dp3, dpagt2, dpc4, dpd, dpde1, dpde2, dpde3, dpde4, dpep1,

-58-

dph212, dpp, dpp4, dpp6, dpt, dpyd, dpys, dpys11, dpys12, dr1, dr3, dr31g, dr5, dra, drad, drada, dra1, drd1, drd1b, drd1b, drd112, drd2, drd3, drd4, drd5, dr11, drp1, drp1, drp2, drp2, drp3, drp1a, drt, dsc1, dsc2, dsc3, dsc3, dsc4, dscam, dscr, dsg1, dsg2, dsg3, dsp, dspg3, dspp, dss, dss1, dtd, dtdp2, dtdst, dtna, dtr, dts, dus, dusp1, 5 dusp11, dusp2, dusp3, dusp4, dusp5, dusp6, dusp7, dusp8, dut, dv1, dv11, dv11, dv13, dxf68s1e, dxs1272e, dxs128, dxs1283e, dxs423e, dxs435e, dxs522e, dxs648, dxs707, dxs8237e, dxys155e, dylx2, dyrk, dys, dysf, dyt1, dyt3, dyt5, dyt6, dyt7, dyt8, dyt9, dyx1, dyx2, e11s, e14, elb, e2a, e2f1, e2f2, e2f3, e2f4, e3, e4f, e4f1, e4tfla, e4tflb, eal, eaac1, eaat1, eaat2, eac, ead, eag, eap, earl, ear2, ear3, eba1, ebf, 10 ebi1, ebm, ebn1, ebn1, ebn2, ebr2a, ebs1, ebvm1, ebvs1, ec1, ecal, ecb2, ecel, ecgf1, ech1, echs1, eck, ecm1, ecp, ecs1, ect2, ed1, ed2, ed3, ed4, eda, eda3, eddr1, edg3, edg6, edh, edh17b2, edh17b2, edh17b3, edm1, edm2, edm3, edmd, edmd2, edn, edn1, edn2, edn3, ednra, ednrb, eec1, eec2, eef1a1, eef1a2, eef1b1, eef1b2, eef1b3, eef1b4, eef2, eeg1, eegv1, eek, een, efla, ef2, efe2, efemp1, efl6, efmr, 15 efna1, efna3, efna4, efnb1, efnb2, efnb3, efp, eftu, egf, egfr, egi, egr1, egr2, egr3, egr4, ehhadh, ehoc1, ei, eif1a, eif2g A, eif2s3 A, eif3s10, eif3s6, eif4a1, eif4a2, eif4c, eif4e, eif4ebp1, eif4e2, eif4e11, eif4e12, eif4g, eif4g1, eif4g2, eif5a, ejm1, ell, ela1, ela2, elam1, elanh2, elav11, elav12, elav14, elc, ele1, elf3, elk1, elk2, elk3, elk4, ell, eln, em9, emap, emap1, emd, emd2, emk 1, emp1, emp55, emr1, ems1, 20 emt, emtb, emx1, emx2, en1, en2, ena78, end, endog, enfl2, eng, en1, eno1, eno2, eno3, enpep, ent1, entk, enur1, enur2, enx2, eos, ep3, ep300, epa, epb3, epb311, epb41, epb4112, epb42, epb49, epb72, epha1, epha2, epha3, epha8, ephb1, ephb2, ephb3, ephb4, ephb6, epht1, epht2, epht3, ephx1, ephx2, epim, eplg1, eplg2, eplg3, eplg4, eplg5, eplg8, eplm1, eplm2, eplm2a, eplmr, epo, epor, eppk, eprs, eps15, eps8, 25 ept, erba1, erba2, erba12, erba13, erbb2, erbb3, erbb4, erc55, ercc1, ercc2, ercc3, ercc4, ercc5, ercc6, ercm1, erdal, erfl, erg, erg3, ergic53, erh, erk, erk1, erk2, erk3, erm, erp11, erv1, erv1, erv3, ervr, ervt1ervt2, ervt3, ervt4, ervt5, eryf1, es1, es130, esa, esa1, esa4, esat, esb3, esd, esg, esr, esr1, esr2, esr11, esr12, esrra, esrrb, esrrg, ess1, est, est, est2, est25263, esx, etfa, etfb, etfdh, etk1, etk2, etm1, etm2, eto, ets1, 30 ets2, etv1, etv3, etv4, etv5, etv6, evc, evc1, evda, evdb, evi1, evi2, evi2a, evi2b,

-59-

evp1, evr1, evx1, evx2, ews, ewsr1, exlml, ext1, ext2, ext3, extl1, extl2, eya1,  
 eya2, eya3, eyc11, eyc13, ezh1, ezh1, ezh2, fl0, fl1, fl2, fl3a, fl3a1, fl3b, f2, f2r,  
 f2rl2, f2rl3, f3, f5, f5f8d, f7, f7e, f7r, f8a, f8b, f8c, f8vwf, f9, fa, fal, faa, fabp1,  
 fabp2fabp3, fabp4, fabp6, fac1, faca, facc, facd, face, fac11, fac12, fac13, fac14,  
 5 facv11, fad, fadd, fadk, fah, fak2, faldh, fal139, falz, fanca, fancc, fancd, fance,  
 fancg, fap, fapa, farr, fas, fas1, fasn, fast1, fat, fau, fbln1, fbln2, fbn1, fbn2, fbn1,  
 fbp1, fcar, fec1, fce, fce2, fcer1a, fcer1b, fcer1g, fcer2, fcgr1a, fcgr1b, fcgr1c,  
 fcgr2a, fcgr3a, fcgrt, fcnd, fcn1, fcn2, fcp, fcp1, fcpX, fct3a, fdc, fdf1, fdh, fdps11,  
 fdps12, fdps13, fdps14, fdps15, fdx1, fdx, fe65, fe6511, fea, feb1, feb2, fech, fech,  
 10 fen1, feo, feom, feom1, feom2, fer, fes, fet1, fevr, ffm, fga, fgarat, fgb, fgc@, fgd1,  
 fgdy, fgfl, fgfl10, fgfl1, fgfl2, fgfl3, fgfl4, fgf2, fgf2, fgf3, fgf4, fgf5, fgf6, fgf7,  
 fgf8, faf9, fgfa, fgfb, fgfr1, fgfr2, fgfr3, fgfr4, fgg, fgr, fgs1, fh, fh, fh3, fhc, fnf1,  
 fhf3, fhf4, fh2, fh1, fh11, fh12, fhr2, fic1, figf, fih, fim, fim1, fim3, fimg, fkbp12,  
 fkbp1a, fkbp2, fkh2, fkh11, fkh110, fkh112, fkh115, fkh116, fkh117, fkh12, fkh15,  
 15 fkh16, fkh17, fkh18, fkh19, fkh, fkh11, flg, fl1, fl1i, fln1, fln2, flna, flnb, flnms,  
 flot2, flt1, flt2, flt3, flt4, fmf, fmn, fmo1, fmo2, fmo3, fmod, fmr1, fmr2, fms, fl1,  
 fn12, fnra, fnrb, fnrb1, fnta, fntb, folh, folh1, folr1, folr2, folt, fos, fosb, fos11,  
 fos12, fpah, fpc, fpd1, fpdmm, fpf, fpgs, fp1, fpp, fpr1, fprh1, fprh2, fpr11, fpr12,  
 fprp, fps12, fps13, fps14, fps15, fr, frap1, fraxa, fraxe, fraxf, frda, freac2, freac6,  
 20 freac9, frgl, frp1, frv1, frv2, frv3, fsg1, fsgs, fshb, fshd1a, fshmd1a, fshprh1, fshr,  
 fssv, fth1, fth16, ft1, ftz1, ftzf1, fuca1, fuca2, fur, fus, fuse, fut1, fut2, fut3, fut4,  
 fut5, fut6, fut7, fut8, fvt1, fxr1, fxy, fy, fyn, fzd1, fzd2, fzd3, fzd5, fzd6, fzd7, fzf,  
 g0s8, g10p1, g10p2, g17, g17p1, g19p1, g1p1, g1p2, g1p3, g22p1, g6pc, g6pd,  
 g6pd1, g6pd1, g6pt, g6pt1, g6s, g7p1, ga2, gaa, gabatr, gabpa, gabpb1, gabral,  
 25 gabra2, gabra3, gabra4, gabra5, gabra6, gabrb1, gabrb2, gabrb3, gabrd, gabre,  
 gabrg1, gabrg2, gabrg3, gabrr1, gabrr2, gad1, gad2, gad3, gadd153, gadd45, gak,  
 gal, galbp, galc, gale, galgt, galk1, galk2, galn, galnact, galnr, galnr1, galns, galnt1,  
 galnt2, galnt3, galr1, galt, gan, gan1, ganab, ganc, gap, gap1m, gap43, gapd, gar22,  
 garp, gars, gart, gas, gas1, gas2, gas41, gas6, gas7, gasr, gast, gata1, gata2, gata3,  
 30 gata4, gata6, gay1, gba, gbas, gbbb1, gbbb2, gbe1, gbp1, gbx2, gc, gcap, gcap2,

-60-

gcdh, gcf1, gcf2, gcfx, gcg, gcgr, gch1, gck, gckr, gcn511, gcn512, gcnf, gcnt1,  
 gcnt2, gcp, gcp2, gcs, gcs1, gcsf, gcsfr, gcsf, gctg, gcy, gda, gde, gdf5, gdf8, gdh,  
 gdi1, gdi2, gdid4, gdld, gdnf, gdnfr, gdnfra, gdnfrb, gdx, gdxxy, ge, gem, geney, gey,  
 gfl, gfl, gfap, gfer, gfer, gfil, gfpt, gfral, gfra2, ggex, ggt1, ggt2, ggtal, ggtb1,  
 5 ggtb2, gh1, gh2, ghe.RTM., ghdx, ghn, ghr, ghfr, ghrh, ghrhr, ghs, ghv, gif, gifb,  
 gip, gip, gipr, girk1, girk2, girk3, girk4, gja1, gja3, gja4, gja5, gja8, gjb1, gjb2, gjb3,  
 gk, gk2, gla, glat, glb1, glb2, glc1a, glc1b, glc1c, glc1d, glc1f, glc3a, glc3b, glc1c,  
 glc1r, glct2, glct3, glde, glepp1, glg1, gli, gli2, gli3, gli4, glnn, glns, glo1, glo2,  
 glp1r, glra1, glra2, glra3, glrb, glrx, gls, glud1, glud2, glu1, glur1, glur2, glur3,  
 10 glur4, glur5, glur6, glur7, glut1, glut2, glut3, glut4, glut5, glvr1, glvr2, gly96, glya,  
 glyb, glysl, glytl, glytl, glyt2, gm2a, gma, gmcsf, gmds, gm1, gmpr, gmpr, gnai1,  
 gnai5, gnai6, gnai1, gnai2, gnai2a, gnai2b, gnai21, gnai3, gna1, gnao1, gnaq, gnas,  
 gnas1, gnat1, gnat2, gnaz, gnb1, gnb2, gnb3, gng5, gn11, gnpta, gnrl1, gnrl2, gnrlr,  
 gns, gnt1, golga4, got1, got2, gp130, gp1ba, gp1bb, gp2, gp2b, gp39, gp3a, gp75,  
 15 gp78, gp9, gpa, gpam, gpat, gpb, gpc, gpc1, gpc3, gpc4, gpd, gpd1, gpd2, gpds1,  
 gpe, gpi, gpi2, gpm6a, gpm6b, gpoa, gpr1, gpr10, gpr11, gpr12, gpr13, gpr15,  
 gpr17, gpr18, gpr19, gpr2, gpr20, gpr21, gpr22, gpr23, gpr25, gpr29, gpr3, gpr30,  
 gpr31, gpr32, gpr35, gpr37, gpr39, gpr4, gpr5, gpr6, gpr7, gpr8, gpr9, gprcy4,  
 gprk21, gprk4, gprk5, gprk6, gprv28, gpsa, gpse, gpt, gpx1, gpx2, gpx3, gpx4, gr2,  
 20 grb1, grb10, grb2, grf2, gria1, gria2, gria3, gria4, grid2, grik1, grik2, grik3, grik4,  
 grik5, grin1, grin2a, grin2b, grin2c, grin2d, grina, grk1, grk5, grk6, gr1, gr111,  
 grm3, grm8, grmp, gm, gro1, gro2, gro3, grp, grp58, grp78, grpr, grx, gs, gsl, gsas,  
 gsc, gsc1, gse, gshs, gsl, gsm1, gsn, gsp, gspt1, gsr, gss, gst12, gst11, gst2, gst2,  
 gst3, gst4, gst5, gstal, gsta2, gstm1, gstm11, gstm2, gstm3, gstm4, gstm5, gstp1,  
 25 gstt2, gt1, gt335, gta, gtb, gtbp, gtd, gtf2e2, gtf2f1, gtf2h1, gtf2h2, gtf2h4, gtf2i,  
 gtf2s, gtf3a, gtg, gucla2, gucla3, guclb3, guc2c, guc2d, guc2f, gucala, gucalb,  
 guca2, guca2, guca2a, guca2b, guca3, guca3b, gucy1a2, gucy1a3, gucy1b3, gucy2c,  
 gucy2d, gucy2f, guk1, guk2, gulo, gulop, gusb, gusm, gust, gxp1, gypa, gypb, gypc,  
 gype, gys, gys1, gys2, gzma, gzmb, gzmh, gzmm, h, h142t, h19, h1f0, h1f1, h1f2,  
 30 h1f3, h1f4, h1f5, h1fv, h2a, h2ax, h2az, h2b, h2b, h3f2, h3f3b, h3ft, h3t, h4, h4f2,

-61-

h4f5, h4fa, h4fb, h4fe, h4fg, h4fh, h4fi, h4fj, h4fk, h4fl, h4fm, h4m, h6, ha2, habp1,  
 hadha, hadhb, hadhsc, haf, hagh, hah1, haip1, hal, hap, hap1, hap2, hars, has2, hat1,  
 hausp, hb1, hb1, hb6, hba1, hba2, hbac@, hbb, hbbc@, hbd, hbe1, hbegf, hbf2,  
 hbg1, hbg2, hbgr, hbhr, hbm, hbp, hbq1, hbz, hc2, hc3, hca, hcat2, hccs, hedh, hcf2,  
 5 hcfcl, hcg, hck, h11, hc12, hc13, hcls1, hcp, hcp1, hcs, hcv5, hd, hdac1, hdc, hdgf,  
 hdhc7, hdlbp, hdld, hdldt1, hdr, hed, hed, hegfl, hek, hek3, heln1, hem1, hema,  
 hemb, henc, hempas, hen1, hen2, hep, hep10, her2, her4, herg, herv1, hes1, hesx1,  
 het, hexa, hexb, hf1, hf10, hfc1, hfe, hfe2, hfh11, hfsp, hgd, hgf, hgf, hgfl, hg1, hh,  
 hh72, hhc1, hhc2, hhd, hhh, hhmjg, hhr23a, hht1, hht2, hiap2, higm1, hilda, hint,  
 10 hiomt, hip, hip1, hip116, hip2, hir, hira, his1, his2, hive1, hivep1, hivep2, hjcd, hk1,  
 hk2, hk3, hk33, hke4, hke6, hkr1, hkr2, hkr3, hkr4, hl11, hl19, hla-a, hla-b, hla-c,  
 hla-cda12, hla-dma, hla-dmb, hla-dna, hla-dob, hla-dpalhla-dpb1, hla-dqa1, hla-  
 drlb, hla-dra, hla-e, hla-f, hla-g, hla-ha2, hlap, hlaf, hlals, hlcs, hlm2, hlp, hlp3,  
 hlr1, hlr2, hlt, hlxl, hlxb9, hmaa, hmab, hmat1, hmbs, hmcs, hmg1, hmg14, hmg17,  
 15 hmg2, hmgc1, hmger, hmges1, hmges2, hmgic, hmgiy, hmgx, hminr, hmn2, hmox1,  
 hmox2, hmr, hms1, hmsn1, hmx1, hmx2, hrid, hnfla, hnfl2, hnfl3a, hnfl3b, hnfl4a,  
 hnp36, hnpcc6, hnrpa1, hnrpa2b1, hnrpd, hnrpf, hnrpg, hnrph1, hnrph2, hnrph3,  
 hnrpk, homg, hops, hox10, hox11, hox12, hox1@, hox1a, hox1b, hox1c, hox1d,  
 hox1e, hox1f, hox1g, hox1h, hox1i, hox1j, hox2@, hox2a, hox2b, hox2c, hox2d,  
 20 hox2e, hox2f, hox2g, hox2h, hox2i, hox3@, hox3a, hox3b, hox3c, hox3d, hox3e,  
 hox3f, hox3g, hox4@, hox4a, hox4b, hox4c, hox4d, hox4e, hox4f, hox4g, hox4h,  
 hox4i, hox7, hox8, hoxa1, hoxa10, hoxa11, hoxa13, hoxa3, hoxa4, hoxa5, hoxa6,  
 hoxa7, hoxa9, hoxa@, hoxb1, hoxb2, hoxb3, hoxb4, hoxb5, hoxb6, hoxb7, hoxb8,  
 hoxb9, hoxb@, hoxc12, hoxc13, hoxc4, hoxc5, hoxc6, hoxc8, hoxc9, hoxc@,  
 25 hoxd1, hoxd10, hoxd11, hoxd12, hoxd13, hoxd3, hoxd4, hoxd8, hoxd9, hoxd@,  
 hoxhb9, hp, hp4, hpafp, hpc1, hpc2, hpca, hpca11, hpcx, hpd, hpdr1, hpdr2, hpe1,  
 hpe2, hpe3, hpe4, hpe5, hpect1, hpfh, hpfh2, hpgd, hplh1, hplh2, hpn, hpr, hpri,  
 hpri1, hps, hpt, hpt1, hptp, hptx, hpv18i1, hpv18i2, hpx, hr, hras, hrb, hrc, hrc1,  
 hrca1, hrd, hres1, hrf, hrg, hrga, hrh1, hrh2, hrmt11, hrpt2, hrx, hrx, hry, hsa11,  
 30 hsa12, hsan1, hsa1, hscr2, hsd11, hsd11b1, hsd11b2, hsd11k, hsd11l, hsd17b1,

-62-

hsd17b2, hsd17b3, hsd17b4, hsd3b1, hsd3b2, hsh, hsn1, hsorc1, hsp27, hsp73,  
 hspa1a, hspa1b, hspa11, hspa2, hspa3, hspa4, hspa5, hspa6, hspa7, hspa8, hspa9,  
 hspb1, hspb2, hspc2, hspca11, hspca12, hspca13, hspca14, hspcb, hspg1, hspg2,  
 hsr1, hsst, hstd, hstf1, htc2, htf4, htk, htk1, ht1, htlf, htivr, htn1, htn2, htn3, htnb,  
 5 htor, htr1a, htr1b, htr1d, htr1e, htr1e1, htr1f, htr2a, htr2b, htr2c, htr3, htr4, htr5a,  
 htr6, htr7, htrx1, hts1, htt, htx, htx1, hub, hud, hup2, hur, hus, hvls, hvbs1, hvbs6,  
 hvbs7, hvem, hvh2, hvh3, hvh8, hxb, hxb1, hy, hya, hya11, hyd2, hygn1, hy1, hyp,  
 hyplip1, hypp, hypx, hyr, hyrc1, hys, ia1, ia2, iap, iapp, iar, iars, ibd1, ibd2, ibm2,  
 ibsp, ical, icam1, icam2, icam3, icca, ich1, icr2, icr2b, ics1, id1, id2, id3, id4, ida,  
 10 idd, iddm1, iddm10, iddm11, iddm12, iddm13, iddm15, iddm17, iddm2, iddm3,  
 iddm4, iddm5, iddm6, iddm7, iddm8, iddmx, ide, idg2, idh1, idh2, idh3a, idh3g, ido,  
 ids, idua, ier1, ier3, iex1, if, ifcr, ifgr2, ifi16, ifi27, ifi35, ifi4, ifi5111, ifi54, ifi56,  
 ifi616, ifi78, ifna1, ifna10, ifna13, ifna14, ifna16, ifna17, ifna21, ifna6, ifna7, ifna8,  
 ifna@, ifnai1, ifnar1, ifnar2, ifnb1, ifnb2, ifnb3, ifng, ifngr1, ifngr2, ifngt1, ifnr,  
 15 ifnwl1, ifrd2, iga, igat, igb, igbp1, igd1, igda1, igdc1, igds2, iger, iges, igf1, igf1r,  
 igf2, igf2r, igfbp1, igfbp10, igfbp2, igfbp3, igfbp4, igfbp6, igfbp7, igfr1, igfr2, igfr3,  
 igh@, igha1, igha2, ighd, ighdy2, ighe, ighg1, ighg2, ighg3, ighg4, ighj, ighm,  
 ighmbp2, ighr, ighv@, igi, igj, igk@, igkc, igkde1, igkj, igkjr1, igkv, iglc, iglc1,  
 iglj, iglp1, iglp2, iglv, igm, igo1, igsf1, ihh, ik1, ikba, il10, il10r, il11, il11ra, il12a,  
 20 il12b, il12rb1, il12rb2, il13, il13ra1, il13ra2, il15, il15ra, il17, il1a, il1b, il1bc, il1r1,  
 il1r2, il1ra, il1rap, il1rb, il1rm, il2, il2r, il2ra, il2rb, il2rg, il3, il3ra, il3ray, il4, il4r,  
 il4ra, il5, il5ra, il6, il6r, il6st, il7, il7r, il8, il8ra, il8rb, il9, il9r, ila, ilf1, illbp, imd1,  
 imd2, imd4, imd5, imd6, impa1, impdh1, impdh2, impdh11, impg1, impt1, indx,  
 infa2, infa4, infa5, ing1, inha, inhba, inhbb, inhbc, ini1, ink4b, inlu, inp10, inpp1,  
 25 inpp5a, inpp5b, inpp5d, inpp11, ins, insig1, ins1, ins13, ins14, insr, insrr, intl,  
 intl11, int2, int3, int4, int6, iosca, ip2, ipf1, ip1, ipm150, ipox, ipp, ipp2, ipw,  
 iqgap1, ir10, ir20, ireb1, ireb2, irf1, irf2, irf4, irf4, irr, irs1, isa, iscw, is11, islr, isot,  
 issx, it15, itba1, itba2, itf, itf2, itga1, itga2, itga2b, itga4, itga5, itga6, itga7, itgad,  
 itga1, itgam, itgav, itgax, itgb1, itgb2, itgb3, itgb4, itgb6, itgb7, iti, itih1, itih2, itih3,  
 30 itih4, itih11, iti1, itk, itm1, itpa, itpka, itpkb, itpr1, itpr2, itpr3, itsn, ivd, iv1, jag1,

jak1, jak2, jak3, jbs, jcap, jh8, jip, jk, jme, jmj, joag, jpd, jrk, jrk1, jtk14, jtv1, jun,  
 junb, jund, jup, jv18, jws, k12t, kai1, kall, kar, kars, katp1, kcnal, kcnal0, kcnalb,  
 kcnal2b, kcnal3, kcnal4, kcnal5, kcnal6, kcnal7, kcnal8, kcnal9, kcnab1, kcnab2, kcnb1,  
 kcnc1, kcnc2, kcnc3, kcnc4, kcne1, kcnh1, kcnh2, kcnj1, kcnj10, kcnj11, kcnj12,  
 5 kcnj15, kcnj3, kcnj4, kcnj5, kcnj6, kcnj6, kcnj7, kcnj8, kcnjn1, kenk1, kenk2,  
 kenk3, kenma1, kenq1, kenq2, kenq3, kenq4, kens2, kd, kdr, ke1, kera, kfl, kfs,  
 kfsc, kfs1, khk, kias0122, kid, kid1, kif2, kif3c, kif5b, kip1, kip2, kiss1, kit, klc2,  
 klk1, klk2, klk3, klk3, klkb1, klkr, klrb1, klrc1, klrc2, klrc3, klrc4, klrd1, klst, kms,  
 kms, kng, kno, kns1, kns2, kns11, kns14, kox1, kox11, kox12, kox13, kox15, kox16,  
 10 kox18, kox19, kox2, kox2, kox22, kox25, kox30, kox32, kox4, kox5, kox6, kox7,  
 kox9, kpna3, kpps1, kpps2, krag, kras1p, kras2, krev1, krg2, krnl, krnl1, krox20,  
 krt1, krt10, krt12, krt13, krt14, krt15, krt16, krt17, krt18, krt19, krt2a, krt2e, krt3,  
 krt4, krt5, krt6a, krt6b, krt7, krt8, krt9, krtha2, krtha5, krthb1, krthb6, ks, ktn1, ku70,  
 kup, kvlt1, kwe, 11.2, 11 cam, 123mrp, lab7, lab72, lac, laci, lacs, lad, lad, lad1,  
 15 laf4, lag3, lag5, lair1, lak1, lalba, lal1, lam1, lama1, lama2, lama3, lama3, lama4,  
 lama5, lamb1, lamb2, lamb2, lamb2t, lamb3, lambr, lamc1, lamc2, lamm, lamnb2,  
 lamp, lamp1, lamp2, lamr1, lams, lap, lap18, laptm5, lar, lar1, lard, large, lars, lbp,  
 lbr, lca, lca1, lcad, lcam, lcat, lccs, lcfs2, lch, lck, lcn1, lcn2, lco, lcp1, lcp2, let, ld,  
 ld78, ldb1, ldb2, ldc, ldh1, ldh3, ldha, ldhb, ldhc, ldlr, le, lect2, lefl, lefty1, lefty2,  
 20 lep, lepr, lerk5, lerk8, leu1, leu7, leut, lfa1a, lfa3, lfhl1, lfp, lgal1s1, lgal1s3,  
 lgal1s3bp, lgal1s7, lgcr, lgmd1, lgmd1a, lgmd1b, lgmd1c, lgmd1d, lgmd2b, lgmd2c,  
 lgmd2d, lgmd2e, lgmd2f, lgmd2g, lgmd2h, lgs, lgtn, lhb, lhgr, lhs, lhx1, lhx3, li,  
 li2, lif, lifr, lig1, lig3, lig4, lim1, lim2, limab1, limk1, limpii, lip2, lipa, lipb, lipc,  
 lipd, lipe, lipo, lis1, lis2, lisx, litaf, lkb1, lkn1, llg11, lman1, lmn1, lmn2, lmna,  
 25 lmnbl, lmnbl, lmo1, lmo2, lmo3, lmo4, lmo5, lmp10, lmp2, lmp7, lmpx, lms, lmx1,  
 lmx1a, lmx1b, lmyc, lnhr, lnhr, locr, loh11cr2a, lor, lot1, lox, lox1, lox11, lpa, lpaab,  
 lpaata, lpap, lpc1, lpc2d, lpd1, lph, lpi, lp1, lpna3, lpp, lps, lpsa, lqt1, lqt2, lqt3, lqt4,  
 lr3, lrel, lre2, lrp, lrp1, lrp2, lrp5, lrp7, lrp8, lrpap1, lrpr1, lrs1, lsamp, lsirf, ls1, lsn,  
 lsp1, lss, lst1, lta, lta4h, ltb, ltb4r, ltbp1, ltbp2, ltbp2, ltbp3, ltbp3, ltr, ltc4s, ltf, ltk,  
 30 ltn, lu, lum, luxs, luzp, lw, ly64, ly6e, ly9, lyam1, lyb2, lyf1, ly11, lyn, lyp, lyst,

-64-

lyt10, lyz, lztr1, m11s1, m130, m17s1, m17s2, m195, m1s1, m3s1, m4s1, m6a, m6b,  
 m6p2, m6pr, m6s1, m7v1, m7vs1, mab211, mac1a, mac2, mac25, macam1, macs,  
 mad, mad211, madd, madh1, madh2, madh3, madh4, madh5, madh6, madh6,  
 madh7, madh9, madm, madr1, maf, mafd1, mafd2, mag, magel, mageb3, mageb4,  
 5 magell, magoh, magp, magp1, magp2, mak, ma1, mal1, man2a2, mana1, mana2,  
 mana2x, manb, manb1, manba, maoa, maob, map1a, map1alc3, map1b, map1b1c3,  
 map2, map4, map80, map97, mapk1, mapkap3, mapkkk4, mapt, mar, mark3, mars,  
 mas1, masp1, mat1a, mat2a, mata1, mata2, matk, matn1, matn3, max, maz, mb,  
 mbd1, mb1, mb12, mbp, mbp1, mbs, mbs2, mclr, mc2r, mc3r, mc4r, mc5r, mcad,  
 10 mcc, mcdcl, mcdrl, mcf2, mcf3, mcfdl, mch2, mch3, mch4, mch5, mckd, mcl,  
 mcl1, mcm, mcm2, mcm2, mcm3, mcm6, mcm7, mcm7, mcm7, mcm7, mcm7, mcm7, mcm7,  
 mcp3, mcph1, mcr, mcs, mcsf, mcsp, mct1, mdl, mdb, mdc, mder, mdde, mdeg,  
 mdf1, mdg, mdg1, mdh1, mdh2, mdk, mdk, mdm2, mdm4, mdr1, mdr3, mdrsl,  
 mdrv, mds, mds1, mdu1, mdu2, mdu3, mdx, me1, me2, mea, mea6, mec11, mecp2,  
 15 med, mef, mef2a, mef2b, mef2c, mef2d, mefv, mehmo, meis1, meis2, mekk, mekk1,  
 mekk4, mel, mel18, melf, memo1, men1, men2a, meox1, meox2, mep1a, mep1b,  
 mer2, mer6, mest, met, metrs, mfap1, mfap2, mfap3, mfap4, mfd1, mfi2, mfs1,  
 mfs2, mft, mfts, mg50, mga, mgal, mga3, mgat1, mgat2, mgat5, mgcl, mgcn, mgr,  
 mgct, mgdf, mgea, mgf, mgi, mgmt, mgp, mgsa, mgst1, mgst2, mhc, mhc2ta, mhp2,  
 20 mhs, mhs2, mhs3, mhs4, mhs6, mia, mic10, mic11, mic12, mic17, mic18, mic2,  
 mic2x, mic2y, mic3, mic4, mic7, mica, micb, mid1, midas, mif, mif, mig, mip,  
 mip2a, mip2b, mip3b, mipep, mitf, miwc, mjd, mk, mki67, mkks, mkp2, mkp3,  
 mkpx, mks, mks, mks1, mks2, mla1, mlck, mlf1, mlf2, mlh1, mlk1, mlk3, ml1,  
 ml12, ml1t1, ml1t2, ml1t3, ml1t4, ml1t6, ml1t7, mlm, mlm, mln, mlp, mlr, mlrg,  
 25 mlrw, mls, mltn, mlvar, mlvi2, mlvt, mmac1, mme, mmp1, mmp10, mmp11,  
 mmp12, mmp13, mmp14, mmp15, mmp16, mmp17, mmp19, mmp2, mmp21,  
 mmp22, mmp3, mmp7, mmp8, mmp9, mn, mn, mnb, mnbh, mnda, mng1, mnk,  
 mns, mnt, mocod, mocs1, mocs2, mody1, mody3, mog, mok2, mom1, mos, mot2,  
 mov34, mox1, mox2, mox44, moz, mp19, mpb1, mpd1, mpdz, mpe, mpe16, mpg,  
 30 mpi, mpif2, mp1, mp11g, mpo, mpp1, mpp2, mpp3, mppb, mpri, mprn, mps2,

-65-

mps3a, mps3c, mps4a, mpsh, mpts, mpv17, mpz, mrl, mr77, mrbc, mrc1, mre11, mre11a, mrg1, mrgh, mros, mrp, mrp, mrp1, mrp123, mrs, mrtd, mrsr, mrst, mrx1, mrx14, mrx2, mrx20, mrx21, mrx23, mrx29, mrx41, mrx48, mrx49, mrx9, mrxa, mrxs1, mrxs2, mrxs3, mrxs4, mrxs5, mrxs6, mrxs8, ms3315, ms336, msg1, msh2, msh3, msh4, msh6, msil, msk16, msk39, msk41, mslr1, msmb, msn, msr1, mss1, mss4, mss4, msse, mst, mst1, mst1r, mstd, mstn, msud1, msx1, msx2, mt1a, mt1b, mt1e, mt1f, mt1g, mt1h, mt1i, mt1j, mt1k, mt1l, mt1x, mt2, mt2a, mt3, mtacl1, mtap, mtbt1, mtcpl, mterf, mtf1, mth1, mthfc, mthfd, mthfr, mtk1, mtm1, mtmr1, mtmx, mtnr1a, mtnr1b, mtp, mtpa, mtr, mtrns, mtrr, mts, mts, mts1, mts1, mts2, mttf1, mtX, mtXn, mu, muc1, muc2, muc3, muc4, muc5, muc5ac, muc5b, muc6, muc8, mul, mum1, muppl, musk, mut, mvk, mvk, mvwf, mwfe, mx, mx1, mx2, mxil, mxs1, myb, myb11, myb12, mybpc1, mybpc2, mybpc3, mybpcf, mybph, myc, myc11, myc12, mycl1, mycn, myd88, myf3, myf4, myf5, myf6, myh1, myh10, myh11, myh12, myh2, myh3, myh4, myh6, myh7, myh8, myh9, myk1, myl, myl1, myl2, myl3, myl4, myl5, mylk, mymy, myo10, myo15, myo1a, myo1c, myo1d, myo1e, myo5a, myo6, myo7a, myo9b, myoc, myod1, myog, myp1, myp2, myp3, myr5, mzfl, n33, nab1, nab2, nabcl, nacla, naca, naca, nacc, nadmr, naga, nagg@, naglu, nagr1, naip, namsd, nanta3, nap114, nap2, nap21, napb, naptb, nars, nat1, nat1, nat2, nb, nb4s, nbat, nbcc3, nbccs, nbccs, nbia1, nbs, nbs, nbs1, nca, ncad, ncam1, ncan, ncbp, ncc1, ncc2, ncc3, ncc4, ncct, ncf1, ncf2, ncf4, nck, ncl, ncst2, ncx1, ncx2, nd, ndhii, ndn, ndp, ndst1, ndufa1, ndufa2, ndufa5, ndufa6, ndufa7, ndufb8, ndufb9, ndufs1, ndufs2, ndufs4, ndufs7, ndufs8, ndufv1, ndufv2, ndufv3, neb, nec1, nec2, nedd1, nedd2, nedd4, nefh, nef1, negf1, negf2, nel11, nel112, nem1, neol, nep, net, net1, neu, neu, neud4, neurod, neurod2, neurod3, nfl, nfla, nf2, nfatcl, nfatc2, nfatp, nfe1, nfe2, nfe211, nfe212, nfe2u, nfia, nfib, nfic, nfix, nfkb1, nfkb2, nfkb3, nfkb1a, nfkb11, nfrkb, nfya, nfyb, nga1, ngbe, ngfb, ngfg, ngfic, ngfr, ng1, ngn, nhbp, nhcp1, nhcp2, nhe1, nhe3, nhe4, nhe5, nhhl1, nhhl2, nhp211, nhs, nid, niddm1, ninj1, nipp1, nipsnap1, nipsnap2, nis, nk1r, nkcc1, nkcc2, nkg2, nkg2a, nkg2c, nkg2e, nkg2f, nkhe, nkna, nkna, nknb, nknp1a, nks1, nksf2, nktr, nkx2a, nkx3.2, nkx3a, nkx6a, nli, nm, nm1, nm23, nmb, nmbr, nmdar1,

-66-

nmdar2a, nmdar2b, nmdar2c, nmdar2d, nmdara1, nme1, nme2, nme4, nmor1,  
 nmor2, nms1, nmyc, nnat, nmnt, nno1, nog, noll, nos1, nos2a, nos2b, nos2c, nos3,  
 not, notch1, notch2, notch3, notch4, nov, nov, nov2, nova1, nova3, novp, np, np10,  
 npat, npc, npc1, npd, nph1, nph2, nphl2, nphn, nphp1, nphp2, nphs1, npm1, nppa,  
 5 nppb, nppe, npps, npr1, npr2, npr3, nps1, npt1, npt2, nptx2, npy, npy1r, npy2r,  
 npy3r, npy5r, npy6r, nqo2, nramp, nramp1, nramp2, nrap, nras, nrb54, nrcam, nrd1,  
 nrf1, nrf1, nrf2, nrgn, nrp1, nrk2, nr1, nrt1, nru, ns1, nsf, nsp, nsp11, nsrd9, nt4, nt5,  
 nt5, ntcp1, ntcp2, ntf3, ntf4, ntf5, nth11, ntn, ntn, ntn21, ntrk1, ntrk2, ntrk3, ntrk4,  
 ntrkr1, ntrkr3, nts, ntt, ntt, nuc1, nucb1, numal, nup214, nup98, nuir1, nv1, nys1,  
 10 nys2, nysa, oa1, oa2, oa3, oar, oas, oat, oat11, oat22, oat23, oatp, oaz, ob, ob10,  
 obf1, obp, obr, oca2, ocm, ocp2, ocr1, ocr11, oct, oct1, oct1, oct2, oct2, oct3, oct7,  
 octn2, octs3, odc1, odcd, odgl, odod, ofc1, ofc2, ofc3, ofd1, ofe og22, ogdh,  
 oggl, ogr1, ogs1, ogs2, ohds, ohs, oias, oip1, ok, olf1, olfmf, olfr1, olfr2, omg,  
 omgp, omp, on, op2, opa1, opa2, opa3, opca3, opcm1, opd1, opgl, ophn1, op11,  
 15 opn, oppg, oprd1, oprk1, oprm1, oprt, opta2, optb1, oqt1, orld2, orlf1, ore11, orc21,  
 orc41, orc51, orfx, orm1, orm2, orw, osbp, osm, osp, ost, ost48, osx, otc, otfl, otfl2,  
 otfl3, otm, otol, ots, otx1, otx2, ovc, ovcs, ovoll, ox40, oxal1, oxct, oxt, oxtr, ozf, p,  
 p, p1, p15, p16, p167, p28, p2rx3, p2rx4, p2ry1, p2ry2, p2ry4, p2ry7, p2u, p2x3,  
 p2x4, p2y1, p2y2, p2y2, p2y4, p3p40phox, p450c11, p450c17, p450c2a, p450c2d,  
 20 p450c2e, p450sc, p4ha, p4ha1, p4ha1, p4hb, p5cdh, p79r, pa2g4, pab1, pab2,  
 pabp2, pabp11, pac1, pac1, pacapr, pace, pace4, paep, paf1, paf2, pafah, pafah1b1,  
 pafah1b2, pafah1b3, paga, pah, pahx, pai1, pai2, paics, pak1, pak3, palb, pals, pam,  
 pang, pap, papa, papa2, pappa, par1, par1, par2, par3, par4, par4, par5, park1, park2,  
 park3, pawr, pax1, pax2, pax3, pax4, pax5, pax6, pax7, pax8, pax9, pbca, pbca,  
 25 pbfe, pbg pbt, pbx1, pbx2, pbx3, pc, pc1, pc2, pc3, pc3, pcal, pcad, pcap, pcar1,  
 pcbe, pcbe, pcbe, pcca, pccb, pcdh7, pcdx, pche, pche1, pci, pck1, pc1,  
 pc1p, pcm1, pcm1, pcmt1, pcna, pcnt, pcolce, pcp, pcp4, pcs, pcsk1, pcsk2, pcsk3,  
 pcsk4, pcsk5, pcsk6, pck1, pck3, pcy1, pdc, pdc2, pdc, pdc, pdc1, pdc2, pddr,  
 pde1a, pde1b, pde1b1, pde3b, pde4a, pde4b, pde4c, pde4d, pde5a, pde6a, pde6b,  
 30 pde6c, pde6d, pde6g, pde6h, pde7a, pdea, pdea2, pdeb, pdeb, pdeg, pdeslb, pdgb,

-67-

pdgfa, pdgfb, pdgfr, pdgfra, pdgfrb, pdha1, pdha2, pdhb, pdj, pdk4, pdnp1, pdnp2,  
 pdnp3, pdr, pds, pds1, pdx1, pdyn, pel, peal5, pebp2a1, pebp2a3, pecam1, ped, ped,  
 pedf, pee, peg1, peg3, pemp, penk, pent, peo, peo1, peo2, pepa, pepb, pepc, pepd,  
 pepe, pepn, peps, per, per2, peta3, pets1, pex1, pex5, pex6, pex7, pf4, pf4v1, pfas,  
 5 pfb1, pfc, pfd, pfhb1, pfic1, pfic2, pfkfb1, pfkfb2, pfk1, pfk-mn, pfkp, pfkx, pfl,  
 pfm, pfn1, pfn2, pfrx, pga3, pga4, pga5, pgam1, pgam2, pgamm, pgc, pgd, pgf, pgft,  
 pgk1, pgk2, pgka, pg1, pgl1, pgl2, pgm1, pgm2, pgm3, pgm5, pgn, pgp, pgp1, pgr,  
 pgs, pgt, pgy1, pgy3, pha1, pha2, pha2a, pha2b, phap1, phb, phc, phe1a, phe3, phex,  
 phf1, phhi, phhi, phk, phka1, phka2, phkb, phkd, phkg1, phkg2, ph1, phl11, phog,  
 10 phox1, phox2a, php, php1b, phpX, phyh, pi, pi10, pi3, pi4, pi5, pi6, pi7, pi8, pi9,  
 piga, pigc, pigf, pigh, pigr, pik3c2b, pik3ca, pik3r1, pik4cb, pi1, pim1, pin, pin1,  
 pin11, pip, pip5k1b, pir1, pir51, pit, pit1, pitpn, pitx1, pitx2, pitx3, pjs, pk1, pk120,  
 pk3, pk428, pkca, pkcb, pkcc, pkcg, pkcs1, pkd1, pkd2, pkd4, pkdts, pkhd1, pklr,  
 pkm2, pkp1, pks1, pks1, pks2, pkul, pl, pla2, pla2a, pla2b, pla2g1b, pla2g2a,  
 15 pla2g4, pla2g4a, pla2g5, pla21, pla21, plag1, plag11, planh1, planh2, planh3, plat,  
 plau, plaur, plb, plc, plc1, plc3, plc3b, plc3c, plc3d, plce, plcg1, plcg2, plcl, pld1, plec1,  
 plg, plgf, plg1, pli, pln, plod, plod2, plos1, plp, pls, pls1, plt1, pltn, pltp, plzf, pmca1,  
 pmca2, pmca3, pmca4, pmch, pmch11, pmch12, pmd, pme117, pmi1, pm1, pmm1,  
 pmm2, pmp2, pmp22, pmp35, pmp69, pmp70, pms1, pms2, pms11, pms12, pmx1,  
 20 pn1, pnd, pnem, pnkd, pnlip, pnmt, pnoc, pod1, podx1, pof, pof1, pol2rb, pola,  
 polb, pold1, pold2, pole, polg, polr2a, polr2c, polr2e, polr2g, polr2i, polrmt, polz,  
 pomc, pon, pon1, pon2, pon3, por, porc, potx, pou1f1, pou2af1, pou3f1, pou3f2,  
 pou3f3, pou3f4, pou4f1, pou4f3, pou5f1, pp, ppl4, pp2, pp4, pp5, ppac, ppard,  
 pparg, pparg1, pparg2, ppat, ppbp, ppcd, ppd, ppef1, ppef2, ppfia3, ppgb, pph, pph1,  
 25 ppia, ppid, ppil1, ppkb, ppks1, ppks2, pp1, ppl2, ppmx, ppnd, ppnoc, ppol1, ppox,  
 ppp1a, ppp1ca, ppp1cb, ppp1cc, ppp1r2, ppp1r5, ppp1r7, pppd1r8, ppp2b, ppp2ca,  
 ppp2cb, ppp2r1b, ppp2r4, ppp2r5a, ppp2r5b, ppp2r5c, ppp2r5d, ppp2r5e, ppp3ca,  
 ppp3cb, ppp3cc, pp3r1, ppp4c, ppp5c, ppt, ppt2, ppx, ppy, ppyr1, pr@, prad1, prb1,  
 prb2, prb3, prb4, prca1, prca2, prcc, prcp, pre1p, prep, prf1, prg, prg1, prg1, prgs,  
 30 prh1, prh2, prim1, prim2a, prim2b, prip, prk1, prkaa1, prkaa2, prkab1, prkaca,

-68-

prkacb, prkacg, prkag1, prkag2, prkar1a, prkar1b, prkar2b, prkca, prkcb1, prkcd,  
 prkeg, prkci, prkcl1, prkenh1, prkcq, prkesh, prkdc, prkg1, prkg1b, prkg2, prkgr1b,  
 prkgr2, prkm1, prkm3, prkm4, prkm9, prkn, prkr, prkx, prky, prl, prlr, prml, prm2,  
 prmt2, prnp, proa, proc, prodh, prohb, prop1, pros1, pros30, prox1, prp8, prph,  
 5 prps1, prps2, pipsap1, prr1, prr2, prs, prsc1, prss1, prssl1, prss2, prss7, prss8, prssl1,  
 prtn3, prts, psa, psa, psach, psap, psbg1, psbg2, psc2, psc5, psca, psd, psen1, psen2,  
 psf1, psf2, psg1, psg11, psg12, psg13, psg2, psg3, psg4, psg5, psg6, psg7, psg8,  
 psg11, pskh1, psm, psma1, psma2, psma3, psma5, psmb1, psmb10, psmb2, psmb3,  
 psmb4, psmb5, psmb8, psmb9, psmc1, psmc2, psmc3, psmc5, psmd7, psmd9,  
 10 psmel, psme2, psors1, psors2, psors3, psp, psps1, psps2, pss1, psst, pst, pst, pst1,  
 psti, ptafr, ptc, ptc, ptc, ptch, ptd, pten, ptgds, ptger1, ptger2, ptger3, ptgir, ptgfrn,  
 ptgir, ptgs1, ptgs2, pth, pthlh, pthr, pthr1, pthr2, ptk1, ptk2, ptk2b, ptk3, ptk7, ptlah,  
 ptma, ptms, ptn, ptos1, ptp18, ptp1b, ptp4a1, ptp4a2, ptpa, ptpa, ptpd, ptpg, ptpg1,  
 ptpgmcl, ptpn1, ptpn10, ptpn11, ptpn12, ptpn13, ptpn14, ptpn2, ptpn5, ptpn6,  
 15 ptpn7, ptpra, ptprb, ptprc, ptprcap, ptprd, ptpre, ptprf, ptprg, ptprh, ptpri, ptpri,  
 ptpri1, ptpri2, ptpri, ptpri, ptpri, ptpri, ptpri, ptpri, ptpri, ptpri, ptpri, ptpri,  
 pum, pur1, pur1, pura, pvalb, pvr, pvr11, pvr12, pvr1, pvr2, pvs, pvt1, pwer, pwp2,  
 pwp2h, pws, pxaal1, pxe, pxe1, pxf, pxmp1, pxmp11, pxmp3, pxr1, pyer1, pyes,  
 pygb, pyg1, pygm, pyk2, pyst1, pyst2, pzp, qars, qdpr, qin, qm, qpc, qprs, rab, rab1,  
 20 rab13, rab1a, rab21, rab3a, rab3b, rab4, rab5, rab5a, rab6, rab7, rabgdl, rabgdib,  
 rabggt, rabggtb, rabif, rac2, rac3, rad1, rad17, rad23a, rad23b, rad51a, rad51c,  
 rad51d, rad5311, rad52, rad54, rad6a, rad6b, raf1, rafal, rag1, rag2, rage, rala, ralb,  
 ralgds, ramp, ranbp211, ranbp3, rao, rap1a, rap1b, rap1ga1, rap1gds1, rap2a, rap74,  
 rapsn, rara, rarb, rarg, rars, rasal, rasa2, rasgr3, rask2, rb1, rbbp2, rbbp5, rbbp6,  
 25 rb11, rb12, rbm1, rbm2, rbm3, rbmyl1, rbp1, rbp2, rbp3, rbp4, rbp5, rbp56, rbp6,  
 rbq3, rbtl1, rbtl11, rbtl12, rc1, rcac, rccl, rccl1, rccl2, red1, red2, redp1, rcn1,  
 rcn2, rcn, rcv1, rd, rdbp, rdc7, rdp, rdpa, rdrc, rds, rdt, rdx, reca, reccl1, recq1, red1,  
 red2, reg, reg1a, reg1, rel, rela, reln, ren, renbp, rens1, rent1, rep8, req, ret, rev3,  
 rev31, rfc1, rfc2, rfc3, rfc4, rfc5, rfp, rfx1, rfx2, rfx5, rfxank, rfxap, rgcl, rgr, rgs,  
 30 rgs1, rgs14, rgs16, rgs2, rgs2, rgs3, rgs5, rh50a, rhag, rhbd1, rhc, rhce, rhd, rheb2,

-69-

rho, rho7, rhogap2, rhogap3, rhoh12, rhoh6, rhoh9, rhok, rhom1, rhom2, rhom3,  
 rieg1, rieg2, rige, rigui, ring1, ring10, ring11, ring12, ring3, ring31, ring4, ring5,  
 ring6, ring7, rip, rip140, riz, rk, r1, rlb1, rlf, rln1, rln2, rmch1, rmd1, rmrp, rmrpr,  
 rn5s1@, mase1, mase2, mase3, mase4, mase5, mase6, mase1, maseli, mel, mfl,  
 5 mfl3, mfl4, mfl5, mnh, mpep, mpulz, mnr1, mnr2, mnr3, mnr4, mnr5, ms1, ms2, ms3, ms4,  
 ms4, ms4i, mntmi, mu1, mu15a, mu17a, mu17b, muna, mu2, mu3, ro52, rom1,  
 romk1, ron, ror1, rora, rorb, rorc, rorg, ros1, rosp1, rox, rp1, rp10, rp105, rp11, rp12,  
 rp13, rp14, rp15, rp17, rp18, rp19, rp2, rp22, rp24, rp25, rp3, rp4, rp6, rp7, rp9,  
 rpa1, rpa2, rpa3, rpd311, rpe, rpe65, rpe119rp122, rp123a, rp1231, rp129, rp130,  
 10 rp135a, rp136a, rp17a, rpms12, rpn1, rpn2, rpo12, rps11, rps14, rps17, rps17a,  
 rps17b, rps1711, rps1712, rps18, rps20a, rps20b, rps24, rps25, rps3, rps4x, rps4y,  
 rps6, rps6ka1, rps6ka2, rps6ka3, rps8, rpsm12, rptpm, rpul, rpx, rrad, rras, rrbp1,  
 rreb1, rrm1, rrm2, rrp, rrp22, rs1, rs1, rscl1, rsk1, rsk2, rsk3, rsn, rss, rsts, rsul, rt6,  
 rtefl, rtkn, rtn1, rtn2, rts, rts, rtt, rws, rxra, rxrb, rxrg, ryr1, ryr2, ryr3, rzrb, rzrg,  
 15 s100a1, s100a10, s100a11, s100a12, s100a13, s100a2, s100a3, s100a4, s100a5,  
 s100a6, s100a7, s100a8, s100a9, s100b, s100d, s100e, s100, s100p, s152, s4, s7,  
 saa1, saa2, saa4, sacs, safb, sag, sah, sahh, sail, sakap84, sal11, sal12, sams1,  
 sams2, sap, sap1, sap1, sap2, sap62, sar, sar1, sar2, sard, sas, sat, satb1, satt, sbma,  
 sc, scl1, sc5d1, sca1, sca10, sca2, sca2, sca3, sca4, sca5, sca6, sca7, sca8, sca8, scar,  
 20 scca1, scca2, sccd, scd, sceh, scg1, scg2, scg3, schad, scida, scidx, scidx1, scl1, sclcl1,  
 scl1, scn, scn1a, scn1b, scn2a, scn2a1, scn2a2, scn2b, scn3a, scn4a, scn5a, scn6a,  
 scn8a, scnn1a, scnn1b, scnn1d, scnn1g, scot, scp, scp1, scp2, scpn, scral, scral, scs,  
 sctr, scya1, scya11, scya13, scya14, scya15, scya16, scya19, scya2, scya21, scya22,  
 scya24, scya25, scya3, scya311, scya4, scya5, scya7, scya8, scyb5, scyb6, scyd1,  
 25 sczd1, sczd2, sczd3, sczd4, sczd5, sczd6, sczd7, sczd8, sdc1, sdc2, sdc4, sdf1, sdf2,  
 sdh1, sdh2, sdha, sdhb, sdhc, sdhd, sdhf, sds22, sdty3, sdys, se, sea, sec1311, sec13r,  
 sec14l, sec7, sed1, sedt, sef2, sel11, sele, sell, selp, selp1g, sema3f, sema4, sema5,  
 semg, semg1, semg2, sen1, sep, seppl, sercal, serca3, serk1, ses1, set, sex, sf, sfl,  
 sfal, sfd, sfmd, sfrs1, sfrs2, sfrs7, sftb3, sftp1, sftp2, sftp4, sftpa1, sftpa2, sftpb,  
 30 sftpc, sftpd, sgb, sgca, sgcb, sgcd, sgcg, sgd, sgk, sgl1, sgl2, sgm1, sgne1, sgp2,

-70-

sgpa, sgsh, sh2d1a, sh3bp2, sh3d1a, sh3gbr, sh3p17, shb, shbg, shc1, shc11, shfd1, shfd2, shfm1, shfm2, shfm3, shh, ship, shmt1, shmt2, shoc2, shot, shox, shox2, shps1, shs, shsf1, si, siah1, siah2, siasd, siat1, siat4, siat4c, siat8, sids, sil, silv, sim1, sim2, sipa1, sis, siv, six1, six5, sja, sjs, ski, ski2, ski2w, skiv21, skp1a, skp1b, skp2, sla, slap, slbp, slc, slc10a1, slc10a2, slc12a1, slc12a2, slc12a3, slc14a1, slc14a2, slc15a1, slc16a1, slc16a2, slc17a1, slc17a2, slc18a1, slc18a2, slc18a3, slc19a1, slc1a1, slc1a2, slc1a3, slc1a4, slc1a5, slc20a1, slc20a2, slc20a3, slc21a2, slc21a3, slc22a1, slc22a2, slc22a5, slc2a1, slc2a2, slc2a3, slc2a4, slc2a5, slc2c, slc3a1, slc4a1, slc4a2, slc4a6, slc5a1, slc5a2, slc5a3, slc5a5, slc6a1, slc6a10, slc6a12, slc6a2, slc6a3, slc6a4, slc6a6, slc6a8, slc6a9, slc7a1, slc7a2, slc7a4, slc7a5, slc7a7, slc8a1, slc8a2, slc9a1, slc9a2, slc9a3, slc9a4, slc9a5, sld, sle1, sleb1, slim1, sln, slo, slos, slp76, sls, slug, sm1, sm22, sma4, smad1, smad1, smad2, smad3, smad4, smad5, smad6, smad7, smad9, sma1, smam1, smarcal, smarca2, smarca3, smarca5, smarcb1, smax2, smc1, smcc, smcr, smcx, smcy, sm11, smn, smn1, smn2, smnr, smo, smoh, smpd1, sms, smt3, smt3h1, smtn, smubp2, sn, snap25, snat, snca, sncb, sncg, snf2h, snf211, snf212, snf213, snf5, sn1, snn, snrp70, snrpa, snrpe, snrpn, snt1, snt2b1, snt2b2, sntb1, snt1, snx, soat, sod1, sod2, sod3, solh, son, sord, sor11, sos1, sos2, sox1, sox10, sox11, sox2, sox20, sox22, sox3, sox4, sox9, sp1, sp1, sp3, sp3, sp4, spa1, spag1, spag4, spam1, sparc, spat, spbp, spch1, spd, spf30, spg3a, spg4, spg5a, spg6, spg7, spg8, spg9, spgp, spgyla, sph2, spil, spink1, spk, spmd, spn, spp1, spp2, sppm, spr, sprk, sprr1a, sprr1b, sprr2a, sprr2b, sprr2c, sprr3, sps1, spsma, sptal, sptan1, sptb, sptbn1, sra1, sra2, src, src1, src1, src2, srd5a1, srd5a2, srebf1, srebf2, sri, srk, srm, sm1, srp14, srp19, srp46, srpr, srpx, srs, srvx, sry, ss, ss, ssa, ssa1, ssa2, ssadh, ssav1, ssbp, ssdd, ssr2, ssr2, sstr1, sstr2, sstr3, sstr4, sstr5, ssx1, ssxt, st2, st3, st4, st5, st6, st8, sta, stac, stam, star, stat, stat1, stat3, stat4, stat5, stx1, stc1, stch, std, std, ste, step, stf1, stfa, stfb, stgd1, stgd2, stgd3, stgd4, sthe, stk1, stk11, stk15, stk2, stk6, st1, stm, stm2, stm7, stmy1, stmy2, stmy3, stp, stp1, stp2, sts, sts1, stx, stxlb, stx7, stxbp1, stxbp2, sultcl1, supt6h, sur, sur1, surf1, surf2, surf3, surf4, surf5, surf6, svct2, svmt, sw, sxi2, syb1, syb2, syb11, sycp1, syk, sym1, syn1, syn2, syn3, syngap, syns1, syp, syt, syt1, syt2, syt3, syt4, syt5, t, t3d, taal6,

-71-

tac1r, tac2, tac2r, tac3, tacr1, tacr2, taf2, taf2a, taf2a, taf2d, taf2h, taf2n, tafii100,  
tagln, tak1, tal1, tal2, taldo1, tam, tan1, tap1, tap2, tapa1, tapbp, tapvr1, tars, tas,  
task, tat, taut, tax, tax1, taz, tbg, tbp, tbp1, tbs, tbx1, tbx2, tbx3, tbx5, tbxa2r, tbxas1,  
tc1, tc2, tcbp, tcd, tceal, tceb11, tceb3, tcfl, tcfl2, tcfl3, tcfl311, tcfl4, tcfl5, tcfl7,  
5 tcfl9, tcfl2, tcfl20, tcfl21, tcfl3, tcfl4, tcfl5, tcfl611, tcfl612, tcfl7, tcfl8, tcfl9, tcfeb, tcfl1,  
tcfl14, tcfl1, tcfl1a, tcfl2, tcfl3, tcfl4, tcfl5, tcn1, tcn2, tco, tcof1, tcp1, tcp10, tcp11,  
top228, topt, toera, toerb, toerd, toerg, tocrz, tocs1, tocta, tocte1, tocte3, tocte11, tdf, tdfa, tdfx,  
tdg, tdgf1, tdn, tdo, tdo2, tdt, tead4, tee, tec, teck, tecta, tef, tegt, tek, tel, tem, tep1,  
terc, terf1, tert, tes1, tesk1, tex28, tf, tf2s, tf6, tfa, tfam, tfap2a, tfap2b, tfap2c, tfap4,  
10 tfcoup1, tfcoup2, tfcp2, tfdp1, tfdp2, tfe3, tff1, tff2, tff3, tfiia, tfn, tfpi, tfpi2, tfr,  
tfrc, tfs1, tft, tg, tg737, tgb1, tgb2, tgd, tgfa, tgfb1, tgfb2, tgfb3, tgfb4, tgfb1, tgfb1,  
tgfb2, tgfb3, tgfbre, tgfr, tgm1, tgm2, tgm3, tgm4, tgn38, tgn46, th, thas, thbd,  
thbp1, thbs1, thbs2, thbs3, thc, thh, th1, thop1, thpo, thr1, thra, thral, thral, thrb,  
thrm, thrsp, thy1, tial1, tiam1, tiar, tic, tie, tie1, tie2, tigr, til, til3, til4, tim, timp,  
15 timp1, timp2, timp3, tinur, titf1, titf2, tjp1, tk1, tk2, tkc, tkcr, tkr, tkt, tkt2, tkt11,  
tla519, tlen, tle1, tle2, tle3, tlh1, tln, tlr1, tlr2, tlr3, tlr4, tlr5, tm4sf1, tm4sf2, tm7sf2,  
tmc, tmd, tmdci, tmem1, tmf1, tmip, tmod, tmp, tmpo, tmprss2, tms, tmsa, tmsb,  
tmvcf, tna, tndm, tnf, tnfa, tnfaip1, tnfaip2, tnfaip4, tnfaip6, tnfar, tnfb, tnfb, tnfc,  
tnfcr, tnfr1, tnfr2, tnfrsf10b, tnfrsf12, tnfrsf14, tnfrsf16, tnfrsf17, tnfrsf1a, tnfrsf1b,  
20 tnfrsf4, tnfrsf5, tnfrsf6, tnfrsf6b, tnfrsf7, tnfrsf8, tnfrsf9, tnfsf11, tnfsf12, tnfsf5,  
tnfsf6, tnfsf7, tnnc1, tnnc2, tnnc1, tnnc2, tnnc3, tnnt1, tnnt2, tnnt3, tnp1, tnp2, tnr,  
tns, tn timer, tn timer, tn timer, tn timer, tn timer, tn timer, tn timer, tn timer, tn timer, tn timer,  
tp1, tp120, tp250, tp53, tp53bp2, tp63, tp73, tpa, tpbg, tpc, tpc, tph, tph2, tp11, tp12, tpm1, tpm2, tpm3, tpm4, tpmt,  
tpo, tpo, tpp2, tpr, tpr1, tprd, tps1, tps2, tpsn, tpst1, tpst2, tpt, tpt1, tptps, tpx, tpx1,  
25 tr, tr2, tr4, tral, traf1, traf5, trail2, tran, trance, trap170, tre3, tre8, tre, treb36, trek,  
trf1, trgl, trh, trhr, tric5, trio, trip1, trip14, trip6, trk, trk1, trka, trkb, trkc, trke, trl1,  
trl2, trm1, trm1, trm2, trma, trmi1, trmi2, trn, trn1, tro, trp1, trp1, trp2, trp3, trpc1,  
trpm2, trpo, trps1, trps2, trq1, trr, trr3, trrap, trsp, trtl, trt2, trv1, trv2, trv3, trv4, trv5,  
try1, try2, ts, ts13, ts546, tsbn51, tsc tsc1, tsc2, tsd, tse1, tsg101, tsg7, tshb, tshr, tsix,  
30 tsp3, tspp, tscc3, tst1, tst1, tsta3, tsy, tte1, tte3, ttf, ttf1, ttf2, ttg2, ttim1, ttin, ttp, ttp1,

-72-

ttpa, ttr, tuba3, tubal1, tubb, tufm, tuft1, tulp1, tuple1, tw, tweak, twik1, twist,  
 txgp11, txk, txn, txnr, txnrd1, tyh, tyk1, tyk2, tyk3, tyms, tyr, tyr1, tyro3, tyrp1,  
 tyrp2, tys, u17hg, ulmp, u22hg, u2afl, u2aflrs1, u2aflrs2, u2aflrs3, uba52, ubb, ubc,  
 ubc4, ubc7, ubc8, ubch2, ubc1, ube1, ube2, ube2a, ube2b, ube2e2, ube2g, ube2g2,  
 5 ube2h, ube2i, ube211, ube2v1, ube3a, ubh1, ubid4, ub11, uch11, ucn, ucp1, ucp2,  
 ucp3, udpgdh, uev1, ufd11, ufs, ugalt, ugb, ugcg, ugdh, ugn, ugp1, ugp2, ugpp2,  
 ugt1, ugt1a1, ugt2b11, ugt2b15, ugt2b17, ugt2b4, ugt2b7, ugt2b8, ugt2b9, ugt1, uhg,  
 uhx1, ukhc, umod, umph2, umpk, umps, unc18, unc18b, und, ung, unr, unr, uox, up,  
 upk1b, ups, uqbp, uqerb, uqerc1, uqerc2, uqerfs1, uqor1, uqor13, uqor22, urk, urkr,  
 10 uroc, urod, uros, usf1, usf2, ush1, ush1a, ush1b, ush1c, ush1d, ush1e, ush1f, ush2a,  
 ush3, uspl1, usp5, usp7, usp9x, usp9y, ut1, ut2, ute, utr, utr, utx, uty, uv20, uv24,  
 uvo, vacht, vacm1, vamp1, vamp2, vars1, vasp, vat1, vat2, vav, vav1, vav2, vbch,  
 vbp1, vcam1, vcf, vcl, vcp, vdac1, vdac2, vdd1, vdi, vdr, vegf, vegfb, vegfd, vegfr3,  
 vgf, vgl, vgr1, vhl, vhr, vill, vil2, vim, vip, vipr1, vipr2, vis1, vla1, vla5a, vlacs,  
 15 vlcad, vldlr, vmat1, vmcm, vmd1, vmd2, vnra, vnt, vp, vpp1, vpp3, vpreb1, vpreb2,  
 vrf, vrk1, vrk2, vrnf, vmi, vsn11, vtn, vwf, vws, waf1, wars, was, wbs, wd1, wdr2,  
 weel, wfis, wfs, wfs1, wgn1, whcr, wi, wispl1, wispl2, wispl3, wnd, wnt1, wnt10b,  
 wnt13, wnt14, wnt15, wnt2, wnt3, wnt5a, wnt7a, wnt7b, wnt8b, wrb, wrn, ws1,  
 ws2a, ws2b, ws4, wsn, wss, wss, wt1, wt2, wt3, wt4, wt5, wts, wts1, wws, x11,  
 20 xbp1, xbp2, xce, xdh, xe169, xe7, xe7y, xg, xgr, xh2, xiap, xic, xist, xk, xla, xla2,  
 xlp, xlpd, xlrs1, xm, xpa, xpb, xpc, xpcc, xpct, xpf, xpf, xpg, xpmc2h, xpnpep2,  
 xpo1, xrcc1, xrcc2, xrcc3, xrcc4, xrcc5, xrcc9, xrs, xs, xwnt2, yb1, yes1, yk140, y11,  
 yrrm1, yt, ywha1, ywhab, ywhah, ywhaz, yy1, zac, zag, zan, zap70, zf87, zfm1,  
 zfp3, zfp36, zfp37, zfx, zfy, zic1, zic2, zic3, zipk, znf1, znf10, znf117, znf11a,  
 25 znf11b, znf12, znf121, znf123, znf124, znf125, znf126, znf13, znf14, znf141,  
 znf144, znf146, znf147, znf157, znf16, znf160, znf162, znf163, znf165, znf169,  
 znf173, znf179, znf189, znf19, znf192, znf193, znf195, znf198, znf2, znf20, znf200,  
 znf204, znf217, znf22, znf23, znf24, znf25, znf26, znf27, znf29, znf3, znf32, znf34,  
 znf35, znf36, znf38, znf4, znf40, znf41, znf42, znf44, znf45, znf46, znf5, znf6,  
 30 znf69, znf7, znf70, znf71, znf72, znf73, znf74, znf75, znf75a, znf75c, znf76, znf77,

-73-

znf79, znf8, znf80, znf81, znf83, znf9, znfc150, znfc25, znfxy, znt3, znt4, zp3a, zp3b, zpk, zws1, and zyx.

Furthermore, genes from bacteria, plants, yeast, and mammals (e.g., mice) can be used with the microorganisms provided herein. Non-limiting examples of *E. coli* genes include:

5 aarF, aas, aat, abpS, abs, accA, accB, accC, accD, acd, aceA, aceB, aceE, aceF, aceK, ackA, ackB, acnA, acnB, acpD, acpP, acpS, acpX, acrA, acrB, acrC, acrD, acrE, acrF, acrR, acs, ada, add, adhB, adhC, adhE, adhR, adiA, adiY, adk, aegA, aer, aes, agaA, agaB, agaC, agaD, agaI, agaR, agaS, agaV, agaW, agaZ, agp, ahpC, ahpF, aidB, ais, alaS, alaT, alaU, alaV, alaW, alaX, aldA, aldB,

10 aldH, alkA, alkB, alpA, alr, alsA, alsB, alsC, alsE, alsK, alx, amiA, amiB, amn, ampC, ampD, ampE, ampG, ampH, amtB, amyA, ansA, ansB, apaG, apaH, aphA, appA, appB, appC, appY, apt, aqpZ, araA, araB, araC, araD, araE, araF, araG, araH, araJ, arcA, arcB, argA, argB, argC, argD, argE, argF, argG, argH, argI, argM, argP, argQ, argR, argS, argT, argU, argV, argW, argX, argY, argZ, aroA, aroB, aroC,

15 aroD, aroE, aroF, aroG, aroH, aroI, aroK, aroL, aroM, aroP, aroT, arsB, arsC, arsR, artI, artJ, artM, artP, artQ, ascB, ascF, ascG, asd, aslA, aslB, asmA, asnA, asnB, asnC, asnS, asnT, asnU, asnV, asnW, aspA, aspC, aspS, aspT, aspU, aspV, asr, asu, atoA, atoB, atoC, atoD, atoS, atpA, atpB, atpC, atpD, atpE, atpF, atpG, atpH, atpI, avtA, azaA, azaB, azi, bacA, baeR, baeS, barA, basR, basS, bax, bcp, bcr, betA,

20 betB, betI, betT, bfd, bfm, bfr, bglA, bglB, bglF, bglG, bglI, bglT, bglX, bioA, bioB, bioC, bioD, bioF, bioH, bioP, bipA, birA, bisC, bisZ, blc, bolA, brnQ, brnR, brnS, brnT, btuB, btuE, btuR, bymA, cadA, cadB, cadC, cafA, caiA, caiB, caiC, caiD, caiE, caiF, caiT, calA, caiC, calD, can, carA, carB, cbl, cbpA, cbt, cca, ccmA, ccmB, ccmC, ccmD, ccmE, ccmF, ccmG, ccmH, cdd, cde, cdh, cdsA, cdsS,

25 cedA, celA, celB, celC, celD, celF, cfa, cfcA, chaA, chaB, chaC, cheA, cheB, cheR, cheW, cheY, cheZ, chpA, chpB, chpR, chpS, cirA, citA, citB, cld, cipA, clpB, clpP, clpX, cls, cmk, cmlA, cmr, cmtA, cmtB, coaA, cobS, cobT, cobU, codA, codB, cof, cog?, corA, cpdA, cpdB, cpsA, cpsB, cpsC, cpsD, cpsE, cpsF, cpsG, cpxA, cpxB, cpxP, cpxR, crcA, crcB, creA, creB, creC, creD, erg, cri, crp, crr, csdA, csgA, csgB,

30 csgD, csgE, csgF, csgG, csiA, csiB, csiC, csiD, csiE, csiF, cspA, cspB, cspC, cspD,

-74-

- cspE, cspG, csrA, csrB, cstA, cstC, cup, cutA, cutC, cutE, cutF, cvaA(ColV),  
 cvaB(ColV), cvaC(Co-IV), cvi(ColV), cvpA, cxm, cyaA, cybB, cybC, cycA, cydA,  
 cydB, cydC, cydD, cynR, cynS, cynT, cynX, cyoA, cyoB, cyoC, cyoD, cyoE, cysA,  
 cysB, cysC, cysD, cysE, cysG, cysH, cysI, cysJ, cysK, cysM, cysN, cysP, cysQ,  
 5 cysS, cysT, cysU, cysW, cysX?, cysZ?, cytR, dacA, dacB, dacC, dacD, dadA, dadB,  
 dadQ, dadX, dam, dapA, dapB, dapD, dapE, dapF, dbpA, ded, dcm, dcp, dcrB, dctA,  
 dctB, dcuA, dcuB, dcuC, ddlA, ddlB, ddpA, ddpB, ddpC, ddpD, ddpF, ddpX, deaD,  
 dedA, dedD, def, degP, degQ, degS, del, deoA, deoB, deoC, deoD, deoR, dfp, dgd,  
 dgkA, dgkR, dgoA, dgoD, dgoK, dgoR, dgoT, dgsA, dgt, dicA, dicB, dicC, dicF,  
 10 dinB, dinD, dinF, dinG, dinI, dinY, dipZ, djlA, dksA, dld, dmsA, dmsB, dmsC,  
 dnaA, dnaB, dnaC, dnaE, dnaG, dnaI, dnaJ, dnaK, dnaL, dnaN, dnaQ, dnaT, dnaX,  
 dppA, dppB, dppC, dppD, dppF, dppG, dps, dsbA, dsbB, dsbC, dsbG, dsdA, dsdC,  
 dsdX, dsrA, dsrB, dut, dvl, dxs, ebgA, ebgB, ebgC, ebgR, ecfa, eco, ecpD, eda, edd,  
 efp, emrA, emrB, emrD, emrE, endA, eno, entA, entB, entC, entD, entE, entF, envN  
 15 envP, envQ, envR, envT, envY, envZ, epd, EppA, minigene, EppB, minigene,  
 EppC, minigene, EppD, minigene, EppE, minigene, EppG, minigene, EppH,  
 minigene, era, esp, evgA, evgS, exbB, exbC, exbD, expA, exuR, exuT, fabA, fabB,  
 fabD, fabF, fabG, fabH, fabI, fabZ, fadA, fadB, fadD, fadE, fadH, fadL, fadR, farR,  
 fatA, fbaA, fbaB, fbp, fcl, fcsA, fdhD, fdhE, fdhF, fdnG, fdnH, fdnI, fdoG, fdoH,  
 20 fdoI, fdrA, fdx, feaB, feaR, fecA, fecB, fecC, fecD, fecE, fecI, fecR, feoA, feoB,  
 fepA, fepB, fepC, fepD, fepE, fepG, fes, fexB, ffh, ffs, fhIA, fhIB, fhuA, fhuB,  
 fhuD, fhuE, fhuF, fic, fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI, fipB,  
 fipC, fis, fiu, fixA, fixB, fixC, fixX, fklB, fkpA, fldA, flgA, flgB, flgC, flgD, flgE,  
 flgF, flgG, flgH, flgI, flgJ, flgK, flgL, flgM, flgN, flhA, flhB, flhc, flhD, fliA, fliC,  
 25 fliD, fliE, fliF, fliG, fliH, fliI, fliJ, fliK, fliL, fliM, fliN, fliO, flip, fliQ, fliR, fliS,  
 fliT, fliY, fliZ, flk, flu, fnt, fnr, focA, focB, folA, folC, folD, folE, folK, folP, folX,  
 fpr, frdA, frdB, frdC, frdD, frr, fruA, fruB, fruK, fruR, fsr, ftn, ftsA, ftsE, ftsI, ftsJ,  
 ftsK, ftsL, ftsN, ftsQ, ftsW, ftsX, ftsY, ftsZ, fucA, fucI, fucK, fucO, fucP, fucR,  
 fumA, fumB, fumC, fur, fusA, fusB, gabC, gabD, gabP, gabT, gadA, gadB, gadR,  
 30 galE, galF, galK, galM, galP, gaiR, galS, galT, galU, gapA, gapC, garA, garB, gata,

-75-

gatB, gatC, gatD, gatR, gatY, gatZ, gcd, gcl, gcpE, gcvA, gcvH, gcvP, gcvR, gcvT,  
 gdhA, gef, ggt, gidA, gidB, gip, glcB, glcC, glcD, glcE, glcG, gldA, glf, glgA, glgB,  
 glgC, glgP, glgS, glgX, glk, glmM, glmS, glmU, glmX, glnA, glnB, glnD, glnE,  
 glnG, glnH, glnK, glhL, glnP, glnQ, glnR, glnS, glnT, glnU, glnV, glnW, glnX,  
 5 gloA, glpA, glpB, glpC, glpD, gipE, gipF, gipG, glpK, glpQ, gipR, glpT, glpX,  
 gltA, gltB, gltD, gltE, gltF, gltH, gltJ, gltK, gltL, gltM, gltP, gltR, gltS, gltT, gltU,  
 gltv, gltW, gltX, glyA, glyQ, glyS, glyT, glyU, glyv, glyW, glyX, glyY, gmd, gmk,  
 gmm, gnd, gntK, gntP, gntR, gntS, gntT, gntU, gntV, goaG, gor, gph, gpmA, gpp,  
 gprA, gprB, gpsA, gpt, greA, greB, groL, groS, grpE, grxA, grxB, grxC, gshA,  
 10 gshB, gsk, gsp, gsp\*, gst, guaA, guaB, guaC, gurB, gurC, gutM, gutQ, gyrA, gyrB,  
 hcaB, hcaC, hcaD, hcaE, hcaF, hcaR, hcaT, hdeA, hdeB, hdeD, hdhA, helD, hemaA,  
 hemB, hemC, hemD, hemE, hemF, hemG, hemH, hemK, hemL, hemM, hemX,  
 hemY, hepA, het, hflB, hflC, hflK, hflX, hfq, hha, hipA, hipB, hisA, hisB, hisC,  
 hisD, hisF, hisG, hisH, hisI, hisJ, hisM, hisP, hisQ, hisR, hisS, hipA, hlyE, hmp, hns,  
 15 holA, holB, holC, holD, holE, hopB, hopC, hopD, hpt, hrpA, hrpB, hrsA, hscA,  
 hscB, hsdM, hsdR, hsdS, hslC, hslD?, hslE-H, hslJ, hslK, hslL-N, hslO-R, hslU,  
 hslV, hslW, htgA, htpG, htpX, htrB, htrC, htrE, htrL, hupA, hupB, hyaA, hyaB,  
 hyaC, hyaD, hyaE, hyaF, hybA, hybB, hybC, hybD, hybE, hybF, hybG, hycA,  
 hycB, hycC, hycD, hycE, hycF, hycG, hycH, hycl, hydA, hydG, hydH, hydN, hyfA,  
 20 hyfB, hyfC, hyfD, hyfE, hyfF, hyfG, hyfH, hyfI, hyfJ, hyfR, hypA, hypB, hypC,  
 hypD, hypE, hypF, iadA, iap, ibpA, ibpB, icd, iclR, ihfA, ihfB, ileR, ileS, ileT, ileU,  
 ileV, ileX, ileY, ilvA, ilvB, ilvC, ilvD, ilvE, ilvF, ilvG, ilvH, ilvI, ilvJ, ilvM, ilvN,  
 ilvR, ilvU, ilvY, imp, inaA, inaR?, infA, infB, infC, inm, insA(IS1), intA, isb(IS1),  
 isfA, ispA, ispB, KanR, katE, katG, kba, kbl, kch, kdgK, kdgR, kdgT, kdpA, kdpB,  
 25 kdpC, kdpD, kdpE, kdpF, kdsA, kdsB, kdtA, kdtB, kefB, kefC, kgtp, ksgA, ksgB,  
 ksgC, ksgD, lacA, lacI, lacY, lacZ, lamB, lar, ldcC, ldhA, lepA, lepB, leuA, leuB,  
 leuC, leuD, leuJ, leuO, leuP, leuQ, leuR, leuS, leuT, leuU, leuV, leuW, leuX, leuY,  
 leuZ, lev, lexA, lgt, lhr, ligA, ligT, linB, lipA, lipB, lit, livF, livG, livH, livJ, livK,  
 livM, lldD, lldP, lldR, lolA, lon, lpcA, lpcB, lpd, lplA, lpp, lpxA, lpxB, lpxC, lpxD,  
 30 lpxK, lrb, lrhA, lrp, Irs lspA, lysA, lysC, lysP, lysQ, lysR, lysS, lysT, lysU, lysV,

-76-

lysW, lysX, lysY, lysZ, lytA, lytB, lyx, maa, mac, mae, mafA, mafB, malE, malF, malG, malI, malK, malM, malP, malQ, malS, malT, malX, malY, malZ, manA, manC, manX, manY, manZ, map, marA, marB, marR, mbrB, mcrA, mcrB, mcrC, mcrD, mdaB, mdh, mdoB, mdoG, mdoH, meb, melA, melB, melR, menA, menB, menC, menD, menE, menF, mepA, mesJ, metA, metB, metC, metD, metE, metF, metG, metH, metJ, metK, metL, metR, metT, metU, metV, metW, metY, metZ, mfd, mglA, mglB, mglC, mglR, mgsA, mgtA, mhpA, mhpB, mhpC, mhpD, mhpE, mhpF, mhpR, miaA, miaD, micF, minC, minD, minE, mioC, mltA, mltB, mltC, mltD, mmrA(rhlB?), mng, mntA, moaA, moaB, moaC, moaD, moaE, mobA, mobB, moc, modA, modB, modC, modE, modF, moeA, moeB, mog, molR, motA, motB, mpl, mppA, mprA, mraA--?, mraY, mrcA, mrcB, mrdA, mrdB, mreB, mreC, mreD, mrr, msbA, msbB, mscL, msrA, msyB, mtg, mtgA, mtlA, mtlD, mtlR, mtr, mttA, mttB, mttC, mukB, mukE, mukF, mul, murA, murB, murC, murD, murE, murF, murG, murH, murI, mutG(putative), mutH, mutL, mutM, mutS, mutT, mutY, nac, nadA, nadB, nadC, nadE, nagA, nagB, nagC, nagD, nagE, nalB, nalD, nanA, nanE, nanK, nanR, nanT, napA, napB, napC, napD, napF, napG, napH, narG, narH, narI, narJ, narK, narL, narP, narQ, narU, narV, narW, narX, narY, narZ, ndh, ndk, neaB, nei, nema, nfi, nfnA, nfnB, nfo, nfrA, nfrB, nfrD, nfsA, nhaA, nhaB, nhaR, nika, nikB, nikC, nikD, nikaE, nirB, nirC, nirD, nlpA, nlpB, nlpC, nlpD, nmpC(qsr'), non, npr, nrdA, nrdB, nrdD, nrdE, nrdF, nrdG, nrfA, nrfB, nrfC, nrfD, nrfE, nrfF, nrfG, nth, ntpA, nuoA, nuoB, nuoC, nuoE, nuoF, nuoG, nuoH, nuoI, nuoJ, nuoK, nuoL, nuoM, nuoN, nupC, nupG, nusA, nusB, nusG, nuvA, nuvC, ogrK, ogt, ompA, ompC, ompF, ompG, ompR, ompT, ompX, oppA, oppB, oppC, oppD, oppE, oppF, opr, ops, oraA, ordL, orf-23(purB, reg)orf195(nikA-reg), orn, osmB, osmC, osmE, osmY, otsA, otsB, oxyR, oxyS, pabA, pabB, pabC, pac, pal, panB, panC, panD, panF, parC, parE, pat, pbpG, pck, pcm, pcnB, pdhR, pdxA, pdxB, pdxH, pdxJ, pdxK, pdxL, pdxY, pepA, pepD, pepE, pepN, pepP, pepQ, pepT, pfkA, pfkB, pflA, pflB, pflC, pflD, pfs, pgi, pgk, pgl, pgm, pgpA, pgpB, pgsA, pheA, pheP, pheS, pheT, pheU, pheV, phnC, phnD, phnE, phnF, phnG, phnH, phnI, phnJ, phnK, phnL, phnM, phnN, phnO, phnP, phoA, phoB, phoE, phoH, phoP, phoQ, phoR, phoU,

-77-

phrB, phxB, pin, pioO, pit, pldA, pldB, plsB, plsC, plsX, pmbA, pncA, pncB, pnp,  
 pntA, pntB, pnuC, poaR, polA, polB, popD, potA, potB, potC, potD, potE, potF,  
 potG, potH, potI, poxA, poxB, ppa, ppc, pphA, pphB, ppiA, ppiB, ppiC, ppk, pppA,  
 pps, ppx, pqiA, pqiB, pqqL, pqqM, prc, prfA, prfB, prfC, priA, priB, priC, prlC,  
 5 prlZ, prmA, prmB, proA, proB, proC, proK, proL, proM, proP, proQ, proS, proT,  
 proV, proW, proX, prpA, prpC, prpR, prr, prs, psd, psiF, pspA, pspB, pspC, pspE,  
 pspF, pssA, pssR, pstA, pstB, pstC, pstS, psu, pta, pth, ptrA, ptrB, ptsG, ptsH, ptsI,  
 ptsN"-", ptsP, purA, purB, purC, purD, purE, purF, purH, purK, purL, purM, purN,  
 purP, purR, purT, purU, pus, putA, putP, pykA, pykF, pyrB, pyrC, pyrD, pyrE,  
 10 pyrF, pyrG, pyrH, pyrI, qmeC, qmeD, qmeE, qor, queA, racC, racR, radA, radC,  
 ranA, rarD, ras, rbfA, rbn, rbsA, rbsB, rbsC, rbsD, rbsK, rbsR, rcsA, rcsB, rcsC,  
 rcsF, rdgA, rdgB, recA, recB, recC, recD, recE, recF, recG, recI, recN, recO, recQ,  
 recR, recT, relA, relB, relE, relF, relX, rep, rer, rfaB, rfaC, rfaD, rfaF, rfaG, rfaH,  
 rfaI, rfaJ, rfaK, rfaL, rfaP, rfaQ, rfaS, rfaY, rfaZ, rfbA, rfbB, rfbC, rfbD, rfbX, rfc,  
 15 rfe, rffA, rffC, rffD, rffE, rffG, rffH, rffM, rffT, rhaA, rhaB, rhaD, rhaR, rhaS, rhaT,  
 rhIB, rhIE, rho, ribA, ribB, ribC, ribD, ribE, ribF, ridA, ridB, rimB, rimC, rimD,  
 rimE, rimG, rimH, rimI, rimJ, rimK, rimL, rimM, rit, rlpA, rlpB, rluA, rluC, rluD,  
 rmf, rna, rnb, rnc, rnd, rne, rnhA, rnhB, rnk, rnpA, rnpB, rnr, rnt, rob, rorB, rpe, rph,  
 rpiA, rpiB, rpiR, rplA, rplB, rplC, rplD, rplE, rplF, rplI, rplJ, rplK, rplL, rplM, rplN,  
 20 rplO, rplP, rplQ, rplR, rplS, rplT, rplU, rplV, rplW, rplX, rplY, rpmA, rpmB, rpmC,  
 rpmD, rpmE, rpmF, rpmG, rpmH, rpmI, rpmJ, rpoA, rpoB, rpoC, rpoD, rpoE, rpoH,  
 rpoN, rpoS, rpoZ, rpsA, rpsB, rpsC, rpsD, rpsE, rpsF, rpsG, rpsH, rpsI, rpsJ, rpsK,  
 rpsL, rpsM, rpsN, rpsO, rpsP, rpsQ, rpsR, rpsS, rpsT, rpsU, rrfA, rrfB, rrfC, rrfD,  
 rrfE, rrfF, rrfG, rrfH, rrlA, rrlB, rrlC, rrlD, rrlE, rrlG, rrlH, rrmA, rrsA, rrsB, rrsC,  
 25 rrsD, rrsE, rrsG, rrsH, rsd, rseA, rseB, rseC, rspA, rspB, rssA, rssB, rsuA, rtcA, rtcB,  
 rtcR, rtn, rus(qsr'), ruvA, ruvB, ruvC, sad, sanA, sapA, sapB, sapC, sapD, sapF,  
 sbaA, sbcB, sbcC, sbcD, sbmA, sbmC(gyrI), sbp, sdaA, sdaB, sdaC, sdhA, sdhB,  
 sdhC, sdhD, sdiA, sds, secA, secB, secD, secE, secF, secG, secY, selA, selB, selC,  
 selD, semA, seqA, serA, serB, serC, serR, serS, serT, serU, serV, serW, serX, sfa,  
 30 sfcA, sfiC, sfsA, sfsB, shiA, sipC, sipD, sir, sixA, slbB, slp, slr, slt, slyD, slyX, smp,

-78-

smtA, sodA, sodB, sodC, sohA, sohB, solA, soxR, soxS, speA, speB, speC, speD, speE, speF, speG, spf, spoT, sppA, spr, srlA, srlB, srlD, srlE, srlR, srmB, srmA, ssaE, ssaG, ssaH, ssb, sseA, sseB, sspA, sspB, ssrA, ssrS, ssyA, ssyD stfZ, stkA, stkB, stkC, stkD, stpA, strC, strM, stsA, sucA, sucB, sucC, sucD, suff, sugE, suhA, 5 suhB, sulA, supQ, surA, surE, syd, tabC, tag, talA, talB, tanA, tanB, tap, tar, tas, tauA, tauB, tauC, tauD, tbaA, tdcA, tdcB, tdcC, tdcD, tdcE, tdcF, tdcG, tdcR, tdk, tdi tdk, tehA, tehB, tesA, tesB, tgt, thdA, thdC, thdD, thiB?, thiC, thiD, thiE, thiF, thiG, thiH, thiI, thiJ, thiK, thiL, thiM, thrA, thrB, thrC, thrS, thrT, thrU, thrV, thrW, thyA, tig, tktA, tktB, tldD, tlnA, tmk, tnaA, tnaB, tnaC, tnm, tol-orf1, tol-orf2, tolA, 10 tolB, tolC, tolD, tolE, toll, tolJ, tolM, tolQ, tolR, tonB, topA, topB, torA, torC, torD, torR, torS, torT, tpiA, tpr, tpx, treA, treB, treC, treF, treR, trg, trkA, trkD, trkG, trkH, trmA, trmB, trmC, trmD, trmE, trmF, trmH, trmU, trnA, trpA, trpB, trpC, trpD, trpE, trpR, trpS, trpT, truA, truB, trxA, trxB, trxC, tsaA, tsf, tsmA, tsr, tsx, ttdA, ttdB, ttk, tufA, tuffB, tus, tynA, tyrA, tyrB, tyrP, tyrR, tyrS, tyrT, tyrU, tyrV, 15 ubiA, ubiB, ubiC, ubiD, ubiE, ubiF, ubiG, ubiH, ubiX, ucpA[], udk, udp, ugpA, ugpB, ugpC, ugpE, ugpQ, uhpA, uhpB, uhpC, uhpT, uidA, uidB, uidR, umuC, umuD, ung, upp, uppS, ups, uraA, usg-1, usbA, uspA, uup, uvh, uvrA, uvrB, uvrC, uvrD, uvs, uxaA, uxaB, uxaC, uxuA, uxuB, uxuR, valS, valT, valU, valV, valW, valX, valY, valZ, vsr, wrbA, xapA, xapB, xapR, xasA, xerC, xerD, xni, xseA, xseB, 20 xthA, xylA, xylB, xylE, xylF, xylG, xylH, xylR, yccA, yhhP, yihG, yjaB, fl47, yjaD, yohF, yqiE, yrfE, zipA, zntA, znuA, znuB, znuC, zur, and zwf.

Non-limiting examples of mouse genes include: Ilr1, Ilr2, Gas10, Tnp1, Inhbb, Inha, Creb1, Mpmv34, Acrd, Acrg, Il110, Otf1, Rab11b-r, Abl1, ald, Amh-rs1, Bcl2B, Cchla3, Ccnb1-rs2, Gpcr16, Htr5b, Idd5, Igfbp2, Igfbp5, Il8rb, Kras2-rs1, Mov7, Mpmv6, Mpmv16, Mpmv22, Mpmv25, Mpmv29, Mpmv42, Mtv7, 25 Mtv27, Mtv39, Oprk1, Otf3-rs1, Otf8, Otf11-rs1, Ptgs2, Ren1, Ren2, Ril3, Sxv, Taz4-rs1, Tgfb2, Wnt6, Xmmv6, Xmmv9, Xmmv36, Xmmv61, Xmmv74, Xmv21, Xmv32, Xmv41, Il12ra, Ab1, Mpmv3, Rap1a-ps2, anx, Mpmv43, Ryr3, Ras12-4, Adra2b, Avp, Glvr1, Il1a, Il1b, Mpmv28, Oxt, Pcsk2, a, Xmv10, Tcf4, Acra, Acra4, 30 Ak1, Bdnf, bs, Cyct, Cyp24, Dbh, Fshb, Geg, Gdf5, Gnass, Gpcr8, Grin1, Hcs4,

-79-

- Hior2, Hsp84-2, Idd12, Ilrn, Jund2, Kras3, Mc3r, Mpmv14, Mtv40, Mxil-rs1, Otf3-rs2, Ptgs1, Ptpa, Rapsn, Src, Svp1, Svp3, Tcf3b, Wt1, Xmmv71, Xmv48, Cena, Fgf2, Fth-rs1, Csfm, Mov10, Egf, Acrb2, Cap1, Crh, Fim3, Fps11, Glut2, Gpcr2, Gria2, Hsd3b-1, Hsd3b-2, Hsd3b-3, Hsd3b-4, Hsp86-ps2, Idd3, I12, I17, Mpvvmv9,
- 5 Mpmv20, Mtv4.8, Ngfb, Npra, Nras, Nras, Ntrk, Otf3-rs3, Otf3-rs4, Rap1a, Tshb, Xmmv22, Xmmv65, Mos, Ras12-7, Lyr, Ifa, Ifb, Jun, azh, db, Ipp, Mp1, Do1, Ak2, Ccnb1-rs4, Cdc211, Cga, Fgr, Foc1, Fps12, Gabrr1, Gabrr2, Gdf6, Glut1, Gnb1, Gpcr14, Grb2-ps, Grik3, Grik5, Hsp86-1ps4, Htr1da, Htr1db, Idd9, Ifa1, Ifa2, Ifa3, Ifa4, Ifa5, Ifa6, Ifa7, Ifa8, Ifa9, Ifa10, Lap18, Lmyc1, Mpmv19, Mpmv44,
- 10 Mtv13, Mtv14, Mtv17, Nppb, Otf6, Otf7, Ri12, Ski, Tnfr2, Wnt4, Xmmv8, Xmmv23, Xmmv62, Xmv1, Xmv2, Xmv8, Xmv9, Xmv14, Xmv44, Xpa, Tec, Fgf5, Nos1, Tcf1, Epo, Gnb2, Flt1, Flt3, Ache, Adra2c, Adrbk2, Afp, Alb1, Ccnb1-rs1, Clock, Cyp3, Cyp3a11, Cyp3a13, Drd1b, Drd5, Fgfr3, Flk1, Gc, Gnrhr, Gpcr1, Hcs5, Hnfl, Htr5a, I15r, I16, Kit, Ltrn3, Mgsa, Mpmv7, Mpmv13, Mpmv23,
- 15 Mtv32, Mtv41, Pdgfa, Pdgfra, Por, Txk, Xmmv3, Xmmv5, Xmmv52, Xmv17, Xmv28, Xmv34, Xmv38, Xmv45, Zp3, Trh, Raf1, Fth-rs2, Ntf3, Kras2, Pthlh, Mov1, Alox5, Braf2, Cfr, Egr4, Fps110, Fgf6, Gdf3, Ghrfr, Glut3, Grin2a, Hior3, Hoxa10, hop, Ica1, I15r, Int41, Itpr1, Krag, Mad, Met, Mi, Mtv8, Mtv23, Mtv29, Mtv33, Mtv34, Nkna, Npy, ob, Otf3-rs5, Tgfa, Tnfr1, Wnt2, Wnt5B, Wnt7A,
- 20 Xmmv27, Xmv24, Xmv61, Fosb, Ryr1, Ngfa, Ufo, Xrcc1, Abpa, Abpga, Gabra4, Gas2, Acra7, Ccnb1-rs7, Egfbp3, Xmv30, Zp2, Fes, Pcsk3, Calc, Ccnb1-rs10, Pth, Ad, Bcl3, Cea, Cea2, Cea3, Cea4, Cea5, Cea6, Cebp, Dm9, Dm15, Drd4, Egfbp1, Egfbp2, Ercc2, Fgf3, Fgfr2, Gabra5, Gabrb3, Gtx, Hcs1, Igflr, Igf2, I14r, Ins2, Int40, Lhb, Mpmv1, Mtv1, Mtv35, Ngfg, Ntf5, Otf2, 2, Pkcc, Ras14, Rras, Ryr,
- 25 Svp2, Tcf3g, Tgfb1, tub, Xmmv31, Xmmv35, Xmmv73, Xmv33, Xmv53, Taz83, Adrb3, Junb, Jund1, Mel, Gpcr19-rs2, Agt, Cadp, Ccnb1-rs9, E, Fgfr1, Gas6, Gnb-rs1, Hcs2, Insr, Maf, Mov34, Mpmv21, Mpmv41, Mtv21, Mtnr1a, Plat, Ras15-2, Ras16, Sntb2, Xmmv29, Xmv12, Xmv26, Xmv62, Epdr, Gpcr13, Otf11, Pthr, Acra3, Acra5, Acrb4, Camk1, Cdc25Mm, Crbp, Crbp2, Csk, Cyp11a, Cyp19, Drd2,
- 30 Ets1, Fli1, Gnai2, Gnat1, Gpcr6, Gria4, Hgf1, Hior1, Hpx, Hsp86-1ps3, Hst2, Idd2,

-80-

I11bc, Lag-rs1, Lap18-rs1, M11, Mpmv27, Penk, Pgr, Ras12-2, Tp11, Trf, Xmmv2,  
 Xmmv67, Xmv15, Xmv16, Xmv25, Xmv60, Mgf, Amh, Braf, Cdc2a, Dmd1, Estr,  
 Fps13, Fps14, Fps15, Gli, Gpcr17, Grik2, Ifgr, Igfl, Mpmv5, Mpmv12, Mpmv40,  
 Myb, Oprm, Pg, Pmch, Ros1, Xmv31, Xmv51, Xmv54, Camk2b, Egfr, Int6, Lif,  
 5 Mtv44, Ews, Csfgm, Flt4, I13, I14, I15, Irfl, Gria1, Glut4, Crhr, Csfg, Mov9,  
 Xmv20, Acrb, Mpmv4, Mpmv15, Ngfr, Nos2, Rara, Taz4, Tcf2, Xmv42, Mtv3,  
 Adra1, Crko, df, Erbb2, Gabra1, Gabra6, Gabrg2, Gh, Glra1, Grb2, Hnflb, Hsp86-  
 ps1, Idd4, Igfbp1, Igfbp3, I113, Int4, Mpmv2, Mpmv8, Mpmv18, Mtv45, nu, Pkca,  
 Rab1, Rel, Shbg, Tcf7, Thra, Tnz1, Trp53, Wnt3, Wnt3A, Xmv4, Xmv5, Xmv47,  
 10 Xmv49, Xmv63, Akt, Amh-rs4, Ccs1, Fps16, Fos, Gdf7, Hcs3, Hsp70-2, Hsp84-3,  
 Hsp86-1, hyt, Ltrm1, Max, Mpmv11, Mpmv24, Mtv9, Mtv30, Pomc1, Tcf3a, Tda2,  
 Tgfb3, Tpo, Tshr, Xmmv21, Xmmv25, Xmmv34, Xmmv50, Gli3, Xmv55, Ryr2,  
 Inhba, Gas1, Pcsk1, Amh-rs2, Ccnb1-rs6, Ccnb1-rs13, Crhpb, Dat1, Drd1a, Fgfr4,  
 Fps17, Fim1, Gpcr15, Gpcr18, Hbvi, Hilda, Htr1a, Idd11, I19, Ltrm4, Mak, mes,  
 15 P11, P12, Pr1, Ra1, Rasa, Srd5a1, Tpbp, Xmv13, Xmv27, Rarb, Rbp3, Htr2, Rb1,  
 Aera2, Camkg, Ceh11a2, Ccnb1-rs5, Ccnb1-rs12, Gnrh, Mtv11, Nras-ps, Otf3-rs6,  
 Plau, Ptprg, Trp53-ps, Wnt5A, Xmv19, Ghr, I17r, Lifr, Mlvi2, Prr, Myc, Rill,  
 cog, Amh-rs7, I12rb, Pdgb, Acr, CP2, Rarg, Sp1-1, Wnt1, Afr1, Atf4, Bzrp, Ccnb1-  
 rs11, Cyp11b, I13rb1, I13rb2, Ins3, Itga, Mlvi1, Mlvi3, Mtv36, Pdgc, Svp5, Tef,  
 20 Trhr, Wnt7B, Xmmv55, Xmmv72, Xmv37, Tnp2, Ets2, Casr, Chuck-rs1, din, Drd3,  
 Erg, G22p1, Gap43, Gas4, Grik1, Htr1f, Ifgt, Int53, Ltrm2, Mpmv17, Mtv6, Mtv1,  
 Pit1, Xmv3, Xmv35, Xmv50, Igf2r, Mas, Tcd3, Glp1r, Idd1, Tla, Aeg1, Ccnb1-rs3,  
 Cdc2b, Csi, Cyp21, Cyp21-ps1, Fps18, Gna-rs1, Gpcr19-rs1, Grr1, Grr2, Hom1,  
 Hsc70t, Hsp70, Hsp70-1, Hsp70-3, Hsp84-1, Hst1, Hst4, Hst5, Hst6, Hye, Int3,  
 25 Itpr3, Lap18-rs2, Otf3, Ptprs, Rab11b, Ras12-1, Ras12-3, Ras13, Rrs, Rxrb, Tas,  
 Tcd1, Tcd2, Teral, Tla-rs, Tnfa, Tnfb, Tpx1, Tpx2, Xmmv15, Xmv36, Xmv57,  
 Csfr, Pdgrb, Adrb2, Apc, Camk2a, Camk4, Dcc, Fgfl, Gna1, Gpcr7, Gr11, Grp,  
 Hsp74, Mcc, Mtv2, Mtv38, Ptpn2, Tp12, Xmv22, Xmv23, Xmv29, Fth, Csfgmra,  
 Mxi1, Adra2a, Adrb1, Adrbk1, Chuck, Cyp17, Gna14, Gnb-ps1, Hcs6, Htr7, Ide,  
 30 Ins1, Lpc1, Pomc2, Seao, Tlx1, Xmmv42, Xmv18, Tcf3, Araf, Avpr2, mdx, Ar,

-81-

Zfx, Otf9, Ccgl, Ccnb1-rs8, Fps19, Gabra3, Glra2, Glra4, Gria3, Grpr, Hsp74-ps1, Hst3, Htr1c, Il2rg, Mov14, Mov15, Mtv28, Otf3-rs8, Sts, Sxa, Sxr, Xta, Tdy, Hya, Zfy1, Zfy2, Mov15, Mov24, Mtv31, Mtv42, Sdma, Spy, Sts, Sxa, Sxr, XmmvY, Xmv7, Xmv11, and Xmv40.

- 5 Non-limiting examples of *Phaseolus vulgaris* genes include: Acc, ace, Adk, Am, Amv-1, Amv-2, Ane, aph, Arc, Are, arg, Arl (Arc), asp, B, bc-u, bc-1.sup.1, bc-1.sup.2, bc-2.sup.1, bc-2.sup.2, bc-3, Bcm, Beg, Bip, blu, Bpm, Bsm, By-1, By-2, C, C/c, c.sup.cr, C.sup.cir, C.sup.ma (M, R.sup.ma), C.sup.r, C.sup.res, C.sup.rho, C.sup.st, [C.sup.st R Acc] (Aeq), c.sup.u (inh, i.sub.e), [c.sup.u Prp.sup.i] (Prp, c.sup.ui, Nud), [c.sup.uprp.sup.st] (prp.sup.st), [C Prp] (Prp), c.sup.v, [C R] (R), [C r] (r), Ca, Cam, Cav, cc, chl, cl, cm1, Co-1 (A), Co-2 (Are), Co-3 (Mexique 1), Co-3.sup.2, Co-4 (Mexique 2), Co-5 (Mexique 3), Co-6, Co-7, cr-1 cr-2, cry, cs, Ct, ctv-1 ctv-2, cyv (by-3), D (Can, Ins), Da, Db, def, dgs (gl, le), dia, Diap-1, Diap-2, diff, dis, D1-1 D1-2 (DL.sub.1 DL.sub.2), do, ds (te), dt-1.sup.a dt-2.sup.a, dt-1.sup.b dt-2.sup.b, dw-1 dw-2, Ea Eb, ers (restr), ers-2, Est-1, Est-2, exp, F, Fa, fast, Fb Fc, fa fb fc, Fcr, Fcr-2, fd, Fe-1 Fe-2, Fin (in), Fop-1, Fop-2, Fr, Fr-2, G (Flav, Ca, Och), Ga, gas, glb, Gpi-cl, Gr, Hb1 (L.sub.HB-1), Hbnc (SC.sub.HB-1), Hbp (PD.sub.HB-1), hmb, Hss, Hsw, Ht-1 Ht-2 (L-1 L-2), I, Ia Ib, ian-1 ian-2 (ia), lbd, ico, Igr (Ih), ilo, ip, iter, iv, iw, J (Sh), Ke, L, Ia, Lan, Ld, Lds (Ds), Lec, Li (L), lo, Ir-1 Ir-2, mar, Me, Mel (Me), Mel-2 (Me-2), mel-3 (me-3), Mf, mi, mia, Mic (Mip), miv, Mrf, Mrf.sup.2, mrf, ms-1, Mue, mu mutator, Nag, Nd-1 Nd-2 (D-1 D-2), nie, nnd (sym-1), nnd-2, No, nts (nod), Nudus, ol, P, p.sup.gri (Gri, v.sup.Pal), pa, pc, pg (pa.sub.1), Pha, Pmv, ppd (neu), Pr, prc (pc), Prx, punc, ram, Rbcs (rbcS), rf-1, rf-2, rf-3, rfi (i), Rfs (m), Rk, rk, rk.sup.d (lin), rn-1 rn-2 (r r), rnd, Ro, Sal, sb, sb.sup.ms, sb-2, sb-3, si1, Skdh, s1, Smv, St, Sur, sw-1 sw-2, T, t (z-1), Th-1 Th-2, Tm, To, Tor (T), Tr, tri, trv, Ts, tw, uni, Uni-2, uni.sup.nde, uni.sup.nie, Ur-1, Ur-2, Ur-2.sup.2, Ur-3 (Ur-3, Ur-4), Ur-3.sup.2, Ur-4, (Up-2, Ur-C), Ur-5, (B-190), Ur-6 (Ur.sub.a, Ur-G), Ur-7 (R.sub.B11), Ur-8 (Up-1), Ur-9 (Ur.sub.p), us, V (B1), v.sup.lae (Cor), v, var, vi (vir.sub.f), wb, Wmv, X.sup.su, y, and Z.

-82-

Non-limiting examples of *Saccharomyces cerevisiae* genes include: PRE3, PUP1, PUP3, PRE2, PRE10, PRE1, PRE8, SCL1, PUP2, PRE5, PRE7, PRE4, RPT2, RPT3, RPN3, RPN11, RPN12, RPT6, RPN1, RPN2, RPT1, RPT5, RPT4, SKI6, RRP4, DIS3, TSC10, RAT1, GND1, EXO70, ERG10, ACC1, RPP0, ACT1, 5 ARP100, ARP3, PAN1, ARP2, ARP4, ARP9, SPE2, CYR1, ALA1, TPS1, TUB1, ABF1, DED81, NIP1, YHC1, SNU71, ATM1, MAK5, ROK1, DED1, SPB4, AUR1, PSE1, ALG1, TUB2, BPL1, MSL5, ERG24, ERG26, ERG25, CMD1, HCA4, SHE9, SHE10, CAK1, PIS1, CHO1, CDS1, ESR1, NUD1, CDC47, CDC13, CDC37, CDC1, CDC4, CDC20, CDC6, CDC46, CDC3, KAR1, BBP1, HRP1, 10 CCT2, CCT3, HSP10, SMC1, SMC2, CHC1, CFT2, CLP1, COP1, SEC26, SEC27, RET2, SEC21, COF1, CCT4, CCT1, CCT6, SEC24, SEC7, PCF11, RNA15, RNA14, FIP1, YSH1, TFB4, TSM1, APC2, APC5, SEC31, TAF47, TAP42, MPP10, CDC53, CKS1, CDC28, KIN28, CNS1, ERG11, DBP10, DBP8, PRO3, DYS1, ALR1, TID3, DNA2, SSL2, RAD3, RFA3, RFA2, RFA1, RFC4, RFC5, 15 RFC3, RFC2, RFC1, TOP2, RAP1, RPC25, PRI2, PRI1, POL1, POL12, HUS2, CDC2, POL2, DPB2, RPB10, RPA135, RPA190, RPA43, RPB8, RPO26, RPB5, RPC40, RPC19, SRB7, SRB4, RGR1, RPB11, SRB6, RPB2, RPB7, RPO21, RET1, RPO31, RPC31, RPC34, RPC53, RPC82, RPB12, RPB3, DPM1, DIP2, RNT1, CDC8, CDC14, DUT1, UBA2, UBA1, UBC9, CDC34, ENP1, ERD2, SSS1, 20 SEC61, SEC63, SEC62, GNA1, GPI8, DAM1, DUO1, IRR1, PRP3, TIM9, HSH49, SUP35, EXM2, MEX67, ERG9, ERG20, FAS2, FAS1, NOP1, FAD1, AOS1, FBA1, NCB2, BRN1, TUB4, GDI1, GOG5, SRM1, CDC25, SPT16, YIF2, BET4, CDC43, MRS6, BET2, PRO1, GLN1, GLN4, GRS1, YIP1, FOL2, GPA1, CDC42, SAR1, YPT1, SEC4, GSP1, TEM1, RHO1, CDC24, RNA1, GUK1, VMA16, 25 PMA1, HKR1, SIS1, MGE1, HSP60, HSF1, HAS1, MOT3, HTS1, ESA1, HSL7, HOM6, RIB7, SLY1, CSL4, PUR5, CSE1, IPP1, MDM1, USO1, SOF1, MAK11, LAS1, TEL2, DPB11, SGD1, FAL1, MTR3, MTR4, SPP2, SIK1, RRP7, POP4, RRP1, POP3, BFR2, CDC5, NRD1, MET30, MCM6, RRP46, SAS10, SCC2, ECO1, PRP43, BET3, BET5, STN1, NFS1, IDI1, SRP1, KAP95, CBF2, SKP1, 30 CEP3, CTF13, ERG7, KRS1, PSA1, PMI40, ALG2, SSF1, MED7, RSC4, CDC54,

-83-

MCM2, AFG2, ERG12, MVD1, CDC48, MHP1, ERV1, SSC1, TIM44, TIM17,  
 TIM23, TOM22, TOM40, MAS1, MCD1, MMC1, STU1, JAC1, ABD1, CEG1,  
 PAB1, MTR2, SEC16, ROT1, INO1, MLC1, MYO2, GPI2, SPT14, NAT2, NMT1,  
 TRM1, NCP1, NBP1, ACF2, SPP41, NUT2, LCP5, PRP19, NMD3, RFT1, NNF1,  
 5 NDC1, CRM1, KAR2, NIP29, NAB2, NIC96, NUP145, NUP49, NUP57, NUP159,  
 NSP1, NUP82, CDC39, NPL4, POP7, NTF2, MAK16, NPL3, NOP2, NOP4, NHP2,  
 NOP10, GAR1, NBP35, WBP1, STT3, SWP1, OST2, OST1, ORC1, ORC6, ORC5,  
 ORC4, ORC3, RRR1, SAT2, PWP2, PEX3, TOR2, PIK1, SEC14, STT4, MSS4,  
 PCM1, GPM1, SEC53, ERG8, YPD1, PAPI, NAB3, RRN7, SEN1, CFT1, PRP11,  
 10 PRP21, PRP39, PRP24, PRP9, SLU7, PRP28, PRP31, IFH1, PTA1, SUB2, FMH1,  
 MAS2, ESS1, PFY1, POL30, POP1, PDI1, RAM2, CDC7, SMP3, CDC15, YTH1,  
 QRI2, YAE1, SFI1, SEC1, BET1, SEC6, SEC13, SEC2, SEC8, CBF5, CDC19,  
 YRB1, RHC18, DBF4, SDS22, MCM3, CEF1, ALG11, GAA1, MOB1, NIP7,  
 TIP20, SEC5, SEC10, GPI10, RRP3, CDC45, DIB1, MIF2, HOP2, PBN1, NOP5,  
 15 RPP1, POP5, POP8, POP6, ERO1, MPT1, DNA43, ESP1, SMC3, LST8, STS1,  
 RPM2, RNR1, RNR2, RNR4, RPS20, RPL25, RPL3, RPL30, RPL32, RPL37A,  
 RPL43A, RPL5, RPL10, RPS3, CET1, YRA1, SNM1, GLE1, DBP5, DRS1, DBP6,  
 BRR2, RRN3, RRN6, RRN11, MED6, PRP16, RPR2, DIM1, RRP43, RRP42,  
 RRP45, SEC20, BOS1, CDC12, GLC7, PKC1, IPL1, SGV1, NRK1, RAD53,  
 20 LCB2, LCB1, MPS1, SES1, SPC3, SEC11, RIO1, ARP7, NEO1, YJU2, POB3,  
 ARH1, IQG1, HRT1, HYM1, MAK21, FUN20, FUN9, NBN1, STB5, YIF1,  
 SMX4, YKT6, SFT1, SMD1, PRP6, LSM2, NUF1, SPC97, SPC42, SPC98,  
 CDC31, SPC19, SPC25, SPC34, SPC24, NUF2, PRP40, MCD4, ERG1, SMC4,  
 CSE4, KRR1, SME1, TRA1, RLP7, SCH9, SMD3, SNP2, SSF2, SPC72, CDC27,  
 25 CDC23, CDC16, APC1, APC11, APC4, ARC19, RPN6, RPN5, RSC6, RSC8,  
 STH1, SFH1, TIM12, TIM22, TIM10, SQT1, SLS1, JSN1, STU2, SCD5, SSU72,  
 ASM4, SED5, UFE1, SYF1, SYF2, CCT5, TBF1, TOA2, TOA1, SUA7, TAF90,  
 TAF61, TAF25, TAF60, TAF17, TAF145, TAF19, TAF40, TAF67, TFA2, TFA1,  
 FCP1, TFG1, TFG2, TFB1, CCL1, SSL1, TFB3, TFB2, PZF1, BRF1, TFC5, TFC4,  
 30 TFC3, TFC7, TFC6, TFC1, SPT15, THI80, THS1, SPT6, SPT5, ROX3, REB1,

-84-

MCM1, MED4, MOT1, MED8, EFB1, YEF3, SUI1, CDC95, TIF11, SUI3, GCD11, SUI2, GCD6, GCD7, GCD2, GCD1, RPG1, GCD10, PRT1, TIF34, CDC33, TIF5, SUP45, GCD14, TIM54, SEC17, TPT1, TRL1, CCA1, SEN54, SEN2, SEN15, SEN34, WRS1, SLN1, TYS1, SNU56, PRP42, CUS1, PRP4, PRP8, SNU114,  
5 USS1, UFD1, SMT3, RSP5, QRI1, ALG7, UGP1, VTI1, VAS1, SEC18, CTR86, and ZPR1.

## 2. Viruses

The microorganisms provided herein include viruses. Such viruses typically have one or more of the microorganism characteristics provided herein. For  
10 example, viruses provided herein can have attenuated pathogenicity, reduced toxicity, preferential accumulation in immunoprivileged cells and tissues, such as tumor, ability to activate an immune response against tumor cells, immunogenic, replication competent, and are able to express exogenous proteins, and combinations thereof. In some embodiments, the viruses have an ability to activate an immune  
15 response against tumor cells without aggressively killing the tumor cells.

The viruses provided herein can be cytoplasmic viruses, such as poxviruses, or can be nuclear viruses such as adenoviruses. The viruses provided herein can have as part of their life cycle lysis of the host cell's plasma membrane. Alternatively, the viruses provided herein can have as part of their life cycle exit of  
20 the host cell by non-lytic pathways such as budding or exocytosis. The viruses provided herein can cause a host organism to develop an immune response to virus-infected tumor cells as a result of lysis or apoptosis induced as part of the viral life cycle. The viruses provided herein also can be genetically engineered to cause a host organism to develop an immune response to virus-infected tumor cells as a  
25 result of lysis or apoptosis, regardless of whether or not lysis or apoptosis is induced as part of the viral life cycle. In some embodiments, the viruses provided herein can cause the host organism to mount an immune response against tumor cells without lysing or causing cell death of the tumor cells.

One skilled in the art can select from any of a variety of viruses, according to  
30 a variety of factors, including, but not limited to, the intended use of the virus (e.g.,

-85-

exogenous protein production, antibody production or tumor therapy), the host organism, and the type of tumor.

**a. Cytoplasmic viruses**

The viruses provided herein can be cytoplasmic viruses, where the life cycle of the virus does not require entry of viral nucleic acid molecules in to the nucleus of the host cell. A variety of cytoplasmic viruses are known, including, but not limited to, pox viruses, African swine flu family viruses, and various RNA viruses such as picorna viruses, calici viruses, toga viruses, corona viruses and <sup>rhabdo</sup> ~~rhabdo~~ viruses. In some embodiments, viral nucleic acid molecules do not enter the host cell nucleus throughout the viral life cycle. In other embodiments, the viral life cycle can be performed without use of host cell nuclear proteins. In other embodiments, the virulence or pathogenicity of the virus can be modulated by modulating the activity of one or more viral proteins involved in viral replication.

**i. Poxviruses**

In one embodiment, the virus provided herein is selected from the pox virus family. Pox viruses include Chordopoxvirinae such as orthopoxvirus, parapoxvirus, avipoxvirus, capripoxvirus, leporipoxvirus, suipoxvirus, molluscipoxvirus and yatapoxvirus, as well as Entomopoxvirinae such as entomopoxvirus A, entomopoxvirus B, and entomopoxvirus A. Chordopoxvirinae are vertebrate poxviruses and have similar antigenicities, morphologies and host ranges; thus, any of a variety of such poxviruses can be used herein. One skilled in the art can select a particular genera or individual chordopoxvirinae according to the known properties of the genera or individual virus, and according to the selected characteristics of the virus (*e.g.*, pathogenicity, ability to elicit an immune response, preferential tumor localization), the intended use of the virus, the tumor type and the host organism. Exemplary <sup>chordopoxvirinae</sup> ~~chordopoxvirinae~~ genera are orthopoxvirus and avipoxvirus.

Avipoxviruses are known to infect a variety of different birds and <sup>have</sup> ~~has~~ been administered to humans. Exemplary avipoxviruses include canarypox, fowlpox, juncopox, mynahpox, pigeonpox, psittacinepox, quailpox, peacockpox, penguinpox, sparrowpox, starlingpox, and turkeypox viruses.

-86-

Orthopoxviruses are known to infect a variety of different mammals including rodents, domesticated animals, primates and humans. Several orthopoxviruses have a broad host range, while others have narrower host range. Exemplary orthopoxviruses include buffalopox, camelpox, <sup>cowpox</sup>~~cowpox~~, ectromelia, monkeypox, raccoon pox, skunk pox, tatera pox, uasin gishu, vaccinia, variola and volepox viruses. In some embodiments, the orthopoxvirus selected can be an orthopoxvirus known to infect humans, such as cowpox, monkeypox, vaccinia or variola virus. Optionally, the orthopoxvirus known to infect humans can be selected from the group of orthopoxviruses with a broad host range, such as cowpox, monkeypox, or vaccinia virus.

a. **Vaccinia Virus**

One exemplary orthopoxvirus is vaccinia virus. A variety of vaccinia virus strains are available, including Western Reserve (WR), Copenhagen, Tashkent, Tian Tan, Lister, Wyeth, IHD-J, and IHD-W, Brighton, Ankara, MVA, Dairen I, L-IPV, LC16M8, LC16MO, L1VP, WR 65-16, Connaught, New York City Board of Health. Exemplary vaccinia viruses are Lister or L1VP vaccinia viruses. Any known vaccinia virus, or modifications thereof that correspond to those provided herein or known to those of skill in the art to reduce toxicity of a vaccinia virus. Generally, however, the mutation will be a multiple mutant and the virus will be further selected to reduce toxicity.

The linear dsDNA viral genome of vaccinia virus is approximately 200 kb in size, encoding a total of approximately 200 potential genes. Viral gene expression can be divided into three stages. In the early stage, gene expression is mainly for viral replication, and for defense against the host's immune system. In the intermediate stage, genes not available for expression in the early stage can be expressed, including late stage transactivators. In the late stage, active transcription is mainly for viral structural components for building mature viruses.

Vaccinia virus possesses a variety of features for use in cancer gene therapy and vaccination. It has a broad host and cell type range. Vaccinia is a cytoplasmic virus, thus, it does not insert its genome into the host genome during its life cycle.

-87-

Unlike many other viruses that require the host's transcription machinery, vaccinia virus can support its own gene expression in the host cell cytoplasm using enzymes encoded in the viral genome. The vaccinia virus genome has a large carrying capacity for foreign genes, where up to 25 kb of exogenous DNA fragments (approximately 12% of the vaccinia genome size) can be inserted. The genomes of several of the vaccinia strains have been completely sequenced, and many essential and nonessential genes identified. Due to high sequence homology among different strains, genomic information from one vaccinia strain can be used for designing and generating modified viruses in other strains. Finally, the techniques for production of modified vaccinia strains by genetic engineering are well established (Moss, Curr. Opin. Genet. Dev. 3 (1993), 86-90; Broder and Earl, Mol. Biotechnol. 13 (1999), 223-245; Timiryasova *et al.*, Biotechniques 31 (2001), 534-540).

Historically, vaccinia virus was used to immunize against smallpox infection. More recently, modified vaccinia viruses are being developed as vaccines to combat a variety of diseases. Attenuated vaccinia virus can trigger a cell-mediated immune response. Strategies such as prime/boost vaccination, vaccination with nonreplicating vaccinia virus or a combination of these strategies, have shown promising results for the development of safe and effective vaccination protocols. Mutant vaccinia viruses from previous studies exhibit a variety of shortcomings, including a lack of efficient delivery of the viral vehicle to the desired tissue only (e.g., specific accumulation in a <sup>tumors</sup> ~~tumor~~), a lack of safety because of possible serious complications (e.g., in young children, eczema vaccinatum and encephalitis, and in adults disseminated or progressive vaccinia may result if the individual is severely immunodeficient).

#### **b. Modified Vaccinia Viruses**

Provided herein are vaccinia viruses with insertions, mutations or deletions, as described more generally elsewhere herein. The vaccinia viruses are modified or selected to have low toxicity and to accumulate in the target tissue. Exemplary of such viruses are those from the LIVP strain.

-88-

Exemplary insertions, mutations or deletions are those that result in an attenuated vaccinia virus relative to the wild type strain. For example, vaccinia virus insertions, mutations or deletions can decrease pathogenicity of the vaccinia virus, for example, by reducing the toxicity, reducing the infectivity, reducing the ability to replicate, or reducing the number of non-tumor organs or tissues to which the vaccinia virus can accumulate. Other exemplary insertions, mutations or deletions include, but are not limited to, those that increase antigenicity of the microorganism, those that permit detection or imaging, those that increase toxicity of the microorganism (optionally, controlled by an inducible <sup>promoter</sup> ~~promoter~~). For example, modifications can be made in genes that are involved in nucleotide metabolism, host interactions and virus formation. Any of a variety of insertions, mutations or deletions of the vaccinia virus known in the art can be used herein, including insertions, mutations or deletions of: the thymidine kinase (TK) gene, the hemagglutinin (HA) gene, the VGF gene (as taught in U.S. Pat. Pub. No. 20030031681); a hemorrhagic region or an A type inclusion body region (as taught in U.S. Pat. No. 6,596,279); Hind III F, F13L, or Hind III M (as taught in U.S. Pat. No. 6,548,068); A33R, A34R, A36R or B5R genes (see, e.g., Katz *et al.*, J. Virology 77:12266-12275 (2003)); SalF7L (see, e.g., Moore *et al.*, EMBO J. 1992 11:1973-1980); N1L (see, e.g., Kotwal *et al.*, Virology 1989 171:579-587); M1 lambda (see, e.g., Child *et al.*, Virology. 1990 174:625-629); HR, HindIII-MK, HindIII-MKF, HindIII-CNM, RR, or BamF (see, e.g., Lee *et al.*, J Virol. 1992 66:2617-2630); or C21L (see, e.g., Isaacs *et al.*, Proc Natl Acad Sci U S A. 1992 89:628-632).

#### c. The F3 Gene

In addition to the mutations known in the art, the vaccinia viruses provided herein can have <sup>an</sup> ~~and~~ insertion, mutation or deletion of the F3 gene (SEQ ID No: 1; an exemplary F3 gene is provided in GenBank Accession No. M57977, which contains the nucleotide and predicted amino acid sequences for LIVP strain F3; see also Mikryukov *et al.*, Biotekhnologiya 4:442-449 (1988)). For example, the F3 gene has been modified at the unique single NotI restriction site located within the F3 gene at position 35 or at position 1475 inside of the HindIII-F fragment of

-89-

vaccinia virus DNA strain LIVP (Mikryukov *et al.*, Biotekhnologiy 4 (1988), 442-449) by insertion of a foreign DNA sequence into the NotI digested virus DNA. As provided herein, an insertion of a nucleic acid molecule containing lacZ or <sup>luciferase</sup> luciferase/GFP into the NotI site of the F3 gene of the LIVP strain (nucleotides 1473-1480 in M57977, or nucleotides 33-40 of SEQ ID NO: 1) can result in decreased accumulation of vaccinia viruses in non-tumorous organs of nude mice, including brain and heart, relative to wild type vaccinia virus. Thus for use in the methods provided herein, vaccinia viruses can contain an insertion, mutation or deletion of the F3 gene or a mutation of a corresponding locus.. For example, as provided herein, F3-interrupted modified LIVP vaccinia virus can selectively replicate in tumor cells in vivo. Therefore, modified vaccinia viruses (*e.g.*, modified strain LIVP) with the interrupted F3 gene can be used in the methods provided herein, such as methods of tumor-directed gene therapy and for detection of tumors and metastases.

Thus, provided herein are vaccinia viruses having a modification of the F3 gene. For example, the vaccinia viruses provided herein can contain an insertion of foreign DNA into the F3 gene. An exemplary insertion of foreign DNA is an insertion at a site equivalent to the NotI site of the F3 gene in vaccinia strain LIVP, or at position 35 of SEQ ID No:1. An F3-modified vaccinia virus provided herein can colonize in tumors specifically, and therefore, can be used <sup>for</sup> tumor-specific therapeutic gene delivery. A GenBank data analysis with BLAST (Basic Local Alignment Search Tool) on nucleotide sequences of different strains of vaccinia virus was performed. Based on this analysis, it was found that in vaccinia virus strain Copenhagen (Goebel *et al.*, Virology 179 (1990), 247-266) the NotI restriction site is located between two open reading frames (ORF) encoding F14L and F15L genes. Therefore, insertion of foreign genes into NotI site of the VV genome strain Copenhagen will not interrupt any vital genes. In VV strain LIVP, the NotI restriction site is located in the ORF encoding the F3 gene with unknown function (Mikryukov *et al.*, Biotekhnologiya 4 (1988), 442-449). Thus, the insertion of foreign genes into the NotI site of the F3 gene interrupted the F3 gene. The ability

-90-

to modify the F3 gene suggests that it may have a nonessential role for virus replication. Although the F3 gene is likely nonessential for virus replication, the results of the animal experiments suggest that interruption of the F3 gene is correlated with decreased viral virulence, the inability to replicate in brain or ovary,  
5 and the ability to replicate preferentially in tumor tissue.

The F3 gene is conserved in a variety of different vaccinia virus strains, including WR (nucleotides 42238-42387 of GenBank Accession No. AY243312.1, Ankara (nucleotides 37155-37304 of GenBank Accession No. U94848.1), Tian Tan (nucleotides 41808-41954 of GenBank Accession No. AF095689), Acambis 3000  
10 (nucleotides 31365-31514 of GenBank Accession No. AY603355.1) and Copenhagen (nucleotides 45368-45517 of GenBank Accession No. M35027.1) strains. The F3 gene also is conserved in the larger family of poxviruses, particularly among orthopoxviruses such as cowpox (nucleotides 58498-58647 of GenBank Accession No. X94355.2), rabbitpox (nucleotides 46969-47118 of  
15 GenBank Accession No. AY484669.1), camelpox (nucleotides 43331-43480 of GenBank Accession No. AY009089.1), ectromelia (nucleotides 51008-51157 of GenBank Accession No. AF012825.2), monkeypox (nucleotides 42515-42660 of GenBank Accession No. AF380138.1), and variola viruses (nucleotides 33100-33249 of GenBank Accession No. X69198.1). Accordingly, also provided are  
20 modifications of the equivalent of the F3 gene in poxviruses, such as orthopoxviruses including a variety of vaccinia virus strains. One skilled in the art can identify the location of the equivalent F3 gene in a variety of poxviruses, orthopoxviruses and vaccinia viruses. For example, an equivalent of the F3 gene in poxviruses, orthopoxviruses and vaccinia viruses can include a gene that contains at  
25 least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with the nucleotide sequence of the F3 gene in SEQ ID No:1. In another example, an equivalent of the F3 gene in poxviruses, orthopoxviruses and vaccinia viruses can include a gene that contains at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least  
30 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with the amino

-91-

acid sequence of F3 in SEQ ID No:2. In another example, the equivalent to the F3 gene in LIVP can be determined by its structural location in the viral genome: the F3 gene is located on the HindIII-F fragment of vaccinia virus between open reading frames F14L and F15L as defined by Goebel *et al.*, Virology (1990) 179:247-266, and in the opposite orientation of ORFs F14L and F15L; one skilled in the art can readily identify the gene located in the structurally equivalent region in a large variety of related viruses, such as a large variety of pox viruses.

Comparative protein sequence analysis revealed some insight into protein function. The closest match with the protein encoded by the F3 gene (strain LIVP) is a prolyl 4-hydroxylase alpha subunit precursor (4-PH alpha) from the nematode *Caenorhabditis elegans* (Veijola *et al.*, J. Biol. Chem. 269 (1994), 26746-26753). This alpha subunit forms an active alpha-beta dimer with the human protein disulfide isomerase beta subunit. Prolyl 4-hydroxylase (EC 1.14.11.2) catalyzes the formation of 4-hydroxyproline in collagen. The vertebrate enzyme is an alpha 2-beta 2 tetramer, the beta subunit of which is identical to the protein disulfide-isomerase (PDI). The importance of this protein for vaccinia viral replication is unknown, but a deficiency of this protein can result in retargeting vaccinia virus to tumor tissue.

#### d. Multiple Modifications

The vaccinia viruses provided herein also can contain two or more insertions, mutations or deletions. Thus, included are vaccinia viruses containing two or more insertions, mutations or deletions of the loci provided herein or other loci known in the art. In one embodiment, a vaccinia virus contains an insertion, mutation or deletion in the F3 gene, and one or more additional insertions, mutations or deletions. In one embodiment of the modified vaccinia virus, at least the F3 gene has been modified by insertion of a foreign nucleotide sequence. Modifications such as modification of the F3 gene will typically result in at least partial inactivation of the gene or gene product. In one example, the F3 gene and the TK gene have been modified by insertion of a foreign nucleotide sequence. In another example, the F3 gene and the HA gene have been modified by insertion of a foreign nucleotide

-92-

sequence. In another example, the F3 gene and both the TK and HA genes have been modified by insertion of a foreign nucleotide sequence. In another example, the HA gene and the TK gene have been modified by insertion of a foreign nucleotide sequence. Accordingly, the present compositions and methods include a modified vaccinia virus wherein two or more of (a) the F3 gene, (b) the TK gene, and (c) the HA gene have been modified. In one embodiment, at least two of the F3 gene, TK gene and HA gene have been inactivated, for example by insertion, deletion and/or replacement of nucleotide(s) within the coding region, or regulatory sequences of two or more of these genes have been inactivated by insertion, deletion or mutation.

**e. The Lister Strain**

In another embodiment, the viruses and methods provided herein can be based on modifications to the Lister strain of vaccinia virus. Lister (also referred to as Elstree) vaccinia virus is available from any of a variety of sources. For example, the Elstree vaccinia virus is available at the ATCC under Accession Number VR-1549. The Lister vaccinia strain has high transduction efficiency in tumor cells with high levels of gene expression.

In one embodiment, the Lister strain can be an attenuated Lister strain, such as the LIVP (Lister virus from the Institute of Viral Preparations, Moscow, Russia) strain, which was produced by further attenuation of the Lister strain. The LIVP strain was used for vaccination throughout the world, particularly in India and Russia, and is widely available.

The LIVP strain has a reduced pathogenicity while maintaining a high transduction efficiency. For example, as provided herein, F3-interrupted modified LIVP vaccinia virus can selectively replicate in tumor cells in vivo. In one embodiment, provided herein are modified LIVP viruses, including viruses having a modified TK gene, viruses having a modified HA gene, viruses having a modified F3 gene, and viruses having two or more of: modified HA gene, modified TK gene, and modified F3 gene.

30

-93-

**ii. Other cytoplasmic viruses**

Also provided herein are cytoplasmic viruses that are not poxviruses.

Cytoplasmic viruses can replicate without introducing viral nucleic acid molecules into the nucleus of the host cell. A variety of such cytoplasmic viruses are known in the art, and include African swine flu family viruses and various RNA viruses such as arenaviruses, picornaviruses, caliciviruses, togaviruses, coronaviruses, paramyxoviruses, flaviviruses, reoviruses, and rhaboviruses. Exemplary togaviruses include Sindbis viruses. Exemplary arenaviruses include lymphocytic choriomeningitis virus. Exemplary rhaboviruses include vesicular stomatitis viruses. Exemplary paramyxo viruses include Newcastle Disease viruses and measles viruses. Exemplary picornaviruses include polio viruses, bovine enteroviruses and rhinoviruses. Exemplary flaviviruses include Yellow fever virus; attenuated Yellow fever viruses are known in the art, as exemplified in Barrett *et al.*, Biologicals 25:17-25 (1997), and McAllister *et al.*, J. Virol. 74:9197-9205 (2000).

Also provided herein are modifications of the viruses provided above to enhance one or more characteristics relative to the wild type virus. Such characteristics can include, but are not limited to, attenuated pathogenicity, reduced toxicity, preferential accumulation in tumor, increased ability to activate an immune response against tumor cells, increased immunogenicity, increased or decreased replication competence, and are able to express exogenous proteins, and combinations thereof. In some embodiments, the modified viruses have an ability to activate an immune response against tumor cells without aggressively killing the tumor cells. In other embodiments, the viruses can be modified to express one or more detectable genes, including genes that can be used for imaging. In other embodiments, the viruses can be modified to express one or more genes for harvesting the gene products and/or for harvesting antibodies against the gene products.

**b. Adenovirus, Herpes, Retroviruses**

Further provided herein are viruses that include in their life cycle entry of a nucleic acid molecule into the nucleus of the host cell. A variety of such viruses are

-94-

known in the art, and include herpesviruses, papovaviruses, retroviruses, adenoviruses, parvoviruses and orthomyxoviruses. Exemplary herpesviruses include herpes simplex type 1 viruses, cytomegaloviruses, and Epstein-Barr viruses. Exemplary papovaviruses include human ~~papillomaviruses~~ <sup>papillomaviruses</sup> and SV40 viruses.

5 Exemplary retroviruses include lentiviruses. Exemplary orthomyxoviruses include influenza viruses. Exemplary parvoviruses include adeno associated viruses.

Also provided herein are modifications of the viruses provided above to enhance one or more characteristics relative to the wild type virus. Such characteristics can include, but are not limited to, attenuated pathogenicity, reduced  
10 toxicity, preferential accumulation in tumor, increased ability to activate an immune response against tumor cells, increased immunogenicity, increased or decreased replication competence, and are able to express exogenous proteins, and combinations thereof. In some embodiments, the modified viruses have an ability to activate an immune response against tumor cells without aggressively killing the  
15 tumor cells. In other embodiments, the viruses can be modified to express one or more detectable genes, including genes that can be used for imaging. In other embodiments, the viruses can be modified to express one or more genes for harvesting the gene products and/or for harvesting antibodies against the gene products.

### 20 3. Bacteria

Bacteria can also be used in the methods provided herein. Any of a variety of bacteria possessing the desired characteristics can be used. In one embodiment, aerobic bacteria can be used. In another embodiment, anaerobic bacteria can be used. In another embodiment, extracellular bacteria can be used. In another  
25 embodiment, intracellular bacteria can be used.

In some embodiments, the bacteria provided herein can be extracellular bacteria. A variety of extracellular bacteria are known in the art and include vibrio, lactobacillus, streptococcus, escherichia. Exemplary bacteria include Vibrio cholerae, Streptococcus pyogenes, and Escherichia coli. In other embodiments, the  
30 bacteria provided herein can be intracellular bacteria. A variety of intracellular

-95-

bacteria are known in the art and include listeria, salmonella, clostridium, and bifodobacterium. Exemplary intracellular bacteria include *Listeria monocytogenes*, *Salmonella typhimurium*, *Clostridium histolyticus*, *Clostridium butyricum*, *Bifodobacterium longum*, and *Bifodobacterium adolescentis*. Additional bacteria  
5 include plant bacteria such as *Clavibacter michiganensis* subsp. *michiganensis*, *Agrobacterium tumefaciens*, <sup>*Erwinia*</sup> ~~*Erwinia*~~ <sup>*Azorhizobium*</sup> ~~*Azorhizobium*~~ *herbicola*, *Azorhizobium caulinodans*, *Xanthomonas campestris* pv. *vesicatoria*, and *Xanthomonas campestris* pv. *campestris*.

A further example of a bacteria provided herein are magnetic bacteria. Such  
10 bacteria allow tumor detection through the accumulation of iron-based contrast agents. Magnetic bacteria can be isolated from fresh and marine sediments. Magnetic bacteria can produce magnetic particles (Fe<sub>3</sub>O<sub>4</sub>) (Blakemore, Annu. Rev. Microbiol. 36 (1982), 217-238). To do so, the magnetic bacteria have efficient iron uptake systems, which allow them to utilize both insoluble and soluble forms of  
15 iron. *Magnetospirillum magnetic* AMB-1 is an example of such magnetic bacteria that has been isolated and cultured for magnetic particle production (Yang *et al.*, Enzyme Microb. Technol. 29 (2001), 13-19). As provided herein, these magnetic bacteria (naturally occurring or genetically modified), when injected intravenously, can selectively accumulate in tumor. Accordingly, these bacteria can be used for  
20 accumulating iron-based contrast agents in the tumors, which in turn allows tumor detection by MRI. Similarly, other naturally isolated metal accumulating strains of bacteria can be used for tumor targeting, absorption of metals from contrast agents, and tumor imaging.

Also provided herein are modifications of bacteria to enhance one or more  
25 characteristics relative to the wild type bacteria. Such characteristics can include, but are not limited to, attenuated pathogenicity, reduced toxicity, preferential accumulation in tumor, increased ability to activate an immune response against tumor cells, increased immunogenicity, increased or decreased replication competence, and are able to express exogenous proteins, and combinations thereof.  
30 In some embodiments, the modified bacteria have an ability to activate an immune

-96-

response against tumor cells without aggressively killing the tumor cells. In other embodiments, the bacteria can be modified to express one or more detectable genes, including genes that can be used for imaging. In other embodiments, the bacteria can be modified to express one or more genes for harvesting the gene products and/or for harvesting antibodies against the gene products.

**a. Aerobic bacteria**

Previous studies have postulated that anaerobic bacteria are preferred for administration to tumors (Lemmon *et al.*, 1997 Gene Therapy 4:791-796). As provided herein, it has been determined that aerobic bacteria can survive and grow in tumors. Accordingly, a bacteria used in the methods provided herein can include a bacteria that can survive and grow in an oxygenated environment. In some embodiments, the bacteria must be in an oxygenated environment in order to survive and grow. A variety of aerobic bacteria are known in the art, including lactobacilli, salmonella, streptococci, staphylococci, vibrio, ~~listeria~~<sup>listeria</sup>, and escherichia. Exemplary bacteria include Vibrio cholerae, Listeria monocytogenes, Salmonella typhimurium, Streptococcus pyogenes, Escherichia coli, Lactobacillus bulgaricus, Lactobacillus casei, Lacto bacillus acidophilus, Lactobacillus brevis, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus salivarius, Lactobacillus sporogenes, Lactobacillus lactis, Lactobacillus fermentum, Streptococcus thermophilus, Bacillus subtilis, Bacillus megaterium, Bacillus polymyxa, Myobacterium smegmatis, Mycobacterium vaccae, Mycobacterium microti, Mycobacterium habana, Enterococcus faecalis, Pseudomonas fluorescens, and Pseudomonas putida.

**b. Anaerobic bacteria**

A bacteria used in the methods provided herein can include a bacteria that does not require oxygen to survive and grow. In some embodiments, the bacteria must be in an oxygen-free environment in order to survive and grow. A variety of aerobic bacteria are known in the art, including clostridium, bifodobacterium. Exemplary bacteria include Clostridium histolyticus, Clostridium butyricum, Clostridium novyi, Clostridium sordellii, Clostridium absonum, Clostridium

-97-

*Clostridium*

bifermentans, ~~Clostridium~~ difficile, Clostridium histolyticum, Clostridium perfringens, Clostridium beijerinckii, Clostridium sporogenes, Staphylococcus aureus, Staphylococcus epidermidis, Bifidobacterium longum, Bifidobacterium adolescentis, Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium laterosporus, Bifidobacterium animalis, Actinomyces israelii, Eubacterium lentum, 5 Peptostreptococcus anaerobis, Peptococcus prevotti, and Acidaminococcus fermentans.

**4. Eukaryotic cells**

Also encompassed within the microorganisms provided herein and the 10 methods of making and using such microorganisms are eukaryotic cells, including cells from multicellular eukaryotes, including mammals such as primates, where exemplary cells are human cells. Typically the cells are isolated cells. For example, eukaryotic cells can be tumor cells, including mammalian tumor cells such as primate tumor cells, where exemplary primate tumor cells are human tumor cells 15 such as human breast cancer cells. In another example, eukaryotic cells can include fibrosarcoma cells such as human fibrosarcoma cells. Exemplary human fibrosarcoma cells include HT1080 (ATCC Accession Nos. CCL-121, CRL-12011 or CRL-12012). In another example, eukaryotic cells can include stem cells, including mammalian stem cells such as primate stem cells, where exemplary 20 primate stem cells are human stem cells.

Also provided herein are modifications of eukaryotic cells to enhance one or more characteristics relative to the wild type cells. Such characteristics can include, but are not limited to, attenuated pathogenicity, reduced toxicity, preferential accumulation in tumor, increased ability to activate an immune response against 25 tumor cells, increased immunogenicity, increased or decreased replication competence, and are able to express exogenous proteins, and combinations thereof. In some embodiments, the modified eukaryotic cells have an ability to activate an immune response against tumor cells without aggressively killing the tumor cells. In other embodiments, the eukaryotic cells can be modified to express one or more 30 detectable genes, including genes that can be used for imaging. In other

-98-

embodiments, the eukaryotic cells can be modified to express one or more genes for harvesting the gene products and/or for harvesting antibodies against the gene products.

### C. Methods for Making a Modified Microorganism

5 The microorganisms provided herein can be formed by standard methodologies well known in the art for modifying microorganisms such as viruses, bacteria and eukaryotic cells. Briefly, the methods include introducing into microorganisms one or more genetic modification, followed by screening the microorganisms for properties reflective of the modification or for other desired  
10 properties.

#### 1. Genetic Modifications

Standard techniques in molecular biology can be used to generate the modified microorganisms provided herein. Such <sup>techniques</sup> ~~techniques~~ include various nucleic acid manipulation techniques, nucleic acid transfer protocols, nucleic acid  
15 amplification protocols, and other molecular biology techniques known in the art. For example, point mutations can be introduced into a gene of interest through the use of oligonucleotide mediated site-directed mutagenesis. Alternatively, homologous recombination can be used to introduce a mutation or exogenous sequence into a target sequence of interest. Nucleic acid transfer protocols include  
20 calcium chloride transformation/transfection, electroporation, liposome mediated nucleic acid transfer, N-[1-(2,3-Dioloxyloxy)propyl]-N,N,N-trimethylammonium methylsulfate mediated transformation, and others. In an alternative mutagenesis protocol, point mutations in a particular gene can also be selected for using a positive selection pressure. See, e.g., Current Techniques in Molecular Biology,  
25 (Ed. Ausubel, *et al.*). Nucleic acid amplification protocols include but are not limited to the polymerase chain reaction (PCR). Use of nucleic acid tools such as plasmids, vectors, promoters and other regulating sequences, are well known in the art for a large variety of viruses and cellular organisms. Further a large variety of nucleic acid tools are available from many different sources including ATCC, and various  
30 commercial sources. One skilled in the art will be readily able to select the

-99-

appropriate tools and methods for genetic modifications of any particular virus or cellular organism according to the knowledge in the art and design choice.

Any of a variety of modifications can be readily accomplished using standard molecular biological methods known in the art. The modifications will typically be one or more truncations, deletions, mutations or insertions of the microorganismal genome. In one embodiment, the modification can be specifically directed to a particular sequence. The modifications can be directed to any of a variety of regions of the microorganismal genome, including, but not limited to, a regulatory sequence, to a gene-encoding sequence, or to a sequence without a known role. Any of a variety of regions of microorganismal genomes that are available for <sup>modification</sup> ~~modification~~ are readily known in the art for many microorganisms, including the microorganisms specifically listed herein. As a non-limiting example, the loci of a variety of vaccinia genes provided herein elsewhere exemplify the number of different regions that can be targeted for modification in the microorganisms provided herein. In another embodiment, the modification can be fully or partially random, whereupon selection of any particular modified microorganism can be determined according to the desired properties of the modified the microorganism.

In some embodiments, the microorganism can be modified to express an exogenous gene. Exemplary exogenous gene products include proteins and RNA molecules. The modified microorganisms can express a detectable gene product, a therapeutic gene product, a gene product for manufacturing or harvesting, or an antigenic gene product for antibody harvesting. The characteristics of such gene products are described <sup>herein</sup> ~~herein elsewhere~~. In some embodiments of modifying an organism to express an exogenous gene, the modification can also contain one or more regulatory sequences to regulate expression of the exogenous gene. As is known in the art, regulatory sequences can permit constitutive expression of the exogenous gene or can permit inducible expression of the exogenous gene. Further, the regulatory sequence can permit control of the level of expression of the exogenous gene. In some examples, inducible expression can be under the control of cellular or other factors present in a tumor cell or present in a microorganism.

-100-

infected tumor cell. In other examples, inducible expression can be under the control of an administerable substance, including IPTG, RU486 or other known induction compounds. Any of a variety of regulatory sequences are available to one skilled in the art according to known factors and design preferences. In some  
5 embodiments, such as gene product manufacture and harvesting, the regulatory sequence can result in constitutive, high levels of gene expression. In some embodiments, such as anti-(gene product) antibody harvesting, the regulatory sequence can result in constitutive, lower levels of gene expression. In tumor therapy embodiments, a therapeutic protein can be under the control of an internally  
10 inducible ~~promoter~~<sup>promoter</sup> or an externally inducible ~~promoter~~<sup>promoter</sup>.

In other embodiments, organ or tissue-specific expression can be controlled by regulatory sequences. In order to achieve expression only in the target organ, for example, a tumor to be treated, the foreign nucleotide sequence can be linked to a tissue specific promoter and used for gene therapy. Such promoters are well known  
15 to those skilled in the art (see *e.g.*, Zimmermann *et al.*, (1994) *Neuron* 12, 11-24; Vidal *et al.*; (1990) *EMBO J.* 9, 833-840; Mayford *et al.*, (1995), *Cell* 81, 891-904; Pinkert *et al.*, (1987) *Genes & Dev.* 1, 268-76).

In some embodiments, the microorganisms can be modified to express two or more proteins, where any combination of the two or more proteins can be one or  
20 more detectable gene products, therapeutic gene products, gene products for manufacturing or harvesting, or antigenic gene products for antibody harvesting. In one embodiment, a microorganism can be modified to express a detectable protein and a therapeutic protein. In another embodiment, a microorganism can be modified to express two or more gene products for detection or two or more therapeutic gene  
25 products. For example, one or more proteins involved in biosynthesis of a luciferase substrate can be expressed along with luciferase. When two or more exogenous genes are introduced, the genes can be regulated under the same or different regulatory sequences, and the genes can be inserted in the same or different regions of the microorganismal genome, in a single or a plurality of genetic manipulation  
30 steps. In some embodiments, one gene, such as a gene encoding a detectable gene

-101-

product, can be under the control of a constitutive <sup>promoter</sup>~~promoter~~, while a second gene, such as a gene encoding a therapeutic gene product, can be under the control of an inducible <sup>promoter</sup>~~promoter~~. Methods for inserting two or <sup>more</sup>~~more~~ genes in to a microorganism are known in the art and can be readily performed for a wide variety of microorganisms using a wide variety of exogenous genes, regulatory sequences, and/or other nucleic acid sequences.

In an example of performing microorganismal modification methods, vaccinia virus strain L1VP was modified to contain insertions of exogenous DNA in three different locations of the viral genome. Using general methods known in the art, known molecular biology tools, and sequences known in the art or disclosed herein can be used to create modified vaccinia virus strains, including viruses containing insertions in the F3 gene, TK gene and/or HA gene. See, e.g., Mikryukov, *et al.*, *Biotekhnologiya* 4 (1998), 442-449; Goebel *et al.*, *Virology* 179 (1990), 247-266; Antoine *et al.*, *Virology* 244 (1998), 365-396; Mayr *et al.*, *Zentbl. Bakterirol. Hyg. Abt. 1 Orig. B* 167 (1978), 375-390; Ando and Matumoto, *Jpn. J. Microbial.* 14 (1979), 181-186; Sugimoto *et al.*, *Microbial. Immunol.* 29 (1985), 421-428; Takahashi-Nishimaki *et al.*, *J. Gen. Virol.* 68 (1987), 2705-2710). These methods include, for example, in vitro recombination techniques, synthetic methods and in vivo recombination methods as described, for example, in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY (1989), and in the Examples disclosed herein. The person skilled in the art can isolate the gene encoding the gene product of F3 (or a related gene product) from any vaccinia strain using, for example, the nucleotide sequence of the F3 gene of SEQ ID No:1 or SEQ ID NOs:10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or 32, or a fragment thereof as a probe for screening a library.

Methods of producing recombinant microorganisms are known in the art. Provided herein for exemplary purposes are methods of producing a recombinant vaccinia virus. A recombinant vaccinia virus with an insertion in the F3 gene can be prepared by the following steps: (a) generating (i) a vaccinia shuttle plasmid

-102-

containing the modified F3 gene inserted at restriction site X and (ii) a dephosphorylated wt VV (VGL) DNA digested at restriction site X; (b) transfecting host cells infected with PUV-inactivated helper VV (VGL) with a mixture of the constructs of (i) and (ii) of step a; and (c) isolating the recombinant vaccinia viruses from the transfectants. One skilled in the art knows how to perform such methods, for example by following the instructions given in Example 1 and the legend to Figure 1; see also Timiryasova *et al.*, *Biotechniques* 31 (2001), 534-540. In one embodiment, restriction site X is a unique restriction site. A variety of suitable host cells also are known to the person skilled in the art and include many mammalian, avian and insect cells and tissues which are susceptible for vaccinia virus infection, including chicken embryo, rabbit, hamster and monkey kidney cells, for example, HeLa cells, RK<sub>13</sub>, CV-1, Vero, BSC40 and BSC-1 monkey kidney cells.

## 2. Screening for above characteristics

Modified microorganisms can be screened for any desired characteristics, including the characteristics described herein such as attenuated pathogenicity, reduced toxicity, preferential accumulation in tumor, increased ability to activate an immune response against tumor cells, increased immunogenicity, increased or decreased replication competence, and are able to express exogenous proteins, and combinations thereof. For example, the modified microorganisms can be screened for the ability to activate an immune response against tumor cells without aggressively killing the tumor cells. In another example, the microorganisms can be screened for expression of one or more detectable genes, including genes that can be used for imaging, or for expression of one or more genes for manufacture or harvest of the gene products and/or for harvest of antibodies against the gene products.

Any of a variety of known methods for screening for such characteristics can be performed, as demonstrated in the Examples provided herein. One Exemplary method for screening for desired characteristics includes, but is not limited to, monitoring growth, replication and/or gene expression (including expression of an exogenous gene) in cell culture or other invitro medium. The cell culture can be from any organism, and from any tissue source, and can include tumorous tissues.

-103-

Other exemplary methods for screening for desired characteristics include, but are not limited to, administering a microorganism to animal, including non-human animals such as a mouse, monkey or ape, and optionally also including humans, and monitoring the microorganism, the tumor, and or the animal; monitoring can be

5 performed by in vivo imaging of the microorganism and/or the tumor (e.g., low light imaging of microorganismal gene expression or ultrasonic tumor imaging), external monitoring of the tumor (e.g., external measurement of tumor size), monitoring the animal (e.g., monitoring animal weight, blood panel, antibody titer, spleen size, or liver size). Other exemplary methods for screening for desired characteristics

10 include, but are not limited to, harvesting a non-human animal for the effects and location of the microorganism and expression by the microorganism, including methods such as harvesting a variety of organs including a tumor to determine presence of the microorganism and/or gene expression by the microorganism in the organs or tumor, harvesting of organs associated with an immune response or

15 microorganismal clearance such as the spleen or liver, harvesting the tumor to determine tumor size and viability of tumor cells, harvesting antibodies or antibody producing cells. Such screening and monitoring methods can be used in any of a variety of combinations, as is known in art. In one embodiment, a microorganism can be screened by administering the microorganism to an animal such as a non-

20 human animal or a human, followed by monitoring by in vivo imaging. In another embodiment, a microorganism can be screened by administering the microorganism to an animal such as a non-human animal, monitoring by in vivo imaging, and then harvesting the animal. Thus, provided herein are methods for screening a microorganism for desired characteristics by administering the microorganism to an

25 animal such as an animal with a tumor, and monitoring the animal, tumor (if present), and/or microorganism in the animal for one or more characteristics. Also provided herein are methods for screening a microorganism for desired characteristics by administering the microorganism to a non-human animal such as a non-human animal with a tumor, harvesting the animal, and assaying the animal's

30 organs, antibody titer, and/or tumor (if present) for one or more characteristics.

-104-

Provided herein are methods for screening a microorganism for attenuated pathogenicity or reduced toxicity, where the pathogenicity or toxicity can be determined by a variety of techniques, including, but not limited to, assessing the health state of the subject, measuring the body weight of a subject, blood or urine analysis of a subject, and monitoring tissue distribution of the microorganism within the subject; such techniques can be performed on a living subject in vivo, or can be performed post mortem. Methods also can include the ability of the microorganisms to lyse cells or cause cell death, which can be determined in vivo or in vitro.

When a subject drops below a threshold body weight, the microorganism can be considered pathogenic to the subject. Exemplary thresholds can be a drop of about 5% or more, a drop of about 10% or more, or a drop of about 15% or more in body weight relative to a reference. A body weight reference can be selected from any of a variety of references used in the art; for example, a body weight reference can be the weight of the subject prior to administration of the microorganism, the body weight reference can be a control subject having the same condition as the test subject (e.g., normal or tumor-injected), where the change in weight of the control is compared to the change in weight of the test subject for the time period after administration of the microorganism.

Blood or urine analysis of the subject can indicate level of immune response, level of toxins in the subject, or other levels of stress to cells, tissues or organs of the subject such as kidneys, pancreas, liver and spleen. Levels increased above established threshold levels can indicate pathogenicity of the microorganism to the subject. Threshold levels of components of blood or urine for indicating microorganismal pathogenicity are well known in the art, and any such thresholds can be selected herein according to the desired tolerance of pathogenicity or toxicity of the microorganism.

Tissue distribution of a microorganism in a subject can indicate pathogenicity or toxicity of the microorganism. In one embodiment, tissue distribution of a microorganism that is not pathogenic or toxic can be mostly in tumor relative to other tissues or organs. Microorganisms located mostly in tumor

-105-

can accumulate, for example, at least about 2-fold greater, at least about 5-fold greater, at least about 10-fold greater, at least about 100-fold greater, at least about 1,000-fold greater, at least about 10,000-fold greater, at least about 100,000-fold greater, or at least about 1,000,000-fold greater, than the microorganism accumulate  
5 in any other particular organ or tissue.

Provided herein are methods for screening a microorganism for tissue distribution or accumulation, where the tissue distribution can be determined by a variety of techniques, including, but not limited to, harvesting a non-human subject, in vivo imaging a detectable gene product in subject. Harvesting can be  
10 accomplished by euthanizing the non-human subject, and determining the accumulation of microorganisms in tumor and, optionally, the accumulation in one or more additional tissues or organs. The accumulation can be determined by any of a variety of methods, including, but not limited to, detecting gene products such as detectable gene products (*e.g.*, gfp or beta galactosidase), histological or  
15 microscopic evaluation of tissue, organ or tumor samples, or measuring the number of plaque or colony forming units present in a tissue, organ or tumor sample. In one embodiment, the desired amount of tissue distribution of a microorganism can be mostly in tumor relative to other tissues or organs. Microorganisms located mostly in tumor can accumulate, for example, at least about 2-fold greater, at least about 5-  
20 fold greater, at least about 10-fold greater, at least about 100-fold greater, at least about 1,000-fold greater, at least about 10,000-fold greater, at least about 100,000-fold greater, or at least about 1,000,000-fold greater, than the microorganism accumulate in any other particular organ or tissue.

Also provided herein are methods of screening for microorganisms that can  
25 elicit an immune response, where the immune response can be against the tumor cells or against the microorganisms. A variety of methods for measuring the ability to elicit an immune response are known in the art, and include measuring an overall increase in immune activity in a subject, measuring an increase in anti-microorganism or anti-tumor antibodies in a subject, testing the ability of a  
30 microorganism-treated (typically a non-human) subject to prevent later

-106-

infection/tumor formation or to rapidly eliminate microorganisms or tumor cells. Methods also can include the ability of the microorganisms to lyse cells or cause cell death, which can be determined in vivo or in vitro.

Also provided herein are methods for determining increased or decreased replication competence, by monitoring the speed of replication of the microorganisms. Such measurements can be performed in vivo or in vitro. For example, the speed of replication in a cell culture can be used to determine replication competence of a microorganism. In another example, the speed of replication in a tissue, organ or tumor in a subject can be used to measure replication competence. In some embodiments, decreased replication competence in non-tumor tissues and organs can be the characteristic to be selected in a screen. In other embodiments, increased replication competence in tumors can be the characteristic to be selected in a screen.

Also provided herein are methods for determining the ability of a microorganism to express genes, such as exogenous gene. Such methods can be performed in vivo or in vitro. For example, the microorganisms can be screened on selective plates for the ability to express a gene that permits survival of the microorganism or permits the microorganism to provide a detectable signal, such as turning X-gal blue. Such methods also can be performed in vivo, where expression can be determined, for example, by harvesting tissues, organs or tumors a non-human subject or by in vivo imaging of a subject.

Also provided herein are methods for determining the ability of a microorganism to express genes toward which the subject can develop antibodies, including exogenous genes toward which the subject can develop antibodies. Such methods can be performed in vivo using any of a variety of non-human subjects. For example, gene expression can be determined, for example, by bleeding a non-human subject to which a microorganism has been administered, and assaying the blood (or serum) for the presence of antibodies against the microorganism-expressed gene, or by any other method generally used for polyclonal antibody harvesting, such as production bleeds and terminal bleeds.

-107-

Also provided herein are methods for screening a microorganism that has two or more characteristics provided herein, including screening for attenuated pathogenicity, reduced toxicity, preferential accumulation in tumor, increased ability to activate an immune response against tumor cells, increased immunogenicity, increased or decreased replication competence, ability to express exogenous proteins, and ability to elicit antibody production against a microorganismally expressed gene product. A single monitoring technique, such as in vivo imaging, can be used to verify two or more characteristics, or a variety of different monitoring techniques can be used, as can be determined by one skilled in the art according to the selected characteristics and according to the monitoring techniques used.

#### D. Therapeutic Methods

Provided herein are therapeutic methods, including methods of treating or preventing ~~immunoprivileged~~ <sup>immunoprivileged</sup> cells or tissue, including cancerous cells, tumor and metastasis. The methods provided herein include administering a microorganism to a subject containing a tumor and/or metastases. The methods provided herein do not require the microorganism to kill tumor cells or decrease the tumor size. Instead, the methods provided herein include administering to a subject a microorganism that can cause or enhance an anti-tumor immune response in the subject. In some embodiments, the microorganisms provided herein can be administered to a subject without causing microorganism-induced disease in the subject. In some embodiments, the microorganisms can accumulate in tumors or metastases. In some embodiments, the microorganisms can elicit an anti-tumor immune response in the subject, where typically the microorganism-mediated anti-tumor immune response can develop over several days, such <sup>as</sup> a week or more, 10 days or more, two weeks or more, or a month or more, as a result of little or no microorganism-cause tumor cell death. In some exemplary methods, the microorganism can be present in the tumor, and can cause an anti-tumor immune response without the microorganism itself causing enough tumor cell death to prevent tumor growth.

In some embodiments, provided herein are methods for eliciting or enhancing antibody production against a selected antigen or a selected antigen type

-108-

in a subject, where the methods include administering to a subject a microorganism that can accumulate in a tumor and/or metastasis, and can cause release of a selected antigen or selected antigen type from the tumor, resulting in antibody production against the selected antigen or selected antigen type. The administered  
5 microorganisms can possess one or more characteristics including attenuated pathogenicity, low toxicity, preferential accumulation in tumor, ability to activate an immune response against tumor cells, immunogenicity, replication competence, ability to express exogenous genes, and ability to elicit antibody production against a microorganismally expressed gene product.

10 Any of a variety of antigens can be targeted in the methods provided herein, including a selected antigen such as an exogenous gene product expressed by the microorganism, or a selected antigen type such as one or more tumor antigens release from the tumor as a result of microorganism infection of the tumor (e.g., by lysis, apoptosis, secretion or other mechanism of causing antigen release from the  
15 tumor). In at least some embodiments, it can be desirable to maintain release of the selected antigen or selected antigen type over a series of days, for example, at least a week, at least ten days, at least two weeks or at least a month.

Also provided herein are methods for providing a sustained antigen release within a subject, where the methods include administering to a subject a  
20 microorganism that can accumulate in a tumor and/or metastasis, and can cause sustained release of an antigen, resulting in antibody production against the antigen. The sustained release of antigen can last for several days, for example, at least a week, at least ten days, at least two weeks or at least a month. The administered microorganisms can possess one or more characteristics including attenuated  
25 pathogenicity, low toxicity, preferential accumulation in tumor, ability to activate an immune response against tumor cells, immunogenicity, replication competence, ability to express exogenous genes, and ability to elicit antibody production against a microorganismally expressed gene product. The sustained release of antigen can result in an immune response by the microorganism-infected host, in which the host  
30 can develop antibodies against the antigen, and/or the host can mount an immune

-109-

response against cells expressing the antigen, including an immune response against tumor cells. Thus, the sustained release of antigen can result in immunization against tumor cells. In some embodiments, the microorganism-mediated sustained antigen release-induced immune response against tumor cells can result in complete  
5 removal or killing of all tumor cells.

Also provided herein are methods for inhibiting tumor growth in a subject, where the methods include administering to a subject a microorganism that can accumulate in a tumor and/or metastasis, and can cause or enhance an anti-tumor immune response. The anti-tumor immune response induced as a result of tumor or  
10 metastases-accumulated microorganisms can result in inhibition of tumor growth. The administered microorganisms can possess one or more characteristics including attenuated pathogenicity, low toxicity, preferential accumulation in tumor, ability to activate an immune response against tumor cells, immunogenicity, replication competence, ability to express exogenous genes, and ability to elicit antibody  
15 production against a microorganismally expressed gene product.

Also provided herein are methods for inhibiting growth or formation of a metastasis in a subject, where the methods include administering to a subject a microorganism that can accumulate in a tumor and/or metastasis, and can cause or enhance an anti-tumor immune response. The anti-tumor immune response induced  
20 as a result of tumor or metastasis-accumulated microorganisms can result in inhibition of metastasis growth or formation. The administered microorganisms can possess one or more characteristics including attenuated pathogenicity, low toxicity, preferential accumulation in tumor, ability to activate an immune response against tumor cells, immunogenicity, replication competence, ability to express exogenous  
25 genes, and ability to elicit antibody production against a microorganismally expressed gene product.

Also provided herein are methods for decreasing the size of a tumor and/or metastasis in a subject, where the methods include administering to a subject a microorganism that can accumulate in a tumor and/or metastasis, and can cause or  
30 enhance an anti-tumor immune response. The anti-tumor immune response induced

-110-

as a result of tumor or metastasis-accumulated microorganisms can result in a decrease in the size of the tumor and/or metastasis. The administered microorganisms can possess one or more characteristics including attenuated pathogenicity, low toxicity, preferential accumulation in tumor, ability to activate an immune response against tumor cells, immunogenicity, replication competence, ability to express exogenous genes, and ability to elicit antibody production against a microorganismally expressed gene product.

Also provided herein are methods for eliminating a tumor and/or metastasis from a subject, where the methods include administering to a subject a microorganism that can accumulate in a tumor and/or metastasis, and can cause or enhance an anti-tumor immune response. The anti-tumor immune response induced as a result of tumor or metastasis-accumulated microorganisms can result in elimination of the tumor and/or metastasis from the subject. The administered microorganisms can possess one or more characteristics including attenuated pathogenicity, low toxicity, preferential accumulation in tumor, ability to activate an immune response against tumor cells, immunogenicity, replication competence, ability to express exogenous genes, and ability to elicit antibody production against a microorganismally expressed gene product.

Methods of reducing inhibiting tumor growth, inhibiting metastasis growth and/or formation, decreasing the size of a tumor or metastasis, eliminating a tumor or metastasis, or other tumor therapeutic methods provided herein include causing or enhancing an anti-tumor immune response in the host. The immune response of the host, being anti-tumor in nature, can be mounted against tumors and/or metastases in which microorganisms have accumulated, and can also be mounted against tumors and/or metastases in which microorganisms have not accumulated, including tumors and/or metastases that form after administration of the microorganisms to the subject. Accordingly, a tumor and/or metastasis whose growth or formation is inhibited, or whose size is decreased, or that is eliminated, can be a tumor and/or metastasis in which the microorganisms have accumulated, or also can be a tumor and/or metastasis in which the microorganisms have not accumulated. Accordingly,

-111-

provided herein are methods of reducing inhibiting tumor growth, inhibiting metastasis growth and/or formation, decreasing the size of a tumor or metastasis, eliminating a tumor or metastasis, or other tumor therapeutic methods, where the method includes administering to a subject a microorganism, where the

5 microorganism accumulates in at least one tumor or metastasis and causes or enhances an anti-tumor immune response in the subject, and the immune response also is mounted against a tumor and/or metastasis in which the microorganism cell did not accumulate. In another embodiment, methods are provided for inhibiting or preventing recurrence of a neoplastic disease or inhibiting or preventing new tumor

10 growth, where the methods include administering to a subject a microorganism that can accumulate in a tumor and/or metastasis, and can cause or enhance an anti-tumor immune response, and the anti-tumor immune response can inhibit or prevent recurrence of a neoplastic disease or inhibit or prevent new tumor growth.

The tumor or neoplastic disease therapeutic methods provided herein, such as

15 methods of reducing inhibiting tumor growth, inhibiting metastasis growth and/or formation, decreasing the size of a tumor or metastasis, eliminating a tumor or metastasis, or other tumor therapeutic methods, also can include administering to a subject a microorganism that can cause tumor cell lysis or tumor cell death. Such a microorganism can be the same microorganism as the microorganism that can cause

20 or enhance an anti-tumor immune response in the subject. Microorganisms, such as the microorganisms provided herein, can cause cell lysis or tumor cell death as a result of expression of an endogenous gene or as a result of an exogenous gene. Endogenous or exogenous genes can cause tumor cell lysis or inhibit cell growth as

25 a result of direct or indirect actions, as is known in the art, including lytic channel formation or activation of an apoptotic pathway. Gene products, such as exogenous gene products can function to activate a prodrug to an active, cytotoxic form, resulting in cell death where such genes are expressed.

Such methods of antigen production or tumor and/or metastasis treatment can include administration of a modified microorganism described herein or a

30 microorganism having modifications with a functional equivalence to the vaccinia

-112-

virus provided herein containing a modification of the F3 gene and the TK gene and/or the HA gene, for therapy, such as for gene therapy, for cancer gene therapy, or for vaccine therapy. Such a microorganism can be used to stimulate humoral and/or cellular immune response, induce strong cytotoxic T lymphocytes responses in subjects who may benefit from such responses. For example, the microorganism can provide prophylactic and therapeutic effects against a tumor infected by the microorganism or other infectious diseases, by rejection of cells from tumors or lesions using microorganisms that express immunoreactive antigens (Earl *et al.* (1986), *Science* 234, 728-831; Lathe *et al.* (1987), *Nature (London)* 326, 878-880), cellular tumor-associated antigens (Bernards *et al.*, (1987), *Proc. Natl. Acad. Sci. USA* 84, 6854-6858; Estin *et al.* (1988), *Proc. Natl. Acad. Sci. USA* 85, 1052-1056; Kantor *et al.* (1992), *J. Natl. Cancer Inst.* 84, 1084-1091; Roth *et al.* (1996), *Proc. Natl. Acad. Sci. USA* 93, 4781-4786) and/or cytokines (*e.g.*, IL-2, IL-12), costimulatory molecules (B7-1, B7-2) (Rao *et al.* (1996), *J. Immunol.* 156, 3357-3365; Chamberlain *et al.* (1996), *Cancer Res.* 56, 2832-2836; Oertli *et al.* (1996), *J. Gen. Virol.* 77, 3121-3125; Qin and Chatterjee (1996), *Human Gene Ther.* 7, 1853-1860; McAneny *et al.* (1996), *Ann. Surg. Oncol.* 3, 495-500), or other therapeutic proteins.

Provided herein, solid tumors can be treated with microorganisms, such as vaccinia viruses, resulting in an enormous tumor-specific microorganism replication, which can lead to tumor protein antigen and viral protein production in the tumors. As provided herein, vaccinia virus administration to mice resulted in lysis of the infected tumor cells and a resultant release of tumor-cell-specific antigens. Continuous leakage of these antigens into the body led to a very high level of antibody titer (in approximately 7-14 days) against tumor proteins, viral proteins, and the virus encoded engineered proteins in the mice. The newly synthesized antitumor antibodies and the enhanced macrophage, neutrophils count were continuously delivered via the vasculature to the tumor and thereby provided for the recruitment of an activated immune system against the tumor. The activated immune system then eliminated the foreign compounds of the tumor including the

-113-

viral particles. This interconnected release of foreign antigens boosted antibody production and continuous response of the antibodies against the tumor proteins to function like an autoimmunizing vaccination system initiated by vaccinia viral infection and replication, followed by cell lysis, protein leakage and enhanced antibody production. Thus, the present methods can provide a complete process that can be applied to all tumor systems with immunoprivileged tumor sites as site of privileged viral, bacterial, and mammalian cell growth, which can lead to tumor elimination by the host's own immune system.

In other embodiments, methods are provided for immunizing a subject, where the methods include administering to the subject a microorganism that expresses one or more antigens against which the subject will develop an immune response. The immunizing antigens can be endogenous to the microorganism, such as vaccinia antigens on a vaccinia virus used to immunize against smallpox. Or the immunizing antigens can be exogenous antigens expressed by the microorganism, such as influenza or HIV antigens expressed on a viral capsid or bacterial cell surface. Thus, the microorganisms provided herein, including the modified vaccinia viruses can be used as vaccines.

#### 1. Administration

In performing the methods provided herein, a microorganism can be administered to a subject, including a subject having a tumor or having neoplastic cells, or a subject to be immunized. An administered microorganism can be a microorganism provided herein or any other microorganism known for administration to a subject, for example, any known microorganism known for therapeutic administration to a subject, including antigenic microorganisms such as any microorganism known to be used for vaccination. In some embodiments, the microorganism administered is a microorganism containing a characteristic such as attenuated pathogenicity, low toxicity, preferential accumulation in tumor, ability to activate an immune response against tumor cells, high immunogenicity, replication competence, and ability to express exogenous proteins, and combinations thereof.

30

-114-

a. Steps prior to administering the microorganism

In some embodiments, one or more steps can be performed prior to administration of the microorganism to the subject. Any of a variety of preceding steps can be performed, including, but not limited to diagnosing the subject with a condition appropriate for microorganismal ~~administration~~ <sup>administration</sup>, determining the immunocompetence of the subject, immunizing the subject, treating the subject with a chemotherapeutic agent, treating the subject with radiation, or surgically treating the subject.

For embodiments that include administering a ~~subject~~ <sup>microorganism</sup> to a tumor-bearing subject for therapeutic purposes, the subject has typically been previously diagnosed with a neoplastic condition. Diagnostic methods also can include determining the type of neoplastic condition, determining the stage of the neoplastic conditions, determining the size of one or more tumors in the subject, determining the presence or absence of metastatic or neoplastic cells in the lymph nodes of the subject, or determining the presence of metastases of the subject. Some embodiments of therapeutic methods for administering a microorganism to a subject can include a step of determination of the size of the primary tumor or the stage of the neoplastic disease, and if the size of the primary tumor is equal to or above a threshold volume, or if the stage of the neoplastic disease is at or above a threshold stage, a microorganism is administered to the subject. In a similar embodiment, if the size of the primary tumor is below a threshold volume, or if the stage of the neoplastic disease is at or below a threshold stage, the microorganism is not yet administered to the subject; such methods can include monitoring the subject until the tumor size or neoplastic disease stage reaches a threshold amount, and then administering the microorganism to the subject. Threshold sizes can vary according to several factors, including rate of growth of the tumor, ability of the microorganism to infect a tumor, and immunocompetence of the subject. Generally the threshold size will be a size sufficient for a microorganism to accumulate and replicate in or near the tumor without being completely removed by the host's immune system, and will typically also be a size sufficient to sustain a microorganismal infection for a time long

-115-

enough for the host to mount an immune response against the tumor cells, typically about one week or more, about ten days or more, or about two weeks or more.

Exemplary threshold tumor sizes for viruses such as vaccinia viruses are at least about 100 mm<sup>3</sup>, at least about 200 mm<sup>3</sup>, at least about 300 mm<sup>3</sup>, at least about 400 mm<sup>3</sup>, at least about 500 mm<sup>3</sup>, at least about 750 mm<sup>3</sup>, at least about 1000 mm<sup>3</sup>, or at least about 1500 mm<sup>3</sup>. Threshold neoplastic disease stages also can vary according to several factors, including specific requirement for staging a particular neoplastic disease, aggressiveness of growth of the neoplastic disease, ability of the microorganism to infect a tumor or metastasis, and immunocompetence of the subject. Generally the threshold stage will be a stage sufficient for a microorganism to accumulate and replicate in a tumor or metastasis without being completely removed by the host's immune system, and will typically also be a size sufficient to sustain a microorganismal infection for a time long enough for the host to mount an immune response against the neoplastic cells, typically about one week or more, about ten days or more, or about two weeks or more. Exemplary threshold stages are any stage beyond the lowest stage (e.g., Stage I or equivalent), or any stage where the primary tumor is larger than a threshold size, or any stage where metastatic cells are detected.

In other embodiments, prior to administering to the subject a microorganism, the immunocompetence of the subject can be determined. The methods of administering a microorganism to a subject provided herein can include causing or enhancing an immune response in a subject. Accordingly, prior to administering a microorganism to a subject, the ability of a subject to mount an immune response can be determined. Any of a variety of tests of immunocompetence known in the art can be performed in the methods provided herein. Exemplary immunocompetence tests can examine ABO hemagglutination titers (IgM), leukocyte adhesion deficiency (LAD), granulocyte function (NBT), T and B cell quantitation, tetanus antibody titers, salivary IgA, skin test, tonsil test, complement C3 levels, and factor B levels, and lymphocyte count. One skilled in the art can determine the desirability to administer a microorganism to a subject according to the level of

-116-

immunocompetence of the subject, according to the immunogenicity of the microorganism, and, optionally, according to the ~~immunogenicity~~<sup>immunogenicity</sup> of the neoplastic disease to be treated. Typically, a subject can be considered immunocompetent if the skilled artisan can determine that the subject is sufficiently competent to mount an immune response against the microorganism.

In some embodiments, the subject can be immunized prior to administering to the subject a microorganism according to the methods provided herein. Immunization can serve to increase the ability of a subject to mount an immune response against the microorganism, or increase the speed at which the subject can mount an immune response against a microorganism. Immunization also can serve to decrease the risk to the subject of pathogenicity of the microorganism. In some embodiments, the immunization can be performed with an immunization microorganism that is similar to the therapeutic microorganism to be administered. For example, the immunization microorganism can be a replication-incompetent variant of the therapeutic microorganism. In other embodiments, the immunization material can be digests of the therapeutic microorganism to be administered. Any of a variety of methods for immunizing a subject against a known microorganism are known in the art and can be used herein. In one example, vaccinia viruses treated with, for example, 1 microgram of psoralen and ultraviolet light at 365 nm for 4 minutes, can be rendered replication incompetent. In another embodiment, the microorganism can be selected as the same or similar to a microorganism against which the subject has been previously immunized, e.g., in a childhood vaccination.

In another embodiment, the subject can have administered thereto a microorganism without any previous steps of cancer treatment such as chemotherapy, radiation therapy or surgical removal of a tumor and/or metastases. The methods provided herein take advantage of the ability of the microorganisms to enter or localize near a tumor, where the tumor cells can be protected from the subject's immune system; the microorganisms can then proliferate in such an immunoprotected region and can also cause the release, typically a sustained release, of tumor antigens from the tumor to a location in which the subject's immune

-117-

system can recognize the tumor antigens and mount an immune response. In such methods, existence of a tumor of sufficient size or sufficiently developed immunoprotected state can be advantageous for successful administration of the microorganism to the tumor, and for sufficient tumor antigen production. If a tumor is surgically removed, the microorganisms may not be able to localize to other neoplastic cells (*e.g.*, small metastases) because such cells may not yet have matured sufficiently to create an immunoprotective environment in which the microorganisms can survive and proliferate, or even if the microorganisms can localize to neoplastic cells, the number of cells or size of the mass may be too small for the microorganisms to cause a sustained release of tumor antigens in order for the host to mount an anti-tumor immune response. Thus, for example, provided herein are methods of treating a tumor or neoplastic disease in which microorganisms are administered to a subject with a tumor or neoplastic disease without removing the primary tumor, or to a subject with a tumor or neoplastic disease in which at least some tumors or neoplastic cells are intentionally permitted to remain in the subject. In other typical cancer treatment methods such as chemotherapy or radiation therapy, such methods typically have a side effect of weakening the subject's immune system. This treatment of a subject by chemotherapy or radiation therapy can reduce the subject's ability to mount an anti-tumor immune response. Thus, for example, provided herein are methods of treating a tumor or neoplastic disease in which microorganisms are administered to a subject with a tumor or neoplastic disease without treating the subject with an immune system-weakening therapy, such as chemotherapy or radiation therapy.

In an alternative embodiment, prior to administration of a microorganism to the subject, the subject can be treated in one or more cancer treatment steps that do not remove the primary tumor or that do not weaken the immune system of the subject. A variety of more sophisticated cancer treatment methods are being developed in which the tumor can be treated without surgical removal or immune-system weakening therapy. Exemplary methods include administering a compound that decreases the rate of proliferation of the tumor or neoplastic cells without

-118-

weakening the immune system (e.g., by administering tumor suppressor compounds or by administering tumor cell-specific compounds) or administering an angiogenesis-inhibiting compound. Thus, combined methods that include administering a microorganism to a subject can further improve cancer therapy.

- 5 Thus, provided herein are methods of administering a microorganism to a subject, along with prior to or subsequent to, for example, administering a compound that slows tumor growth without weakening the subject's immune system or a compound that inhibits vascularization of the tumor.

**b. Mode of administration**

- 10 Any mode of administration of a microorganism to a subject can be used, provided the mode of administration permits the microorganism to enter a tumor or metastasis. Modes of administration can include, but are not limited to, intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intratumor, multipuncture (e.g., as used with smallpox vaccines), inhalation, intranasal, oral, intracavity (e.g.,  
15 administering to the bladder via a <sup>catheter</sup> ~~catheter~~, administering to the gut by suppository or enema), aural, or ocular administration. One skilled in the art can select any mode of administration compatible with the subject and the microorganism, and that also is likely to result in the microorganism reaching tumors and/or metastases. The route of administration can be selected by one skilled in the art according to any of a  
20 variety of factors, including the nature of the disease, the kind of tumor, and the particular microorganism contained in the pharmaceutical composition. Administration to the target site can be performed, for example, by ballistic delivery, as a colloidal dispersion system, or systemic administration can be performed by injection into an artery.

25 **c. Dosage**

- The dosage regimen can be any of a variety of methods and amounts, and can be determined by one skilled in the art according to known clinical factors. As is known in the medical arts, dosages for any one patient can depend on many factors, including the subject's species, size, body surface area, age, sex,  
30 immunocompetence, and general health, the particular microorganism to be

-119-

administered, duration and route of administration, the kind and stage of the disease, for example, tumor size, and other compounds such as drugs being administered concurrently. In addition to the above factors, such levels can be affected by the infectivity of the microorganism, and the nature of the microorganism, as can be determined by one skilled in the art. At least some of the viruses used in the methods provided herein can be more infectious than the bacteria used herein. Thus, in some embodiments of the present methods, virus can be administered at lower levels than bacteria. In the present methods, appropriate minimum dosage levels of microorganisms can be levels sufficient for the microorganism to survive, grow and replicate in a tumor or metastasis. Exemplary minimum levels for administering a virus to a 65 kg human can include at least about  $5 \times 10^5$  plaque forming units (pfu), at least about  $1 \times 10^6$  pfu, at least about  $5 \times 10^6$  pfu, at least about  $1 \times 10^7$  pfu, or at least about  $1 \times 10^8$  pfu. Exemplary minimum levels for administering a bacterium to a 65 kg human can include at least about  $5 \times 10^6$  colony forming units (cfu), at least about  $1 \times 10^7$  cfu, at least about  $5 \times 10^7$  cfu, at least about  $1 \times 10^8$  cfu, or at least about  $1 \times 10^9$  cfu. In the present methods, appropriate maximum dosage levels of microorganisms can be levels that are not toxic to the host, levels that do not cause splenomegaly of 3x or more, levels that do not result in colonies or plaques in normal tissues or organs after about 1 day or after about 3 days or after about 7 days. Exemplary maximum levels for administering a virus to a 65 kg human can include no more than about  $5 \times 10^{10}$  pfu, no more than about  $1 \times 10^{10}$  pfu, no more than about  $5 \times 10^9$  pfu, no more than about  $1 \times 10^9$  pfu, or no more than about  $1 \times 10^8$  pfu. Exemplary maximum levels for administering a bacterium to a 65 kg human can include no more than about  $5 \times 10^{11}$  pfu, no more than about  $1 \times 10^{11}$  pfu, no more than about  $5 \times 10^{10}$  pfu, no more than about  $1 \times 10^{10}$  pfu, or no more than about  $1 \times 10^9$  pfu.

**d. Number of administrations**

The methods provided herein can include a single administration of a microorganism to a subject or multiple administrations of a microorganism to a subject. In some embodiments, a single administration is sufficient to establish a

-120-

microorganism in a tumor, where the microorganism can proliferate and can cause or enhance an anti-tumor response in the subject; such methods do not require additional administrations of a microorganism in order to cause or enhance an anti-tumor response in a subject, which can result, for example in inhibition of tumor growth, inhibition of <sup>metastasis</sup> ~~metasis~~ growth or formation, reduction in tumor or <sup>metastasis</sup> ~~metasis~~ size, elimination of a tumor or metastasis, inhibition or prevention of recurrence of a neoplastic disease or new tumor formation, or other cancer therapeutic effects. In other embodiments, a microorganism can be administered on different occasions, separated in time typically by at least one day. Separate administrations can increase the likelihood of delivering a microorganism to a tumor or metastasis, where a previous administration may have been ineffective in delivering a microorganism to a tumor or metastasis. Separate administrations can increase the locations on a tumor or metastasis where microorganism proliferation can occur or can otherwise increase the titer of microorganism accumulated in the tumor, which can increase the scale of release of antigens or other compounds from the tumor in eliciting or enhancing a host's anti-tumor immune response, and also can, optionally, increase the level of microorganism-based tumor lysis or tumor cell death. Separate administrations of a microorganism can further extend a subject's immune response against microorganismal antigens, which can extend the host's immune response to tumors or metastases in which microorganisms have accumulated, and can increase the likelihood <sup>of</sup> a host mounting an anti-tumor immune response.

When separate administrations are performed, each administration can be a dosage amount that is the same or different relative to other administration dosage amounts. In one embodiment, all administration dosage amounts are the same. In other embodiments, a first dosage amount can be a larger dosage amount than one or more subsequent dosage amounts, for example, at least 10x larger, at least 100x larger, or at least 1000x larger than subsequent dosage amounts. In one example of a method of separate administrations in which the first dosage amount is greater than

-121-

one or more subsequent dosage amounts, all subsequent dosage amounts can be the same, smaller amount relative to the first administration.

Separate administrations can include any number of two or more administrations, including two, three, four, five or six administrations. One skilled in the art can readily determine the number of administrations to perform or the desirability of performing one or more additional administrations according to methods known in the art for monitoring therapeutic methods and other monitoring methods provided herein. Accordingly, the methods provided herein include methods of providing to the subject one or more administrations of a microorganism, where the number of administrations can be determined by monitoring the subject, and, based on the results of the monitoring, determining whether or not to provide one or more additional administrations. Deciding of whether or not to provide one or more additional administrations can be based on a variety of monitoring results, including, but not limited to, indication of tumor growth or inhibition of tumor growth, appearance of new metastases or inhibition of metastasis, the subject's anti-microorganism antibody titer, the subject's anti-tumor antibody titer, the overall health of the subject, the weight of the subject, the presence of microorganism solely in tumor and/or metastases, the presence of microorganism in normal tissues or organs.

The time period between administrations can be any of a variety of time periods. The time period between administrations can be a function of any of a variety of factors, including monitoring steps, as described in relation to the number of administrations, the time period for a subject to mount an immune response, the time period for a subject to clear microorganism from normal tissue, or the time period for microorganismal proliferation in the tumor or metastasis. In one example, the time period can be a function of the time period for a subject to mount an immune response; for example, the time period can be more than the time period for a subject to mount an immune response, such as more than about one week, more than about ten days, more than about two weeks, or more than about a month; in another example, the time period can be less than the time period for a subject to

-122-

mount an immune response, such as less than about one week, less than about ten days, less than about two weeks, or less than about a month. In another example, the time period can be a function of the time period for a subject to clear microorganism from normal tissue; for example, the time period can be more than the time period for a subject to clear microorganism from normal tissue, such as more than about a day, more than about two days, more than about three days, more than about five days, or more than about a week. In another example, the time period can be a function of the time period for microorganismal proliferation in the tumor or metastasis; for example, the time period can be more than the amount of time for a detectable signal to arise in a tumor or metastasis after administration of a microorganism expressing a detectable marker, such as about 3 days, about 5 days, about a week, about ten days, about two weeks, or about a month.

**e. Co-administrations**

Also provided are methods in which an additional therapeutic substance, such as a different therapeutic microorganism or a therapeutic compound is administered. These can be administered simultaneously, sequentially or intermittently with the first microorganism. The additional therapeutic substance can interact with the microorganism or a gene product thereof, or the additional therapeutic substance can act independently of the microorganism.

**i. Administration of a plurality of microorganisms**

Methods are provided for administering to a subject two or more microorganisms. <sup>Administration</sup> ~~Administration~~ can be effected simultaneously, sequentially or intermittently. <sup>Ⓢ</sup> The plurality of microorganisms can be administered as a single composition or as two or more compositions. The two or more microorganisms can include at least two bacteria, at least two viruses, at least two eukaryotic cells, or two or more selected from among bacteria, viruses and eukaryotic cells. The plurality of microorganisms can be provided as combinations of compositions containing and/or as kits that include the microorganisms <sup>packaged</sup> ~~packaged~~ for administration and optionally including <sup>instructions</sup> ~~instructions~~ therefore. The <sup>compositions</sup> ~~compositions~~ can contain the

-123-

microorganisms formulated for single dosage administration (*i.e.*, for direct administration) can require dilution or other additions.

In one embodiment, at least one of the microorganisms is a modified microorganism such as those provided herein, having a characteristic such as low pathogenicity, low toxicity, preferential accumulation in tumor, ability to activate an immune response against tumor cells, immunogenic, replication competent, ability to express exogenous proteins, and combinations thereof. The microorganisms can be administered at approximately the same time, or can be administered at different times. The microorganisms can be administered in the same composition or in the same administration method, or can be administered in separate composition or by different administration methods.

In one example, a bacteria and a virus can be administered to a subject. The bacteria and virus can be administered at the same time, or at different times. For example, the virus can be administered prior to administering the bacteria, or the bacteria can be administered prior to administering the virus; typically the virus is administered prior to administering the bacteria. As provided herein, administering to a subject a virus prior to administering to the subject a bacterium can increase the amount of bacteria that can accumulate and/or proliferate in a tumor, relative to methods in which bacteria alone are administered.

Accordingly, the methods provided herein that include administration of virus prior to administration of bacteria permit the administration of a lower dosage amount of bacteria than would otherwise be administered in a method in which bacteria alone are administered or a method in which bacteria are administered at the same time as or prior to administration of a virus. For example, in some embodiments, a bacterium to be administered can have one or more properties that limit the ability of the bacterium to be used, such properties can include, but are not limited to toxicity, low tumor specificity of accumulation, and limited proliferation capacity. A bacterium to be administered that has one or more limiting properties can require administration in lower dosage amounts, or can require assistance in tumor-specific accumulation and/or proliferation. Provided herein are methods of

-124-

administering such a bacterium with limiting properties, where prior to administering the bacterium, a virus is administered such that the limited bacterium can be administered in smaller quantities, can accumulate in tumor with increased specificity, and/or can have an increased ability to proliferate in a tumor.

- 5           The time period between administrations can be any time period that achieves the desired effects, as can be determined by one skilled in the art. Selection of a time period between administrations of different microorganisms can be determined according to parameters similar to those for selecting the time period between administrations of the same microorganism, including results from
- 10   monitoring steps, the time period for a subject to mount an immune response, the time period for a subject to clear microorganism from normal tissue, or the time period for microorganismal proliferation in the tumor or metastasis. In one example, the time period can be a function of the time period for a subject to mount an immune response; for example, the time period can be more than the time period for
- 15   a subject to mount an immune response, such as more than about one week, more than about ten days, more than about two weeks, or more than about a month; in another example, the time period can be less than the time period for a subject to mount an immune response, such as less than about one week, less than about ten days, less than about two weeks, or less than about a month. In another example, the
- 20   time period can be a function of the time period for a subject to clear microorganism from normal tissue; for example, the time period can be more than the time period for a subject to clear microorganism from normal tissue, such as more than about a day, more than about two days, more than about three days, more than about five days, or more than about a week. In another example, the time period can be a
- 25   function of the time period for microorganismal proliferation in the tumor or metastasis; for example, the time period can be more than the amount of time for a detectable signal to arise in a tumor or metastasis after administration of a microorganism expressing a detectable marker, such as about 3 days, about 5 days, about a week, about ten days, about two weeks, or about a month. In one example a
- 30   virus can first be administered, and a bacteria can be administered about 5 days after

-125-

administration of the virus. In another example, a virus can be first administered, and a bacterium can be administered upon detection of a virally-encoded detectable gene product in the tumor of the subject, optionally when the virally-encoded detectable gene product is detected only in the tumor of the subject.

5                                   ii.       **Therapeutic compounds**

The methods can include administering one or more therapeutic compounds to the subject in addition to administering a microorganism or plurality thereof to a subject. Therapeutic compounds can act independently, or in conjunction with the microorganism, for tumor therapeutic ~~effects~~ <sup>effects</sup>. Therapeutic compounds that can act independently include any of a variety of known chemotherapeutic compounds that can inhibit tumor growth, inhibit metastasis growth and/or formation, decrease the size of a tumor or metastasis, eliminate a tumor or metastasis, without reducing the ability of a microorganism to accumulate in a tumor, replicate in the tumor, and cause or enhance an anti-tumor immune response in the subject.

15           Therapeutic compounds that act in conjunction with the microorganisms include, for example, compounds that alter the expression of the microorganism or compounds that can interact with a microorganism-expressed gene, or compounds that can inhibit microorganismal proliferation, including compounds toxic to the microorganism. Therapeutic compounds that can act in conjunction with the  
20   microorganism include, for example, therapeutic compounds that increase the proliferation, toxicity, tumor cell killing, or immune response eliciting properties of a microorganism, and also can include, for example, therapeutic compounds that decrease the proliferation, toxicity, or cell killing properties of a microorganism. Thus, provided herein are methods of administering to a subject one or more  
25   therapeutic compounds that can act in conjunction with the microorganism to increase the proliferation, toxicity, tumor cell killing, or immune response eliciting properties of a microorganism. Also provided herein are methods of administering to a subject one or more therapeutic compounds that can act in conjunction with the microorganism to decrease the proliferation, toxicity, or cell killing properties of a  
30   microorganism.

-126-

In one embodiment, therapeutic compounds that can act in conjunction with the microorganism to increase the proliferation, toxicity, tumor cell killing, or immune response eliciting properties of a microorganism are compounds that can alter gene expression, where the altered gene expression can result in an increased killing of tumor cells or an increased anti-tumor immune response in the subject. A gene expression-altering compound can, for example, cause an increase or decrease in expression of one or more microorganismal genes, including endogenous microorganismal genes and/or exogenous microorganismal genes. For example, a gene expression-altering compound can induce or increase transcription of a gene in a microorganism such as an exogenous gene that can cause cell lysis or cell death, that can provoke an immune response, that can catalyze conversion of a prodrug-like compound, or that can inhibit expression of a tumor cell gene. Any of a wide variety of compounds that can alter gene expression are known in the art, including IPTG and RU486. Exemplary genes whose expression can be up-regulated include proteins and RNA molecules, including toxins, enzymes that can convert a prodrug to an anti-tumor drug, cytokines, transcription regulating proteins, siRNA, and ribozymes. In another example, a gene expression-altering compound can inhibit or decrease transcription of a gene in a microorganism such as an exogenous gene that can reduce microorganismal toxicity or reduces microorganismal proliferation. Any of a variety of compounds that can reduce or inhibit gene expression can be used in the methods provided herein, including siRNA compounds, transcriptional inhibitors or inhibitors of transcriptional activators. Exemplary genes whose expression can be down-regulated include proteins and RNA molecules, including microorganismal proteins or RNA that suppress lysis, nucleotide synthesis or proliferation, and cellular proteins or RNA molecules that suppress cell death, immunoreactivity, lysis, or microorganismal replication.

In another embodiment, therapeutic compounds that can act in conjunction with the microorganism to increase the proliferation, toxicity, tumor cell killing, or immune response eliciting properties of a microorganism are compounds that can interact with a microorganism-expressed gene product, and such interaction can

-127-

result in an increased killing of tumor cells or an increased anti-tumor immune response in the subject. A therapeutic compound that can interact with a microorganism-expressed gene product can include, for example a prodrug or other compound that has little or no toxicity or other biological activity in its subject-administered form, but after interaction with a microorganism-expressed gene product, the compound can develop a property that results in tumor cell death, including but not limited to, cytotoxicity, ability to induce apoptosis, or ability to trigger an immune response. A variety of prodrug-like substances are known in the art and an exemplary set of such compounds are disclosed elsewhere herein, where such compounds can include gancyclovir, 5-fluorouracil, 6-methylpurine deoxyriboside, cephalosporin-doxorubicin, 4-[(2-chloroethyl)(2-mesuloxyethyl)amino]benzoyl-L-glutamic acid, acetaminophen, indole-3-acetic acid, CB1954, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, bis-(2-chloroethyl)amino-4-hydroxyphenylaminomethanone 28, 1-chloromethyl-5-hydroxy-1,2-dihydro-3H-benz[e]indole, epirubicin-glucuronide, 5'-deoxy-5-fluorouridine, cytosine arabinoside, and linamarin.

In another embodiment, therapeutic compounds that can act in conjunction with the microorganism to decrease the proliferation, toxicity, or cell killing properties of a microorganism are compounds that can inhibit microorganismal replication, inhibit microorganismal toxins, or cause microorganismal death. A therapeutic compound that can inhibit microorganismal replication, inhibit microorganismal toxins, or cause microorganismal death can generally include a compound that can block one or more steps in the microorganismal life cycle, including, but not limited to, compounds that can inhibit microorganismal DNA replication, microorganismal RNA transcription, viral coat protein assembly, outer membrane or polysaccharide assembly. Any of a variety of compounds that can block one or more steps in a microorganismal life cycle are known in the art, including any known antibiotic, microorganismal DNA polymerase inhibitors, microorganismal RNA polymerase inhibitors, inhibitors of proteins that regulate microorganismal DNA replication or RNA transcription. In one example, when a

-128-

microorganism is a bacteria, a compound can be an antibiotic. In another example, a microorganism can contain a gene encoding a microorganismal life cycle protein, such as DNA polymerase or RNA polymerase that can be inhibited by a compound that is, optionally, non-toxic to the host organism.

5                   f.       **State of subject**

In another embodiment, the methods provided herein for administering a microorganism to a subject can be performed on a subject in any of a variety of states, including an anesthetized subject, an alert subject, a subject with elevated body temperature, a subject with reduced body temperature, or other state of the subject that is known to affect the accumulation of microorganism in the tumor. As provided herein, it has been determined that a subject that is anesthetized can have a decreased rate of accumulation of a microorganism in a tumor relative to a subject that is not anesthetized. Further provided herein, it has been determined that a subject with decreased body temperature can have a decreased rate of accumulation of a microorganism in a tumor relative to a subject with a normal body temperature. Accordingly, provided herein are methods of administering a microorganism to a subject, where the methods can include administering a microorganism to a subject where the subject is not under anesthesia, such as general anesthesia; for example, the subject can be under local anesthesia, or can be unanesthetized. Also provided herein are methods of administering a microorganism to a subject, where the methods can include administering a microorganism to a subject with altered body temperature, where the alteration of the body temperature can influence the ability of the microorganism to accumulate in a tumor; typically, a decrease in body temperature can decrease the ability of a microorganism to accumulate in a tumor. Thus, in one exemplary embodiment, a method is provided for administering a microorganism to a subject, where the method includes elevating the body temperature of the subject to a temperature above normal, and administering a microorganism to the subject, where the microorganism can accumulate in the tumor more readily in the subject with higher body temperature relative to the ability of the

-129-

microorganism to accumulate in a tumor of a subject with a normal body temperature.

## 2. Monitoring

The methods provided herein can further include one or more steps of  
5 monitoring the subject, monitoring the tumor, and/or monitoring the microorganism administered to the subject. Any of a variety of monitoring steps can be included in the methods provided herein, including, but not limited to, monitoring tumor size, monitoring anti-(tumor antigen) antibody titer, monitoring the presence and/or size of metastases, monitoring the subject's lymph nodes, monitoring the subject's  
10 weight or other health indicators including blood or urine markers, monitoring anti-(microorganismal antigen) antibody titer, monitoring microorganismal expression of a detectable gene product, and directly monitoring microorganismal titer in a tumor, tissue or organ of a subject.

The purpose of the monitoring can be simply for assessing the health state of  
15 the subject or the progress of therapeutic treatment of the subject, or can be for determining whether or not further administration of the same or a different microorganism is warranted, or for determining when or whether or not to administer a compound to the subject where the compound can act to increase the efficacy of the therapeutic method, or the compound can act to decrease the  
20 pathogenicity of the microorganism administered to the subject.

### a. Monitoring microorganismal gene expression

In some embodiments, the methods provided herein can include monitoring one or more microorganismally expressed genes. Microorganisms, such as those provided herein or otherwise known in the art, can express one or more detectable  
25 gene products, including but not limited to, detectable proteins.

As provided herein, measurement of a detectable gene product expressed in a microorganism can provide an accurate determination of the level of microorganism present in the subject. As further provided herein, measurement of the location of the detectable gene product, for example, by imaging methods including  
30 tomographic methods, can determine the localization of the microorganism in the

-130-

subject. Accordingly, the methods provided herein that include monitoring a detectable microorganismal gene product can be used to determine the presence or absence of the microorganism in one or more organs or tissues of a subject, and/or the presence or absence of the microorganism in a tumor or metastases of a subject.

5 Further, the methods provided herein that include monitoring a detectable microorganismal gene product can be used to determine the titer of microorganism present in one or more organs, tissues, tumors or metastases. Methods that include monitoring the localization and/or titer of microorganisms in a subject can be used for determining the pathogenicity of a microorganism; since microorganismal

10 infection, and particularly the level of infection, of normal tissues and organs can indicate the pathogenicity of the probe, methods of monitoring the localization and/or amount of microorganisms in a subject can be used to determine the pathogenicity of a microorganism. Since methods provided herein can be used to monitor the amount of microorganisms at any particular location in a subject, the

15 methods that include monitoring the localization and/or titer of microorganisms in a subject can be performed at multiple time points, and, accordingly can determine the rate of microorganismal replication in a subject, including the rate of microorganismal replication in one or more organs or tissues of a subject; accordingly, the methods of monitoring a microorganismal gene product <sup>can</sup> be used

20 for determining the replication competence of a microorganism. The methods provided herein also can be used to quantitate the amount of microorganism present in a variety of organs or tissues, and tumors or <sup>metastases</sup> ~~metastases~~, and can thereby indicate the degree of preferential accumulation of the microorganism in a subject; accordingly, the microorganismal gene product monitoring methods provided herein

25 can be used in methods of determining the ability of a microorganism to accumulate in tumor or metastases in preference to normal tissues or organs. Since the microorganisms used in the methods provided herein can accumulate in an entire tumor or can accumulate at multiple sites in a tumor, and can also accumulate in metastases, the methods provided herein for monitoring a microorganismal gene

30 product can be used to determine the size of a tumor or the number of metastases are

-131-

present in a subject. Monitoring such presence of microorganismal gene product in tumor or metastasis over a range of time can be used to assess changes in the tumor or metastasis, including growth or shrinking of a tumor, or development of new metastases or disappearance of metastases, and also can be used to determine the rate of growth or shrinking of a tumor, or development of new metastases or disappearance of metastases, or the change in the rate of growth or shrinking of a tumor, or development of new metastases or disappearance of metastases. Accordingly, the methods of monitoring a microorganismal gene product can be used for monitoring a neoplastic disease in a subject, or for determining the efficacy of treatment of a neoplastic disease, by determining rate of growth or shrinking of a tumor, or development of new metastases or disappearance of metastases, or the change in the rate of growth or shrinking of a tumor, or development of new metastases or disappearance of metastases.

Any of a variety of detectable proteins can be detected in the monitoring methods provided herein; an exemplary, non-limiting list of such detectable proteins includes any of a variety of fluorescence proteins (*e.g.*, green fluorescence proteins), any of a variety of luciferases, transferring or other iron binding proteins; or receptors, binding proteins, and antibodies, where a compound that specifically binds the receptor, binding protein or antibody can be a detectable agent or can be labeled with a detectable substance (*e.g.*, a radionuclide or imaging agent).

**b. Monitoring tumor size**

Also provided herein are methods of monitoring tumor and/or metastasis size and location. Tumor and or metastasis size can be monitored by any of a variety of methods known in the art, including external assessment methods or tomographic or magnetic imaging methods. In addition to the methods known in the art, methods provided herein, for example, monitoring microorganismal gene expression, can be used for monitoring tumor and/or metastasis size.

Monitoring size over several time points can provide information regarding the increase or decrease in size of a tumor or metastasis, and can also provide information regarding the presence of additional tumors and/or metastases in the

-132-

subject. Monitoring tumor size over several time points can provide information regarding the development of a neoplastic disease in a subject, including the efficacy of treatment of a neoplastic disease in a subject.

**c. Monitoring antibody titer**

5       The methods provided herein also can include monitoring the antibody titer in a subject, including antibodies produced in response to administration of a microorganism to a subject. The microorganisms administered in the methods provided herein can elicit an immune response to endogenous microorganismal antigens. The microorganisms administered in the methods provided herein also can  
10       elicit an immune response to exogenous genes expressed by a microorganism. The microorganisms administered in the methods provided herein also can elicit an immune response to tumor antigens. Monitoring antibody titer against microorganismal antigens, microorganismally expressed exogenous gene products, or tumor antigens can be used in methods of monitoring the toxicity of a  
15       microorganism, monitoring the efficacy of treatment methods, or monitoring the level of gene product or antibodies for production and/or harvesting.

      In one embodiment, monitoring antibody titer can be used to monitor the toxicity of a microorganism. Antibody titer against a microorganism can vary over the time period after administration of the microorganism to the subject, where at  
20       some particular time points, a low anti-(microorganismal antigen) antibody titer can indicate a higher toxicity, while at other time points a high anti-(microorganismal antigen) antibody titer can indicate a higher toxicity. The microorganisms used in the methods provided herein can be immunogenic, and can, therefore, elicit an immune response soon after administering the microorganism to the subject.

25       Generally, a microorganism against which a subject's immune system can quickly mount a strong immune response can be a microorganism that has low toxicity when the subject's immune system can remove the microorganism from all normal organs or tissues. Thus, in some embodiments, a high antibody titer against microorganismal antigens soon after administering the microorganism to a subject  
30       can indicate low toxicity of a microorganism. In contrast, a microorganism that is

-133-

not highly immunogenic may infect a host organism without eliciting a strong immune response, which can result in a higher toxicity of the microorganism to the host. Accordingly, in some embodiments, a high antibody titer against microorganismal antigens soon after administering the microorganism to a subject  
5 can indicate low toxicity of a microorganism.

In other embodiments, monitoring antibody titer can be used to monitor the efficacy of treatment methods. In the methods provided herein, antibody titer, such as anti-(tumor antigen) antibody titer, can indicate the efficacy of a therapeutic method such as a therapeutic method to treat neoplastic disease. Therapeutic  
10 methods provided herein can include causing or enhancing an immune response against a tumor and/or metastasis. Thus, by monitoring the anti-(tumor antigen) antibody titer, it is possible to monitor the efficacy of a therapeutic method in causing or enhancing an immune response against a tumor and/or metastasis. The therapeutic methods provided herein also can include administering to a subject a  
15 microorganism that can accumulate in a tumor and can cause or enhance an anti-tumor immune response. Accordingly, it is possible to monitor the ability of a host to mount an immune response against microorganisms accumulated in a tumor or metastasis, which can indicate that a subject has also mounted an anti-tumor immune response, or can indicate that a subject is likely to mount an anti-tumor immune  
20 response, or can indicate that a subject is capable of mounting an anti-tumor immune response.

In other embodiments, monitoring antibody titer can be used for monitoring the level of gene product or antibodies for production and/or harvesting. As provided herein, methods can be used for producing proteins, RNA molecules or  
25 other compounds by expressing an exogenous gene in a microorganism that has accumulated in a tumor. Further provided herein are methods for producing antibodies against a protein, RNA molecule or other compound produced by exogenous gene expression of a microorganism that has accumulated in a tumor. Monitoring antibody titer against the protein, RNA molecule or other compound can  
30 indicate the level of production of the protein, RNA molecule or other compound by

-134-

the tumor-accumulated microorganism, and also can directly indicate the level of antibodies specific for such a protein, RNA molecule or other compound.

**d. Monitoring general health diagnostics**

The methods provided herein also can include methods of monitoring the health of a subject. Some of the methods provided herein are therapeutic methods, including neoplastic disease therapeutic methods. Monitoring the health of a subject can be used to determine the efficacy of the therapeutic method, as is known in the art. The methods provided herein also can include a step of administering to a subject a microorganism. Monitoring the health of a subject can be used to determine the pathogenicity of a microorganism administered to a subject. Any of a variety of health diagnostic methods for monitoring disease such as neoplastic disease, infectious disease, or immune-related disease can be monitored, as is known in the art. For example, the weight, blood pressure, pulse, breathing, color, temperature or other observable state of a subject can indicate the health of a subject. In addition, the presence or absence or level of one or more components in a sample from a subject can indicate the health of a subject. Typical samples can include blood and urine samples, where the presence or absence or level of one or more components can be determined by performing, for example, a blood panel or a urine panel diagnostic test. Exemplary components indicative of a subject's health include, but are not limited to, white blood cell count, hematocrit, c-reactive protein concentration.

**e. Monitoring coordinated with treatment**

Also provided herein are methods of monitoring a therapy, where therapeutic decisions can be based on the results of the monitoring. Therapeutic methods provided herein can include administering to a subject a microorganism, where the microorganism can preferentially accumulate in a tumor and/or <sup>metastasis</sup> metastasis, and where the microorganism can cause or enhance an anti-tumor immune response. Such therapeutic methods can include a variety of steps including multiple administrations of a particular microorganism, administration of a second microorganism, or administration of a therapeutic compound. Determination of the

-135-

amount, timing or type of microorganism or compound to administer to the subject can be based on one or more results from monitoring the subject. For example, the antibody titer in a subject can be used to determine whether or not it is desirable to administer a microorganism or compound, the quantity of microorganism or compound to administer, and the type of microorganism or compound to administer, where, for example, a low antibody titer can indicate the desirability of administering additional microorganism, a different microorganism, or a therapeutic compound such as a compound that induces microorganismal gene expression. In another example, the overall health state of a subject can be used to determine whether or not it is desirable to administer a microorganism or compound, the quantity of microorganism or compound to administer, and the type of microorganism or compound to administer, where, for example, determining that the subject is healthy can indicate the desirability of administering additional microorganism, a different microorganism, or a therapeutic compound such as a compound that induces microorganismal gene expression. In another example, monitoring a detectable microorganismally expressed gene product can be used to determine whether or not it is desirable to administer a microorganism or compound, the quantity of microorganism or compound to administer, and the type of microorganism or compound to administer. Such monitoring methods can be used to determine whether or not the therapeutic method is effective, whether or not the therapeutic method is pathogenic to the subject, whether or not the microorganism has accumulated in a tumor or metastasis, and whether or not the microorganism has accumulated in normal tissues or organs. Based on such determinations, the desirability and form of further therapeutic methods can be derived.

In one embodiment, determination of whether or not a therapeutic method is effective can be used to derive further therapeutic methods. Any of a variety of methods of monitoring can be used to determine whether or not a therapeutic method is effective, as provided herein or otherwise known in the art. If monitoring methods indicate that the therapeutic method is effective, a decision can be made to maintain the current course of therapy, which can include further administrations of

-136-

a microorganism or compound, or a decision can be made that no further administrations are required. If monitoring methods indicate that the therapeutic method is ineffective, the monitoring results can indicate whether or not a course of treatment should be discontinued (*e.g.*, when a microorganism is pathogenic to the subject), or changed (*e.g.*, when a microorganism accumulates in a tumor without harming the host organism, but without eliciting an anti-tumor immune response), or increased in frequency or amount (*e.g.*, when little or no microorganism accumulates in tumor).

In one example, monitoring can indicate that a microorganism is pathogenic to a subject. In such instances, a decision can be made to terminate administration of the microorganism to the subject, to administer lower levels of the microorganism to the subject, to administer a different microorganism to a subject, or to administer to a subject a compound that reduces the pathogenicity of the microorganism. In one example, administration of a microorganism that is determined to be pathogenic can be terminated. In another example, the dosage amount of a microorganism that is determined to be pathogenic can be decreased for subsequent administration; in one version of such an example, the subject can be pre-treated with another microorganism that can increase the ability of the pathogenic microorganism to accumulate in tumor, prior to re-administering the pathogenic microorganism to the subject. In another example, a subject can have administered thereto a bacteria or virus that is pathogenic to the subject; administration of such a pathogenic microorganism can be accompanied by administration of, for example an antibiotic, anti-microorganismal compound, pathogenicity attenuating compound (*e.g.*, a compound that down-regulates the expression of a lytic or apoptotic gene product), or other compound that can decrease the proliferation, toxicity, or cell killing properties of a microorganism, as described herein elsewhere. In one variation of such an example, the localization of the microorganism can be monitored, and, upon determination that the microorganism is accumulated in tumor and/or metastases but not in normal tissues or organs, administration of the antibiotic, anti-microorganismal compound or pathogenicity attenuating compound can be

-137-

terminated, and the pathogenic activity of the microorganism can be activated or increased, but limited to the tumor and/or metastasis. In another variation of such an example, after terminating administration of an antibiotic, anti-microorganismal compound or pathogenicity attenuating compound, the presence of the  
5 microorganism and/or pathogenicity of the microorganism can be further monitored, and administration of such a compound can be reinitiated if the microorganism is determined to pose a threat to the host by, for example, spreading to normal organs or tissues, releasing a toxin into the vasculature, or otherwise having pathogenic effects reaching beyond the tumor or metastasis.

10 In another example, monitoring can determine whether or not a microorganism has accumulated in a tumor or metastasis of a subject. Upon such a determination, a decision can be made to further administer additional microorganism, a different microorganism or a compound to the subject. In one example, monitoring the presence of a virus in a tumor or metastasis can be used in  
15 deciding to administer to the subject a bacterium, where, for example, the quantity of bacteria administered can be reduced according to the presence and/or quantity of virus in a tumor or metastasis. In a similar example, monitoring the presence of a virus in a tumor or metastasis can be used in deciding when to administer to the subject a bacterium, where, for example, the bacteria can be administered upon  
20 detecting to the presence and/or a selected quantity of virus in a tumor or metastasis. In another example, monitoring the presence of a microorganism in a tumor can be used in deciding to administer to the subject a compound, where the compound can increase the pathogenicity, proliferation, or immunogenicity of a microorganism or the compound can otherwise act in conjunction with the microorganism to increase  
25 the proliferation, toxicity, tumor cell killing, or immune response eliciting properties of a microorganism; in one variation of such an example, the microorganism can, for example have little or no lytic or cell killing capability in the absence of such a compound; in a further variation of such an example, monitoring of the presence of the microorganism in a tumor or metastasis can be coupled with monitoring the  
30 absence of the microorganism in normal tissues or organs, where the compound is

-138-

administered if the microorganism is present in tumor or metastasis and not at all present or substantially not present in normal organs or tissues; in a further variation of such an example, the amount of microorganism in a tumor or metastasis can be monitored, where the compound is administered if the microorganism is present in  
5 tumor or metastasis at sufficient levels.

**E. Methods of Producing Gene Products and Antibodies**

Provided herein are microorganisms, and methods for making and using such microorganisms for production products of exogenous genes and/or for production of antibodies specific for exogenous gene products. The methods provided herein  
10 result in efficient recombinant production of biologically active proteins. In EP A1 1 281 772, it is disclosed that when vaccinia virus (LIVP strain) carrying the light emitting fusion gene construct rVV-ruc-gfp was injected intravenously into nude mice, the virus particles were found to be cleared from all internal organs within 4 days, as determined by extinction of light emission. In contrast, when the fate of the  
15 injected vaccinia virus was similarly followed in nude mice bearing tumors grown from subcutaneously implanted C6 rat <sup>glioma</sup> glioma cells, virus particles were found to be retained over time in the tumor tissues, resulting in lasting light emission. The presence and amplification of the virus-encoded fusion proteins in the same tumor were monitored in live animals by observing GFP fluorescence under a  
20 stereomicroscope and by detecting luciferase-catalyzed light emission under a low-light video-imaging camera. Tumor-specific light emission was detected 4 days after viral injection in nude mice carrying subcutaneous C6 glioma implants. Tumor accumulation of rVV-ruc-gfp virus particles was also seen in nude mice carrying subcutaneous tumors developed from implanted PC-3 human prostate cells, and in  
25 mice with orthotopically implanted MCF-7 human breast tumors. Further, intracranial C6 rat glioma cell implants in immunocompetent rats and MB-49 human bladder tumor cell implants in C57 mice were also targeted by the vaccinia virus. In addition to primary breast tumors, small metastatic tumors were also detected externally in the contralateral breast region, as well as in nodules on the exposed  
30 lung surface, suggesting metastasis to the contralateral breast and lung. In summary

-139-

it was shown that light-emitting cells or microorganisms, for example, vaccinia virus can be used to detect and treat metastatic tumors.

Similar results were obtained with light-emitting bacteria (*Salmonella*, *Vibrio*, *Listeria*, *E. coli*) which were injected intravenously into mice and which  
5 could be visualized in whole animals under a low light imager immediately. No light emission was detected twenty four hours after bacterial injection in both athymic (nu/nu) mice and immunocompetent C57 mice as a result of clearing by the immune system. In nude mice bearing tumors developed from implanted C6 glioma cells, light emission was abolished from the animal entirely twenty four hours after  
10 delivery of bacteria, similar to mice without tumors. However, forty eight hours post-injection, a strong, rapidly increasing light emission originated only from the tumor regions was observed. This observation indicated a continuous bacterial replication in the tumor tissue. The extent of light emission was dependent on the bacterial strain used. The homing-in process together with the sustained light  
15 emission was also demonstrated in nude mice carrying prostate, bladder, and breast tumors. In addition to primary tumors, metastatic tumors could also be visualized as exemplified in the breast tumor model. Tumor-specific light emission was also observed in immunocompetent C57 mice, with bladder tumors as well as in Lewis rats with brain glioma implants. Once in the tumor, the light-emitting bacteria were  
20 not observed to be released into the circulation and to re-colonize subsequently implanted tumors in the same animal. Further, mammalian cells expressing the Ruc-GFP fusion protein, upon injection into the bloodstream, were also found to home in to, and propagate in, glioma tumors. These findings opened the way for designing multifunctional viral vectors useful for the detection of tumors based on signals such  
25 as light emission, for suppression of tumor development and angiogenesis signaled by, for example, light extinction and the development of bacterial and mammalian cell-based tumor targeting systems in combination with therapeutic gene constructs for the treatment of cancer. These systems have the following advantages: (a) They target the tumor specifically without affecting normal tissue; (b) the expression and  
30 secretion of the therapeutic gene constructs can be, optionally, under the control of

-140-

an inducible promoter enabling secretion to be switched on or off; and (c) the location of the delivery system inside the tumor can be verified by direct visualization before activating gene expression and protein delivery.

As provided herein, the system described above based on the accumulation of bacteria, viruses and eukaryotic cells in tumors can be used for simple, quick, and inexpensive production of proteins and other biological compounds originating from cloned nucleotide sequences. This system also is useful for the concomitant overproduction of polypeptides, RNA or other biological compounds (in tumor tissue) and antibodies against those compounds (in the serum) in the same animal.

As provided herein, after intravenous injection, a microorganism such as vaccinia virus can enter the tumor of an animal and, due to the immunoprivileged state of the tumor, can replicate preferentially in the tumor tissues and thereby can overproduce the inserted gene encoded protein in the tumors. After harvesting the tumor tissues, the localized and overexpressed protein can be isolated by a simple procedure from tumor homogenates. In addition, based on the findings that only 0.2 to 0.3% of the desired proteins produced in the tumor were found in the blood stream of the same animal, a simultaneous vaccination of the mouse and efficient antibody production against the overproduced protein was achieved. Thus, serum from the same mouse (or any other animal) can be harvested and used as mouse-derived antibodies against the proteins or other products overproduced in the tumor.

Thus, provided herein are methods of producing gene products and or antibodies in a non-human subject, by administering to a subject containing a tumor, a microorganism, where the microorganism expresses a selected protein or RNA to be produced, a protein or RNA whose expression can result in the formation of a compound to be produced, or a selected protein or RNA against which an antibody is to be produced. The methods provided herein can further include administering to a subject containing a tumor, a microorganism expressing an exogenous gene encoding a selected protein or RNA to be produced, a protein or RNA whose expression can result in the formation of a compound to be produced, or a selected protein or RNA against which an antibody is to be produced. The methods provided

-141-

herein can further include administering to a subject containing a tumor, a microorganism expressing an gene encoding a selected protein or RNA to be produced, a protein or RNA whose expression can result in the formation of a compound to be produced, or a selected protein or RNA against which an antibody is to be produced, where such gene expression can be regulated, for example, by a transcriptional activator or inducer, or a transcriptional suppressor. The methods provided herein for producing a protein, RNA, compound or antibody can further include monitoring the localization and/or level of the microorganism in the subject by detecting a detectable protein, where the detectable protein can indicate the expression of the selected gene, or can indicate the readiness of the microorganism to be induced to express the selected gene or for suppression of expression to be terminated or suspended. Also provided herein are methods of producing gene products and or antibodies in a non-human subject, by administering to a subject containing a tumor, a microorganism, where the microorganism expresses a selected protein or RNA to be produced, a protein or RNA whose expression can result in the formation of a compound to be produced, or a selected protein or RNA against which an antibody is to be produced, where the subject to which the microorganism is administered is not a transgenic animal. Also provided herein are methods of producing gene products and or antibodies in a non-human subject, by administering to a subject containing a tumor, a microorganism, where the microorganism expresses a selected protein to be produced, where the tumor within the subject is selected according to its ability to post-translationally process the selected protein.

The advantages of the system, include:

- (a) No production of a transgenic animal carrying the novel polypeptide-encoding cassette is required;
- (b) the tumor system is more efficient than tissue culture;
- (c) proteins interfering with animal development and other toxic proteins can be overproduced in tumors without negative effects to the host animal;
- (d) the system is fast: within 4 to 6 weeks from cDNA cloning to protein and antisera purification;

-142-

- (e) the system is relatively inexpensive and can be scaled up easily;
- (f) correct protein folding and modifications can be achieved;
- (g) high antigenicity can be achieved, which is beneficial for better antibody production; and
- 5 (h) species-specific-cell-based production of proteins in animals such as mice, with tumors as fermentors can be achieved.

Depiction of an exemplary method for production of gene products and/or antibodies against gene products is provided in Figure 2.

- 10 In one embodiment, methods are provided for producing a desired polypeptide, RNA or compound, the method including the following steps: (a) injecting a microorganism containing a nucleotide sequence encoding the desired polypeptide or RNA into an animal bearing a tumor; (b) harvesting the tumor tissue from the animal; and (c) isolating the desired polypeptide, RNA or compound from the tumor tissue.

- 15 Steps of an exemplary method can be summarized as follows (shown for a particular embodiment, *i.e.* vaccinia virus additionally containing a gene encoding a light-emitting protein):

- (1) Insertion of the desired DNA or cDNA into the vaccinia virus genome;
- (2) modification of the vaccinia virus genome with light-emitting protein
- 20 construct as expression marker;
- (3) recombination and virus assembly in cell culture;
- (4) screening of individual viral particles carrying inserts followed by large scale virus production and concentration;
- (5) administration of the viral particles into mice or other animals bearing
- 25 tumors of human, non-human primate or other mammalian origins;
- (6) verification of viral replication and protein overproduction in animals based on light emission;
- (7) harvest of tumor tissues and, optionally, the blood (separately); and
- (8) purification of overexpressed proteins from tumors and, optionally, antisera
- 30 from blood using conventional methods.

-143-

Any microorganism can be used in the methods provided herein, provided that they replicate in the animal, are not pathogenic for the animal, for example, are attenuated, and are recognized by the immune system of the animal. In some embodiments, such microorganisms also can express exogenous genes. Suitable  
5 microorganisms and cells are, for example, disclosed in EP A1 1 281 772 and EP A1 1 281 767. The person skilled in the art also knows how to generate animals carrying the desired tumor (see, *e.g.*, EP A1 1 281 767 or EP A1 1 281 777).

Also provide is a method for simultaneously producing a desired polypeptide, RNA or compound and an antibody directed to the polypeptide, RNA  
10 or compound, the method having the following steps: (a) administering a microorganism containing a nucleotide sequence encoding the desired polypeptide or RNA into an animal bearing a tumor; (b) harvesting the tumor tissue from the animal; (c) isolating the desired polypeptide, RNA or compound from the tumor tissue; and (d) isolating the antibody directed to the polypeptide, RNA or compound  
15 from the serum obtained from the animal. This approach can be used for generating polypeptides and/or antibodies against the polypeptides which are toxic or unstable, or which require species specific cellular environment for correct folding or modifications.

In another embodiment, the microorganism can further contain a nucleotide  
20 sequence encoding a detectable protein, such as a luminescent or fluorescent protein, or a protein capable of inducing a detectable signal.

Typically in methods for transfecting the microorganisms or cells with nucleotide sequences encoding the desired polypeptide or RNA and, optionally, a nucleotide sequence encoding a detectable protein such as a luminescent or  
25 fluorescent protein, or a protein capable of inducing a detectable signal, the nucleotide sequences are present in a vector or an expression vector. A person skilled in the art is familiar with a variety of expression vectors, which can be selected according to the microorganism used to infect the tumor, the cell type of the tumor, the organism to be infected, and other factors known in the art. In some  
30 embodiments, the microorganism can be a virus, including the viruses disclosed

-144-

herein. Thus, the nucleotide sequences can be contained in a recombinant virus containing appropriate expression cassettes. Suitable viruses for use herein, include, but are not limited to, baculovirus, vaccinia, Sindbis virus, Sendai virus, adenovirus, an AAV virus or a parvovirus, such as MVM or H-1. The vector can also be a retrovirus, such as MoMULV, MoMuLV, HaMuSV, MuMTV, RSV or GaLV. For expression in mammalian cells, a suitable promoter is, for example, human cytomegalovirus immediate early promoter (pCMV). Furthermore, tissue and/or organ specific promoters can be used. For example, the nucleotide sequences can be operatively linked with a promoter allowing high expression. Such promoters can include, for example, inducible promoters; a variety of such <sup>promoters</sup> promoters are known to persons skilled in the art.

For generating protein or RNA-encoding nucleotide sequences and for constructing expression vectors or viruses that contain the nucleotide sequences, it is possible to use general methods known in the art. These methods include, for example, in vitro recombination techniques, synthetic methods and in vivo recombination methods as <sup>known</sup> known in the art, and exemplified in Sambrook *et al.*, Molecular Cloning, A Laboratory Manual, 2nd edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Methods of transfecting cells, of phenotypically selecting transfectants cells, of phenotypically selecting transfectants and of expressing the nucleotide sequences by using vectors containing protein or RNA-encoding DNA are known in the art.

In some embodiments, the protein or RNA to be produced in the tumor can be linked to an inducible <sup>promoter</sup> promoter, such as a <sup>promoter</sup> promoter that can be induced by a substance endogenous to the subject, or by a substance that can be administered to a subject. Accordingly, provided herein are methods of producing a protein or RNA in a tumor, where the production can be induced by administration of a substance to a subject, and, optionally, harvesting the tumor and isolating the protein or RNA from the tumor. Such induction methods can be coupled with methods of monitoring a microorganism in a subject. For example, a microorganism can be monitored by detecting a detectable protein. In methods that include monitoring,

-145-

detection of a desired localization and/or level of microorganism in the subject can be coordinated with induction of microorganismal gene expression. For example, when a microorganismally expressed detectable protein is detected in tumor, but not appreciably in normal organs or tissues, an inducer can be administered to the  
5 subject. In another example, when a microorganismally expressed detectable protein is detected in tumor, and also in normal organs or tissues, administration of an inducer can be suspended or postponed until the detectable protein is no longer detected in normal organs or tissues. In another example, when a microorganismally expressed detectable protein is detected at sufficient levels in tumor, an inducer can  
10 be administered to the subject. In another example, when a microorganismally expressed detectable protein is not detected at sufficient levels in tumor administration of an inducer can be suspended or postponed until the detectable protein is detected at sufficient levels in the tumor.

Also provided herein are methods of producing a protein or RNA in a tumor,  
15 by administering a microorganism encoding the protein or RNA, and a suppressor of gene expression. The suppressor of gene expression can be administered for a pre-defined period of time, or until the microorganism accumulated in tumor but not in normal organs or tissues, or until sufficient levels of the microorganism have accumulated in the tumor, at which point administration of the suppressor can be  
20 terminated or suspended, which can result in expression of the protein or RNA. As will be recognized by one skilled in the art, methods similar to those provided herein in regard to monitoring a detectable protein and administering an inducer, can also apply for terminating or suspending administration of a suppressor.

In one embodiment, the microorganism is a bacterium, for example, an  
25 attenuated bacterium, such as those provided herein. Exemplary bacteria include attenuated *Salmonella typhimurium*, attenuated *Vibrio cholerae*, attenuated *Listeria monocytogenes* or *E. coli*. Alternatively, viruses such as vaccinia virus, AAV, a retrovirus can be used in the methods provided herein. In exemplary methods, the virus is vaccinia virus. Other cells that can be used in the present methods include

-146-

mammalian cells, such as fibroma cells, including human cells such as human fibroma cells.

Any of a variety of animals, including laboratory or livestock animals can be used, including for example, mice, rats and other rodents, rabbits, guinea pigs, pigs, sheep, goats, cows and horses. Exemplary animals are mice. The tumor can be generated by implanting tumor cells into the animal. Generally, for the production of a desired polypeptide, RNA, or compound, any solid tumor type can be used, such as a fast growing tumor type. Exemplary fast growing tumor types include C6 rat glioma and HCT116 human colon carcinoma. Generally, for the production of a desired antibody, a relatively slow growing tumor type can be used. Exemplary slow growing tumor types include HT1080 human fibrosarcoma and GI-101A human breast carcinoma. For T-independent antibody production, nu-/nu- mice bearing allogenic tumor or xenografts can be used; while for T-dependent antibody production, immunocompetent mice with syngenic tumors can be used. In some embodiments, such as where the compound to be produced is a protein, the microorganism selected can be a microorganism that uses the translational components (*e.g.*, proteins, vesicles, substrates) of the tumor cells, such as, for example, a virus that uses the translational components of a tumor cell. In such instances, the tumor cell type can be selected according to the desired post-translational processing to be performed on the protein, including proteolysis, glycosylation, lipidylation, disulfide formation, and any refolding or multimer assembly that can require cellular components for completing. In some examples, the tumor cell type selected can be the same species as the protein to be expressed, thus resulting in species-specific post-translational processing of the protein; an exemplary tumor cell type-expressed protein species is human.

#### 1. Production of Recombinant Proteins and RNA molecules

The tumor tissue can be surgically removed from the animal. After homogenization of the tumor tissue, the desired polypeptide, RNA or other biological compound can be purified according to established methods. For example, in the case of a recombinant polypeptide, the polypeptide might contain a

-147-

bindable tag such as a his-tag, and can be purified, for example, via column chromatography. The time necessary for accumulation of sufficient amounts of the polypeptide or RNA in the tumor of the animal depends on many factors, for example, the kind of animal or the kind of tumor, and can be determined by the skilled person by routine experimentation. In general, expression of the desired polypeptide can be detected two days after virus injection. The expression peaks approximately two weeks after injection, and lasts up to two months. In some embodiments, the amount of desired polypeptide or RNA in the tumor can be determined by monitoring a microorganismally expressed detectable substance, where the concentration of the detectable substance can reflect the amount of desired polypeptide or RNA in the tumor.

In another embodiment, the desired polypeptide, RNA or other compound can be manufactured in the subject, and provide a beneficial effect to the subject. In one example, a microorganism can encode a protein or RNA, or a protein that manufactures a compound that is not manufactured by the subject. In one example, a microorganism can encode a peptide hormone or cytokine, such as insulin, which can be released into the vasculature of a subject lacking the ability to produce insulin or requiring increased insulin concentrations in the vasculature. In another example, blood clotting factors can be manufactured in a subject with blood clotting deficiency, such as a hemophiliac. In some embodiments, the protein or RNA to be produced in the tumor can be linked to an inducible promoter, such as a promoter that can be induced by increased glucose concentrations. In such instances, the manufacture of the protein or RNA can be controlled in response to one or more substances in the subject or by one or more substances that can be administered to a subject, such as a compound that can induce transcription, for example, RU486. Thus, in some embodiments, the methods provided herein can include administering to a subject having a tumor, a microorganism that can express one or more genes encoding a beneficial gene product or a gene product that can manufacture a beneficial compound.

-148-

## 2. Production of Antibodies

Also provided are methods for producing a desired antibody, the method comprising the following steps: (a) administering a microorganism containing a nucleotide sequence encoding an antigen into an animal bearing a tumor; and (b) isolating the antibody directed to the antigen from the serum obtained from the animal. The antibodies directed to the antigen can be isolated and purified according to well known methods. Antibodies that are directed against specific contaminating antigens (e.g., bacteria antigens) can be removed by adsorption, and the antibodies directed against the target antigen can be separated from contaminating antibodies by affinity purification, for example, by immuno affinity chromatography using the recombinant antigen as the ligand of the column, by methods known in the art. Antibodies can be collected from the animal in a single harvest, or can be collected over time by collection bleeds, as is known in the art.

## F. Pharmaceutical Compositions, combinations and kits

Provided herein are pharmaceutical compositions, combinations and kits containing a microorganism provided herein and one or more components. Pharmaceutical compositions can include a microorganism and a pharmaceutical carrier. Combinations can include two or more microorganisms, a microorganism and a detectable compound, a microorganism and a microorganism expression modulating compound, a microorganism and a therapeutic compound. Kits can include the pharmaceutical compositions and/or combinations provided herein, and one or more components such as instructions for use, a device for detecting a microorganism in a subject, a device for administering a compound to a subject, and a device for administering a compound to a subject.

### 1. Pharmaceutical Compositions

Also provided herein are pharmaceutical compositions containing a modified microorganism and a suitable pharmaceutical carrier. Examples of suitable pharmaceutical carriers are known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions, etc. Such carriers can be formulated by conventional

-149-

methods and can be administered to the subject at a suitable dose. Colloidal dispersion systems that can be used for delivery of microorganisms include macromolecule complexes, nanocapsules, microspheres, beads and lipid-based systems including oil-in-water emulsions (mixed), micelles, liposomes and lipoplexes. An exemplary colloidal system is a liposome. Organ-specific or cell-specific liposomes can be used in order to achieve delivery only to the desired tissue. The targeting of liposomes can be carried out by the person skilled in the art by applying commonly known methods. This targeting includes passive targeting (utilizing the natural tendency of the liposomes to distribute to cells of the RES in organs which contain sinusoidal capillaries) or active targeting (for example by coupling the liposome to a specific ligand, for example, an antibody, a receptor, sugar, glycolipid, protein etc., by well known methods). In the present methods, monoclonal antibodies can be used to target liposomes to specific tissues, for example, tumor tissue, via specific cell-surface ligands.

## 2. Host Cells

Also provided herein are host cells that contain a microorganism provided herein such as a modified vaccinia virus. These host cells can include any of a variety of mammalian, avian and insect cells and tissues that are susceptible to microorganism, such as vaccinia virus, infection, including chicken embryo, rabbit, hamster and monkey kidney cells, for example, CV-1, BSC40, Vero, BSC40 and BSC-1, and human HeLa cells. Methods of transforming these host cells, of phenotypically selecting transformants etc., are known in the art.

## 3. Combinations

Combinations can include a microorganism and one or more components. Any combination herein also can, in place of a microorganism, contain a pharmaceutical composition and/or a host cell containing a microorganism and one or more components.

Exemplary combinations can contain two or more microorganisms, a microorganism and a detectable compound, a microorganism and a microorganism expression modulating compound, or a microorganism and a therapeutic compound.

-150-

Combinations that contain two or more microorganisms can contain, for example, two or more microorganisms that can both be administered to a subject in performing the methods provided herein, including sequentially administering the two microorganisms. In one example, a combination can contain a virus and a  
5 bacterium, where, for example, the virus can first be administered to the subject, and the bacterium can be subsequently administered to the subject.

Combinations provided herein can contain a microorganism and a detectable compound. A detectable compound can include a ligand or substrate or other compound that can interact with and/or bind specifically to a microorganismally  
10 expressed protein or RNA molecule, and can provide a detectable signal, such as a signal detectable by tomographic, spectroscopic or magnetic resonance techniques. Exemplary detectable compounds can be, or can contain, an imaging agent such as a magnetic resonance, ultrasound or tomographic imaging agent, including a radionuclide. The detectable compound can include any of a variety of compounds  
15 as provided elsewhere herein or are otherwise known in the art. Typically, the detectable compound included with a microorganism in the combinations provided herein will be a compound that is a substrate, a ligand, or can otherwise specifically interact with, a protein or RNA encoded by the microorganism; in some examples, the protein or RNA is an exogenous protein or RNA. Exemplary  
20 microorganisms/detectable compounds include a microorganism encoding luciferase/luciferin,  $\beta$ -galactosidase/(4,7,10-tri(acetic acid)-1-(2- $\beta$ -galactopyranosylethoxy)-1,4,7,10-tetraazacyclododecane) gadolinium (Egad), and other combinations known in the art.

Combinations provided herein can contain a microorganism and a  
25 microorganism gene expression modulating compound. Compounds that modulate gene expression are known in the art, and include, but are not limited to, transcriptional activators, inducers, transcriptional suppressors, RNA polymerase inhibitors, and RNA binding compounds such as siRNA or ribozymes. Any of a variety of gene expression modulating compounds known in the art can be included  
30 in the combinations provided herein. Typically, the gene expression modulating

-151-

compound included with a microorganism in the combinations provided herein will be a compound that is a can bind, inhibit, or react with one or more compounds active in gene expression such as a transcription factor or RNA, of the microorganism of the combination. An exemplary microorganism/expression  
5 modulator can be a microorganism encoding a chimeric transcription factor complex having a mutant human progesterone receptor fused to a yeast GAL4 DNA-binding domain an activation domain of the herpes simplex virus protein VP16 and also containing a synthetic promoter containing a series of GAL4 recognition sequences upstream of the adenovirus major late E1B TATA box, where the compound can be  
10 RU486 (see, *e.g.*, Yu *et al.*, Mol Genet Genomics 2002 268:169-178). A variety of other microorganism/expression modulator combinations known in the art also can be included in the combinations provided herein.

Combinations provided herein can contain a microorganism and a therapeutic compound. Therapeutic compounds can include compounds that are  
15 substrates for microorganismally expressed enzymes, compound that can kill or inhibit microorganism growth or toxicity, or other therapeutic compounds provided herein or known in the art to act in concert with a microorganism. Typically, the therapeutic compound included with a microorganism in the combinations provided herein will be a compound that can act in concert with a microorganism, such as a  
20 substrate of an enzyme encoded by the microorganism, or an antimicroorganismal agent known to be effective against the microorganism of the combination. Exemplary microorganism/therapeutic compound combinations can include a microorganism encoding Herpes simplex virus thymidine kinase/gancyclovir, and streptococcus ~~pyogenes~~ <sup>pyogenes</sup>/penicillin. Any of a variety of known combinations  
25 provided herein or otherwise known in the art can be included in the combinations provided herein.

#### 4. Kits

Kits are packaged in combinations that optionally include other reagents or devices, or instructions for use. Any kit provided herein also can, in place of a

-152-

microorganism, contain a pharmaceutical composition, a host cell containing a microorganism, and/or a combination, and one or more components.

Exemplary  
Exemplary

Exemplary kits can include the microorganisms provided herein, and can optionally include one or more components such as instructions for use, a device for  
5 detecting a microorganism in a subject, a device for administering a compound to a subject, and a device for administering a compound to a subject.

In one example, a kit can contain instructions. Instructions typically include a tangible expression describing the microorganism and, optionally, other components included in the kit, and methods for administration, including methods  
10 for determining the proper state of the subject, the proper dosage amount, and the proper administration method, for administering the microorganism. Instructions can also include guidance for monitoring the subject over the duration of the treatment time.

In another example, a kit can contain a device for detecting a microorganism  
15 in a subject. Devices for detecting a microorganism in a subject can include a low light imaging device for detecting light, for example emitted from luciferase, or fluoresced from green fluorescence protein, a magnetic resonance measuring device such as an MRI or NMR device, a tomographic scanner, such as a PET, CT, CAT, SPECT or other related scanner, an ultrasound device, or other device that can be  
20 used to detect a protein expressed by the microorganism within the subject. Typically, the device of the kit will be able to detect one or more proteins expressed by the microorganism of the kit. Any of a variety of kits containing microorganisms and detection devices can be included in the kits provided herein, for example, a microorganism expressing luciferase and a low light imager, or a microorganism  
25 expressing green fluorescence protein and a low light imager.

Kits provided herein also can include a device for administering a microorganism to a subject. Any of a variety of devices known in the art for administering medications or vaccines can be included in the kits provided herein. Exemplary devices include a hypodermic needle, an intravenous needle, a catheter, a  
30 needle-less injection device, an inhaler, and a liquid dispenser such as an

-153-

eyedropper. Typically, the device for administering a microorganism of the kit will be compatible with the microorganism of the kit; for example, a needle-less injection device such as a high pressure injection device can be included in kits with microorganisms not damaged by high pressure injection, but is typically not included in kits with microorganisms damaged by high pressure injection.

Kits provided herein also can include a device for administering a compound to a subject. Any of a variety of devices known in the art for administering medications to a subject can be included in the kits provided herein. Exemplary devices include a hypodermic needle, an intravenous needle, a catheter, a needle-less injection an inhaler, and a liquid dispenser. Typically the device for administering the compound of the kit will be compatible with the desired method of administration of the compound. For example, a compound to be delivered subcutaneously can be included in a kit with a hypodermic needle and syringe.

#### F. Examples

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

##### Example 1

##### Generation of recombinant viruses

A Wild type vaccinia virus (VV) strain LVP (the well known viral strain, originally derived by <sup>attenuation</sup>attenuation of the strain Lister from the ATCC under Accession Number VR-1549, from the Lister Institute of Viral Preparations, Moscow, Russia; see, Al'tshtein *et al.*, (1983) Dokl. Akad. Nauk USSR 285:696-699) designed as VGL was used as a parental virus for the construction of recombinant viruses designated RVGLX herein. All vaccinia viruses were purified using sucrose gradient (Yoklik). VVs were propagated and titers were determined by plaque assays using CV-1 cells (ATCC No. CCL-70). Methods for constructing recombinant vaccinia viruses are known to those of skill in the art (see, *e.g.*, Chakrabarti *et al.*, (1985 Mol. Cell Biol. 5:3403 and U.S. Patent No.4,722,848. Table 1 summarizes the recombinant VV strains described in this Example.

##### 30 Inactivation of VV by PUV treatment

-154-

LIVP VV (3 x 10<sup>8</sup> pfu/ml) was incubated with 1 µg/ml psoralen (Calbiochem, La Jolla, CA), suspended in Hank's buffer at room temperature for 10 min, and then irradiated for 5 min in Stratalinker 1800 UV crosslinking unit (Stratagent, La Jolla CA) equipped with five 365 nm long wave UV bulb to produce PUV-VV.

#### RVGL8: LacZ insertion into F3 of LIVP

Construction of recombinant vaccinia virus RVGL8 containing a lacZ gene inserted the NotI site was prepared as described in Timiryasova *et al.* (2001), BioTechniques 31, 534-540. Briefly it was prepared as follows. The BamHI/SmaI fragment (3293 bp) of pSC65 (see, Chakrabarti *et al.* (1997), BioTechniques 23, 1094-1097; see, also Current Protocols in Molecular Biology, Green Publishing and Wiley-Interscience Supplement 15:16.17.2 (1992); see also SEQ ID No. 5 herein and SEQ ID No. 57 in PCT International application No. WO 99/32646) containing the lacZ gene under the control of the vaccinia p7.5 promoter and strong synthetic vaccinia pE/L promoter was isolated by digestion with restriction enzymes, blunted with Klenow enzyme, and cloned into SmaI site of pNT8 plasmid (Timiryasova *et al.* (2001), BioTechniques 31: 534-540) to produce pNZ2 a shuttle plasmid.

To construct pNT8, the NotI region of the wild type VV strain LIVP was amplified using the following primers:

Forward: 5'-GGGAATTCTTATACATCCTGTTCTATC - 3' (SEQ ID No. 3);  
Reverse: 5'-CCAAGCTTATGAGGAGTATTGCGGGGCTAC-3' (SEQ ID No. 4)  
with the VV as a template. The resulting 972 bp fragment contained flanking EcoRI and HindIII sites at the 5' and 3' ends, respectively. The PCR product was cleaved with EcoRI and HindIII and inserted in pUC28 (Benes *et al.*, (1993) Gene 130: 151. Plasmid pUC28 is prepared from pUC18 (available from the ATCC under Accession Number 37253 by introducing a synthetic oligo adaptor using primers: pUC28 I: 5'AATTCAGATCTCCATGGATCGATGAGCT 3' (SEQ ID NO.6); pUC28 II: 3'GTCTAGAGGTACCTAGCTAC 5' (SEQ ID No. 7) into the EcoRI and SstI sites of pUC18. This introduces BglII, ClaI, and NcoI sites into the polylinker of pUC18.

-155-

Plasmid pNZ2 contains cDNA encoding the *E. coli* lacZ gene under the control of the vaccinia virus early/late promoter p7.5 and a synthetic early/late vaccinia pE/L promoter derived from the plasmid pSC65 (see, Chakrabarti *et al.* (1997), *BioTechniques* 23, 1094-1097; see, also *Current Protocols in Molecular Biology*, Green Publishing and Wiley-Interscience Supplement 15:16.17.2 (1992); see also SEQ ID No. 5 herein and SEQ ID No. 57 in PCT International application No. WO 99/32646). Plasmid pNZ2 provides for homologous recombination of lacZ into the NotI site of the VGL virus (ATCC VR-1549), to produce in the recombinant vaccinia virus designated RVGL8. The complex of wild type vaccinia virus DNA digested with NotI and not digested plasmid DNA pNZ2 was transfected for in vivo recombination into PUV VV infected cells to produce RVGL8 (see Figure 1A and Figure 1B). RVGL8 and the other recombinant vaccinia viruses described herein are listed in Table 1, below.

#### Mutant Virus Formation/Transfection

CV-1 African green monkey kidney fibroblasts (ATCC No. CCL-70) grown on 60 mm dishes (Corning, Corning, NY, USA) were infected with PUV-VV (strain LIVP treated with psoralen and UV; see, *e.g.*, Tsung *et al.* (1996), *J. Virol.* 70, 165-171; Timiryasova *et al.* (2001), *BioTechniques* 31, 534-540; Timiryasova *et al.* (2001), *J. Gene 3 Med.* 3, 468-477) at multiplicity of infection (MOI) of 1.

Two hours post-infection, the cells were transfected with a mixture of NotI-digested viral DNA (4 µg) and intact plasmid DNA (4 µg). Lipid-mediated transfection of cells was carried out using 5 µl of GenePORTER reagent (Gene Therapy Systems, San Diego, CA, USA) per µg of the DNA according to manufacturers' instructions. Cells were incubated in transfection mixture for 4 h and then supplemented with a medium containing 20% of fetal bovine serum. Cytopathic effects were monitored daily by light microscopy. Cells were incubated for 5-7 days until formation of the virus plaques and complete cytopathic effect. Then, infected cells were harvested, resuspended in 0.5 ml of medium, and frozen and thawed three times to release the virus. Single virus plaques were selected for

-156-

the preparation of small and large recombinant virus stocks and analyzed for the insertion and expression of the genes.

#### Confirm Mutant

Viral DNA was analyzed by Southern blots. Briefly, to isolate viral DNA, 5  
confluent monolayers of CV-1 cells, grown on 10 cm plates, were infected with the  
wild type VV (strain LIVP) or VV of the virus stock obtained from a single  
recombinant plaque. When the cytopathic effect was complete, cells were harvested  
and the pellet was resuspended in 3 ml of 10 mM Tris-HCl, pH 9.0. Viral particles  
were lysed, treated with proteinase K, and the virus DNA was isolated by  
10 phenol/chloroform extraction, followed by ethanol precipitation. The DNA was  
resuspended in 100  $\mu$ l of sterile water. The viral DNA samples were digested by  
NotI overnight at 37°C, followed by phenol-chloroform treatment, precipitated and  
10  $\mu$ g of DNA samples were separated through a 0.8% agarose gel. The DNA was  
transferred to a positively charged nylon membrane (Roche Diagnostics  
15 Corporation, Indianapolis, IN, USA) and fixed to the membrane using a GS Gene  
Linker (Bio-Rad Laboratories, Hercules, CA, USA). The DIG-labeling of DNA was  
performed using a nonradioactive DNA labeling and detection kit (Roche  
Diagnostics Corporation) and incubating for 60 min at 37°C. The membrane was  
hybridized with a denatured DIG-labeled 3357 bp NotI-NotI DNA fragment of the  
20 plasmid pNZ2 encoding the lacZ gene. Hybridization conditions and blot  
development were performed as suggested by the manufacturer.

The predicted size of the band is 3357 bp. The hybridization of NotI  
digested viral DNAs with a 3357 bp DNA probe confirmed the integration of the  
lacZ gene into NotI site of virus genome.

#### 25 Construction of RVGL2 and RVGL23 viruses with a single *TK* gene mutation

Vaccinia virus LIVP was used for the construction of recombinant virus  
RVGL2. Vaccinia virus Western Reserve (WR) was used for the construction of  
recombinant virus RVGL23. The cDNA of *Renilla* luciferase and *Aequorea* GFP  
fusion (*ruc-gfp*; 1788 bp; see, Wang *et al.*, (1996) Bioluminescence  
30 Chemiluminescence 9:419-422; Wang *et al.*, (2002) Mol. Genet. Genomics 268:160-

-157-

168; Wang *et al.* (1997) pp 419-422 in Bioluminescence and Chemiluminescence: molecular reporting with photons, Hastings *et al.*, eds., Wiley, Chichester UK ; see, also U.S. Patent No. 5,976,796; see also SEQ ID No. 8 herein, which sets forth a sequence for a *ruc-gfp* construct) was excised from plasmid pcDNA-*ruc-gfp* (RG), which is described in Wang *et al.*, (1996) Bioluminescence Chemiluminescence 9:419-422 and Wang *et al.*, (2002) Mol. Genet. Genomics 268:160-168 and briefly below, by restriction endonuclease PmeI and inserted into the SmaI site of pSC65 plasmid (see SEQ ID No. 5; see, also herein and SEQ ID No. 57 in PCT International application No. WO 99/32646), resulting in pSC65-RG-1 plasmid DNA.

Briefly to prepare pcDNA-*ruc-gfp*, the *EcoRI-NotI* fragment encoding the modified *Renilla* luciferase-ending DNA (see, Wang *et al.* (1997) pp 419-422 in Bioluminescence and Chemiluminescence: molecular reporting with photons, Hastings *et al.*, eds., Wiley, Chichester UK) was cloned into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA), placing expression of the *Renilla* luciferase under control of the CMV promoter. The stop codon at the end of the *Renilla* luciferase ORF was removed, and the resulting plasmid digested with *NotI*. The *NotI* fragment containing the ORF <sup>encoding</sup> ~~encodes~~ humanized *Aequorea* GFP (Zolotukhin *et al.*, (1996) *J. Virol.* 70:4646-4654) was <sup>excised</sup> ~~excised~~ from the pTR- $\beta$ -actin plasmid and inserted into the *NotI* site of the plasmid encoding the *Renilla* luciferase. The resulting plasmid was designated pcDNA-*ruc-gfp*.

New plasmid pSC65-RG-1 containing *ruc-gfp* fusion under the control of the vaccinia PE/L promoter and *E. coli*  $\beta$ -galactosidase under control of p7.5 promoter of VV was used for the construction of a single *TK* gene interrupted virus RVGL2 of strain L1VP and RVGL23 of strain WR. CV-1 cells were infected with wt L1VP or wt WR virus at MOI of 0.1, and two hours later, pSC65-RG-1 plasmid DNA was transfected using FuGene6 transfection reagent (Roche). After 24 h of incubation, cells were three times frozen and thawed to release the virus. Recombinant viruses were screened on CV-cells in the presence of substrate 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside (X-gal, Stratagene, Cedar Creek, TX, USA). After four

-158-

cycles of virus purification, all virus plaques were positive for  $\beta$ -galactosidase expression. The expression of the *ruc-gfp* fusion protein was confirmed by luminescence assay and fluorescence microscopy, respectively. Schematic maps of the viruses are set forth in Figure 1B

5           **Construction of RVGL5 and RVGL9 viruses with single gene mutations**

Recombinant vaccinia virus RVGL5 contains the *lacZ* gene under the control of the vaccinia late p11 promoter inserted into the HA gene of vaccinia genome (Timiryasova *et al.* (1993) Mol Biol 27:392-402; see, also, Timiryasova *et al.*, (1992) Oncol. Res 11:133-144.). Recombinant vaccinia virus RVGL9 contains a fusion of the Renilla luciferase gene (*ruc*) and cDNA of green fluorescence protein (*gfp*) under the control of a synthetic early/late vaccinia promoter (PE/L) inserted into the F3 gene of the VV genome (Timiryasova *et al.*, (2000) pp. 457-459 in Proceedings of the 11th International Symposium on Bioluminescence and Chemiluminescence, Case *et al.*, eds). RVGLP5 and RVGLP9 were constructed as described for RVGLP2 and RVGLP23.

15           **Construction of RVGL20 virus with double TK and F3 gene mutations**

The cDNA of human <sup>transferrin</sup> ~~transferrin~~ receptor (*hTR*) (2800 bp) with polyA sequence was isolated from pCDTR1 plasmid (ATCC Accession No. 59324 and 59325) by *Bam*HI, treated with *Klenow* and inserted into *Sa*II site of pSC65 plasmid (SEQ ID No. 5 herein and SEQ ID No. 57 in PCT International application No. WO 99/32646), resulting in pSC-TfR and pSC-rTfR. Plasmid pSC-rTfR contains cDNA *hTR* in an orientation opposite to the vaccinia PE/L promoter and *E.coli*  $\beta$ -galactosidase under control of the early/late vaccinia p7.5 promoter flanked by vaccinia sequences for insertion into vaccinia *TK* gene. pSC-rTfR was used for the construction of RVGL20 virus. RVGL9, a recombinant virus with single deletion carrying *ruc-gfp* fusion in the F3 gene locus, which contains a unique *Not*I site in the LIVP strain (see above, see, also, Timiryasova *et al.*, (2000) pp. 457-459 in Proceedings of the 11<sup>th</sup> International Symposium on Bioluminescence and Chemiluminescence, Case *et al.*, eds), was used as a parental virus for the creation

-159-

of RVGL20 virus by homologous recombination as described above. A schematic of RVGL20 virus is set forth in Figure 1B

**Construction of RVGL21 virus with triple *TK*, *F3* and *HA* gene mutations**

5        The cDNA of the  $\beta$ -glucuronidase (*gus*) of *E. coli* (1879 bp) was released from pLacGus plasmid (Invitrogen; see SEQ ID No. 9 herein) with *Xba*I (blunt ended with Klenow fragment) and *Hind*III, and cloned into pSC11 plasmid pSC65 (Chakrabarti *et al.* (1985) Mol. Cell Biol. 5:3403-3409; SEQ ID 5 herein and SEQ ID No. 57 in PCT International application No. WO 99/32646) digested with *Xho*I  
10        (treated with Klenow) and *Hind*III under the control of a vaccinia p11 late promoter, resulting in a plasmid pSC-GUS. The *Sma*I-*Hind*III fragment from pSC-GUS plasmid was inserted into pVY6 plasmid, a vector for inserting antigen genes into the hemagglutinin gene of vaccinia (see, e.g., Flexner *et al.*, (1988) Nature 355:259-262; Flexner *et al.*, (1988) Virology 166: 339-349; see also U.S. Patent No.  
15        5,718,902) digested with *Sma*I and *Bam*HI, resulting in pVY-GUS plasmid. The resulting plasmid, designated pVY-GUS plasmid, contains the cDNA encoding *gus* under the control of the vaccinia late promoter p11 flanked by vaccinia sequences for insertion into the hemagglutinin (*HA*) gene. Recombinant virus RVGL20 with double deletions was used as the parental virus for the construction of RVGL21  
20        virus. CV-1 cells were infected with RVGL20 virus at MOI of 0.1. Two hours after infection, cells were transected with pVY-GUS plasmid DNA using FuGene6 transfection reagent (Roche). Recombinant virus plaques were selected in CV-1 cells by color screening upon addition of  $\beta$ -glucuronidase substrate 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronic acid (X-GlcA) (Research Products Int. Co., Mt.  
25        Prospect, IL, USA) into agar medium. After eight cycles of purification in agar medium in the presence of X-GlcA pure recombinant virus RVGL21 was selected. RVGL21 virus has interruptions of *TK*, *F3* and *HA* genes and is presented schematically in Figure 1B.

***In vitro* virus growth**

-160-

CV-1, C6 (ATCC No. CCL-107), B16-F10 (ATCC No. CRL-6475), and GI-101A (Rumbaugh-Goodwin Institute for Cancer Research Inc. Plantation, FL; U.S. Pat. No. 5,693,533) cells were seeded in 24-well plates at the density of  $1 \times 10^5$ ,  $2 \times 10^5$ ,  $4 \times 10^5$ , and  $2 \times 10^5$  cells/well, respectively. The next day, the cells were

5 simultaneously infected with 0.001 or 0.01 PFU/cell of a wild type LIVP and its mutants. The virus suspension was added to cell monolayer (0.15 ml/well) and incubated at 37°C for 1 h with brief agitation every 10 min. Then, the virus was removed, appropriate complete growth medium was added (1 ml/well), and the cells were then incubated at 37°C for 24, 48, 72 and 96 h after virus infection. To

10 establish resting cell culture, a confluent monolayer of CV-1 cells was incubated for 6 days in DMEM with 5 % FBS at 37°C. These resting cells were infected and harvested at the same time points after infection as described above. Virus from the infected cells was released by one cycle of freezing and thawing. Viral titers were determined in duplicates by plaque assay on CV-1 cells and expressed as PFU/ml.

15

-161-

Table1

## List of recombinant vaccinia viruses (VV)

| Designation | Prior Designation          | Description                                                   | Insertion Locus/loci          | Reference                                                                                                                                                                                           |
|-------------|----------------------------|---------------------------------------------------------------|-------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| VGL         | wt VV                      | strain LIPV VV                                                | No Insertions                 | Publicly available                                                                                                                                                                                  |
| RVGL1       | recVV2                     | (p7.5) Luc-(p11) LacZ of LIPV VV                              | HindIII-N-Interrupted         | Timiryasova TM, Kopylova-Sviridova TN, Fodor I. Mol. Biol. (Russian) 27:392-401 (1993);<br>Timiryasova TM, Li J, Chen B, Chong D, Langridge WHR, Gridley DS, Fodor I. Oncol. Res. 11:133-144 (1999) |
| RVGL5       | recVV8                     | (p11) LacZ of LIPV VV                                         | HA-Interrupted                | Timiryasova TM, Kopylova-Sviridova TN, Fodor I. Mol. Biol. (Russian) 27:392-401 (1993)                                                                                                              |
| RVGL7       | rVV-EGFP or rVV-GFP        | (PE/L) EGFP-(p7.5) LacZ of LIPV VV                            | TK-Interrupted                | Umphress S, Timiryasova T., Arakawa T, Hilliker S, Fodor I, Langridge W. Transgenics 4:19-33 (2003)                                                                                                 |
| RVGL8       | rVV-Not-LacZ or rVV-Not-LZ | (p7.5) LacZ of LIPV VV                                        | NotI (F3)-Interrupted         | Timiryasova TM, Chen B, Fodor N, Fodor I. BioTechniques 31:534-540 (2001)                                                                                                                           |
| RVGL9       | rVV-RG or rVV-ruc-gfp      | (PE/L) Ruc-GFP of LIPV VV                                     | NotI (F3)-Interrupted         | Timiryasova TM, Yu Ya, Shabahang S, Fodor I, Szalay AA. Proceedings of the 11 <sup>th</sup> International Symposium on Bioluminescence & Chemiluminescence pp.457-460 (2000)                        |
| RVGL12      |                            | Same as RVGL7, except that HSV TK is inserted in place of gfp |                               |                                                                                                                                                                                                     |
| RVGL19      |                            | (PE/L) Trf-(p7.5) LacZ in Tk locus (PE/L) Ruc-GFP in          | TK- and NotI (F3)-Interrupted | Herein                                                                                                                                                                                              |

-162-

|        |  |                                                                                                                            |                                                             |        |
|--------|--|----------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|--------|
|        |  |                                                                                                                            |                                                             |        |
|        |  | F3 locus of<br>LIVP VV                                                                                                     |                                                             |        |
| RVGL20 |  | (PE/L)<br>rTrf-(p7.5)<br>LacZ in TK<br>locus (PE/L)<br>Ruc-GFP in<br>F3 locus of<br>LIVP V                                 | Tk- and<br>NotI (F3)-<br>Interrupted                        | Herein |
| RVGL21 |  | (PE/L)<br>rTrf-(p7.5)<br>LacZ in TK<br>locus, (p11)<br>LacZ in HA<br>locus, (PE/L)<br>Ruc-GFP in<br>F3 locus of<br>LIVP VV | Tk-, HA-<br>interrupted<br>and NotI<br>(F3)-<br>Interrupted | Herein |
| RVGL23 |  | (PE/L)<br>rTrf-(p7.5)<br>LacZ in TK<br>locus of WR<br>VV                                                                   | Tk-<br>Interrupted                                          | Herein |

### Example 2

#### In vitro analysis of virus levels

##### LacZ

- 5 Analysis of *lacZ* expression induced by recombinant vaccinia virus was performed as described previously (Timiryasova *et al.* (2001), *BioTechniques* 31, 534-540). Briefly, CV-1 cells grown 6-well plates (Corning, Corning, NY, USA) were infected with ten-fold dilutions of the virus stock. The virus was allowed to absorb for 1 h at 37°C with occasional rocking. Then, the virus inoculum was
- 10 replaced with a complete medium containing 1% of agar, and the incubation was carried out for 48 h. To visualize the virus plaques, 300 µg of X-Gal (Molecular Probes, Eugene, Oregon, USA) per ml and 0.1% of neutral red (Sigma, St. Louis, MO, USA) were added to the second agar overlay, and plaques were counted and isolated after 12 h incubation at 37°C. Levels of vaccinia virus in cells in vitro could
- 15 also be determined by measuring the plaque forming units (PFU) in the cells.

#### In vitro infectivity of VV's measured by Plaque Forming Units

-163-

The ability of wt LIVP virus and its mutants to infect and replicate was analyzed in dividing and resting CV-1 cells as well as in three tumor cell lines (C6, GI-101A, B16-F10). The results demonstrate that vaccinia mutants can efficiently infect and replicate in dividing CV-1 cells at an MOI of 0.001. A significant yield of vaccinia virus was obtained from dividing CV-1 cells. The yield of wt VV and its mutants in dividing CV-1 cells was about 10 times higher than in resting CV-1 cells. There was no significant difference in viral recovery between vaccinia mutants and wt virus *in vitro* studies. The interruption of *TK*, *F3* and *HA* genes made no difference to VV mutants replication in the dividing CV-1 cells. Three tumor cells were tested. The relative sensitivities to cytopathic effects at MOI of 0.001 were follows: CV-1 (dividing, highest), CV-1 (resting), C6, GI-101A, B16-F10 (lowest). Mouse B16-F10 melanoma cells were not sensitive to virus infection at MOI of 0.001. Very low viral titer was recovered from melanoma cells infected at MOI of 0.01. Also observed was that wt WR strain was able to infect melanoma cells *in vitro* more efficiently compared to LIVP strain and virus recovery was higher compared to LIVP strain.

### Example 3

#### Animal models and assays

##### Animal Models

Athymic nude mice (*nu/nu*) and C57BL/6 mice (Harlan Animal Res., Inc., Wilmington, MA) at 6-8 weeks of age were used for animal studies. Mice in groups of five or four were infected i.v. with  $10^7$  PFU of VV in a volume of 0.1 ml i.v. Mice were imaged by low-light imager and fluorescence imager for *ruc* and for *gfp* expression, respectively. The study was approved prior to initiation by the Animal Research Committee of LAB Research International Inc. (San Diego, CA, USA). All animal care was performed under the direction of a licensed veterinarian of LAB Research International Inc. (San Diego, CA, USA).

##### Glioma Model

To establish subcutaneous glioma tumor, rat glioma C6 cells (ATCC No. CCL-107) were collected by trypsinization, and  $5 \times 10^5$  cells/0.1 ml/mouse were

-164-

injected subcutaneously (s.c.) into right hind leg of 6-8 week old male athymic mice. On day 7 after C6 cell implantation when median tumor size was about 150 mm<sup>3</sup>, viruses at the dose of 10<sup>7</sup> PFU/0.1ml/mouse were injected intravenously (i.v.). Mice were sacrificed 14 days after virus injection. In the kinetic studies using of RVGL9 virus, mice were sacrificed at 20 min, 1 h, 4 h, 18 h, 36 h, 3 d, 5 d, 7 d and 14 days after virus injection.

#### Breast Tumor Model

To develop sub cutaneous (s.c). breast tumor, human breast cancer GI-101 A cells (Rumbaugh-Goodwin Institute for Cancer Research Inc. Plantation, FL; U.S. Pat. No. 5,693,533) at the dose of 5 x 10<sup>6</sup> cells/0.1 ml/mouse were injected s.c. into the right hind leg of 6-8 week old female athymic mice. On day 30 after GI-101A cell implantation, when median tumor size was about 500 mm<sup>3</sup>, viruses at the dose of 10<sup>7</sup> PFU/mouse were injected i.v. Mice were sacrificed on day 14 after virus injection. Mice for survival experiments and breast tumor therapy studies were kept for long time periods (more than 100 days after virus injection). Mice that developed tumor with the size about 4000 mm<sup>3</sup>, and/or lost 50% of body weight were sacrificed.

#### Melanomal Model

For a melanoma model, mouse melanoma B16-F10 cells (ATCC No. CRL-6475) at the dose of 2 x 10<sup>5</sup> cells/0.04 ml/mouse were injected into the foot pad of 6-8 week old male C57BL/6 mice. When the tumor was established (median size of tumor about 100 mm<sup>3</sup>), on day 18 after cell implantation, viruses at the dose of 10<sup>7</sup>/mouse were injected i.v. Mice were sacrificed 10 days after virus injection.

#### Vaccinia Virus in Animal Models

25 Vaccinia virus recovery from tumor and organs of nude mice

From sacrificed animals blood was collected, and organs (lung, liver, spleen, kidneys, testes, ovaries, bladder, brain, heart) and tumors were harvested and homogenized in PBS containing a mixture of protease inhibitors. Scissors and forceps were changed after each organ dissection or incision to avoid cross-contamination of the tissues. Samples were frozen and thawed, centrifuged at 1,000

-165-

g for 5 min. Viral titer was determined in the supernatant diluted in serum-free medium on CV-1 cells by plaque assay and staining them with 1% (wt/vol) crystal violet solution after 48 h incubation. Each sample was assayed in duplicate and viral titer was expressed as mean PFU/g of tissue.

#### 5 Assay Measurements

Survival studies were performed on 6-week old nude mice bearing s.c. human breast tumor. Mice were injected i.v. with  $10^7$  of vaccinia viruses and followed for survival. Individual body weight was measured twice a week. Gain/loss of body weight after virus infection was calculated as the percentage: body weight (g) - tumor weight (g) on day of virus injection / body weight (g) - tumor weight (g) on day of monitoring x 100%. Spleens were excised from euthanized animals and weighed. The RSW was calculated as follows:  $RSW = \text{weight of spleen (g)} \times 10^4 / \text{animal body weight (g)} - \text{tumor weight (g)}$ . Mice were euthanized when the mean tumor volume reached  $3000 \text{ mm}^3$  or developed the signs of disease. Rapid  $\text{CO}_2$  euthanasia was humanely performed in compliance with the *NIH Guide for the*

15 *Care and Use of Laboratory Animals*.

#### Reporter genes assays

##### LacZ

*E. coli*  $\beta$ -galactosidase activity in tissue samples and in the serum of the mice was determined using chemiluminescent <sup>Galacto</sup>Galo-Light Plus™ Assay system (Applied Biosystems, Bedford, MA, USA) according to the instructions of the kit manufacturer. Briefly, 1-20  $\mu\text{l}$  of the sample was transferred into the tube with 200  $\mu\text{l}$  of 1:100 diluted Reaction Buffer Diluent and incubated at RT for 30 min. A 300 $\mu\text{l}$  aliquot of accelerator (-II) was added into the tube with the sample, mixed

25 quickly and the signal was read using luminometer.  $\beta$ -galactosidase activity was expressed as relative light units (RLU) per g of tissue. Purified *E. coli*  $\beta$ -galactosidase (Sigma) was used as a positive control and to generate a standard curve.

30

-166-

### Luciferase

*Renilla* luciferase activity was measured in the supernatant of the tissue samples after they had been homogenized using a Turner TD 20e luminometer (Turner Designs, Sunnyvale, CA, USA) as described previously (Yu and Szalay, 2002) with some modifications. In brief, 20  $\mu$ l of the samples was added into 500  $\mu$ l of luciferase assay buffer (0.5 M NaCl, 1 mM EDTA, 0.1 M potassium phosphate pH 7.4) containing a <sup>substrate</sup> ~~substrate~~ coelentraine. Luciferase activity was measured during 10-s interval and expressed as RLU per g of tissue.

### Assay Results

#### 10 Tumor-selective replication of vaccinia virus RVGL8.

Recovery of wt LVP (VGL) and RVGL8 from tumor samples and different organs from six mice and from normal organs. VGL was recovered from tumor, testes, bladder, and liver and as well as from brain. Recombinant virus RVGL8, however, was found mostly in tumor only (in mouse #24 virus was found in testes, 15 bladder and liver; in mouse #22 in testes) and no virus was recovered from brain tissue in six tested animals. This finding demonstrates the safety of RVGL8 with the interruption of the NotI site,

#### Presence of RVGL9 over time

A vaccinia virus RVGL9 with a single *F3* gene mutation and carrying *ruc-gfp* was used to assess the pattern of vector tissue distribution following i.v. administration into immunocompromised athymic mice bearing s.c. glioma tumors. The tissue distribution data using this recombinant virus ~~vxs~~ showed virus distribution and tumor targeting by this VV strain. Kinetics studies were performed by noninvasive imaging of virus replication in the mice based on *ruc* and *gfp* expression. Four to five animals per group bearing s.c. rat glioma C6 tumor were 25 injected with  $10^7$  of RVGL9 virus via the tail vein. The animals were sacrificed at 20 min, 1, 4, 18 and 36 hours, 3, 5, and 14 days after virus injection. No viable viral particles were recovered from brain, bladder or testes at any time point after i.v. injection of virus. Some viral particles were recovered from spleen, heart and lung 30 at early time points after virus injection. After 18 h post-infection, the titer of

-167-

RVGL9 virus in these organs decreased. No virus was recovered in the heart tissue after 18 h; around 156.5 and 44 PFU/g tissue was recovered from spleen and lung, respectively, on day 14 as compared to 3221.0 and 3521.9 PFU/g tissue at 20 min after virus injection, respectively. The pattern of virus recovery from liver and kidneys was different from the pattern in the spleen, heart, or lung. No virus in the kidneys and 174.9 PFU/g tissue of virus was recovered from liver at an early time after virus injection. On day 5 after virus injection, the titer of virus in these organs increased and went down on day 14 post virus injection. In tumor tissue virus was detected starting 18 h after virus administration ( $1.6 \times 10^3$  PFU/g tissue), and dramatically increased over the time of observation ( $1.8 \times 10^8$  PFU/g tissue on day 7). Virus in the tumor tissue was detectable for more than 60 days after a single i.v. virus injection. The results demonstrate tumor-specific replication of these vaccinia mutants. A correlation was observed between the virus recovery and the transgene expression in tumors and in organs. Based on the data of RVGL9 virus kinetics, day 10 or day 14 was used for tissue distribution studies of different vaccinia mutants in melanoma and glioma and breast tumor models, respectively.

#### Presence of various VV in mice bearing a glioma tumor

To examine tissue distribution of vaccinia virus in <sup>immunodeficient</sup> ~~immunodeficient~~ mice bearing an s.c. glioma tumor, viruses were injected i.v. at a dose of  $1 \times 10^7$  PFU/0.1 ml/mouse on day 7 after C6 rat glioma cell implantation. Fourteen days after virus injection, mice were sacrificed and virus titer was determined in different tissues. Mice injected with wt WR virus ~~were~~ were sick and dying due to viral <sup>pathogenicity</sup> ~~pathogenicity~~. Hence, WR-injected mice were sacrificed on day 7 after virus injection. Wild type LIVP virus was recovered from all analyzed tissues as well as from brain. The amount of recovered virus particles from the mice injected with wt LIVP was much lower than wt WR strain of VV. The results presented in Table 1A

**Table 1A. Viral recovery from nude mice tissues in glioma model.<sup>a</sup>**

|         | LIVP<br>Wt        | RVGL2<br>TK-      | RVGL5<br>HA-      | RVGL9<br>F3- | RVGL20<br>TK-, F3- | RVGL21<br>TK-, F3-,<br>HA- | WR <sup>b</sup><br>Wt | RVGL23<br>TK-, WR |
|---------|-------------------|-------------------|-------------------|--------------|--------------------|----------------------------|-----------------------|-------------------|
| Brain   | $1.2 \times 10^3$ | $1.4 \times 10^3$ | 0                 | 0            | 0                  | 0                          | $1.4 \times 10^7$     | $1.9 \times 10^8$ |
| Kidneys | $6.1 \times 10^2$ | $6.7 \times 10^2$ | $1.6 \times 10^2$ | 34.6         | 33.3               | 36.6                       | $5.4 \times 10^6$     | $7.9 \times 10^7$ |

-168-

|                    | LIVP<br>Wt        | RVGL2<br>TK-      | RVGL5<br>HA-      | RVGL9<br>F3-      | RVGL20<br>TK-, F3- | RVGL21<br>TK-, F3-,<br>HA- | WR <sup>b</sup><br>Wt | RVGL23<br>TK-, WR |
|--------------------|-------------------|-------------------|-------------------|-------------------|--------------------|----------------------------|-----------------------|-------------------|
| Lung               | $2.9 \times 10^2$ | 0                 | $1.6 \times 10^2$ | $1.4 \times 10^4$ | $6.7 \times 10^3$  | $2.4 \times 10^3$          | $1.9 \times 10^6$     | $2.1 \times 10^3$ |
| Spleen             | $1.9 \times 10^2$ | 0                 | $1.8 \times 10^2$ | $1.0 \times 10^3$ | $1.0 \times 10^2$  | $1.7 \times 10^2$          | $1.6 \times 10^6$     | $1.8 \times 10^3$ |
| Testes             | $5.8 \times 10^4$ | 64.3              | $6.4 \times 10^2$ | $7.5 \times 10^2$ | 0                  | 0                          | $9.8 \times 10^4$     | $1.7 \times 10^3$ |
| Bladder            | $6.4 \times 10^3$ | 0                 | 0                 | $2.9 \times 10^3$ | 0                  | 0                          | $2.8 \times 10^3$     | $1.2 \times 10^3$ |
| Liver              | $3.4 \times 10^4$ | 63.6              | $4.2 \times 10^2$ | 33.6              | 96.6               | 30.8                       | $7.1 \times 10^3$     | $5.6 \times 10^3$ |
| Heart              | $6.0 \times 10^2$ | 0                 | 0                 | 0                 | 0                  | 0                          | $1.4 \times 10^3$     | 0                 |
| Serum <sup>c</sup> | 0                 | 0                 | 0                 | 0                 | 0                  | 0                          | $6.0 \times 10^2$     | 0                 |
| Tumor              | $5.4 \times 10^7$ | $1.5 \times 10^7$ | $3.8 \times 10^7$ | $2.9 \times 10^7$ | $3.9 \times 10^7$  | $1.9 \times 10^7$          | $1.9 \times 10^8$     | $3.7 \times 10^7$ |

The results demonstrate that 10000-fold more virus was recovered in the brain of mice injected with WR strain versus wt LIVP strain. Wild type WR strain virus was recovered from the serum (600 PFU/20  $\mu$ l) of mice on day 7 after virus injection.

- 5 No virus was recovered in the serum of the mice injected with LIVP mutants on day 14. The level of wt LIVP in serum was not tested on day 7. About  $1.9 \times 10^6$  PFU/g tissue of TK-mutant of WR strain (RVGL23) was found in the brain tissue compared to  $1.4 \times 10^3$  PFU/g tissue for mice injected with the TK- mutant of LIVP strain (RVGL2).

- 10 All other mutants of VV strain LIVP were found mostly in tumor only and no virus was recovered from brain tissue of mice injected with a double or triple mutant (Table 1A). Three times as many virus particles were recovered from the tumors of mice injected with WR compared to wt LIVP. The mean of viral recovery in tumor tissue of the mutants of LIVP strain was similar to the wt LIVP and
- 15 equivalent to TK- mutant of WR strain.

#### Presence of various VV in mice bearing a breast tumor

Data for tissue distribution in immunocompromised mice bearing s.c. GI-101A human breast are presented in Table 1B:

- 20 Table 1B. Viral recovery from nude mice tissues in breast cancer model.

|         | LIVP<br>Wt        | RVGL2<br>TK-      | RVGL5<br>HA- | RVGL9<br>F3-      | RVGL20<br>TK-, F3- | RVGL21<br>TK-, F3-,<br>HA- | WR <sup>b</sup><br>Wt | RVGL23<br>TK-, WR |
|---------|-------------------|-------------------|--------------|-------------------|--------------------|----------------------------|-----------------------|-------------------|
| Brain   | 0                 | 0                 | 0            | 0                 | 0                  | 0                          | $7.2 \times 10^6$     | $1.6 \times 10^4$ |
| Kidneys | $3.6 \times 10^3$ | 38.3              | 27           | $3.3 \times 10^2$ | 25.8               | 0                          | $3.2 \times 10^7$     | $2.8 \times 10^3$ |
| Lung    | $8.6 \times 10^3$ | $5.5 \times 10^2$ | 29.1         | $1.6 \times 10^3$ | $1.6 \times 10^3$  | $1.0 \times 10^3$          | $2.1 \times 10^6$     | $3.7 \times 10^3$ |
| Spleen  | $5.5 \times 10^3$ | 99.5              | 0            | $1.8 \times 10^2$ | 0                  | 0                          | $1.6 \times 10^6$     | $1.8 \times 10^3$ |
| Ovaries | $1.6 \times 10^3$ | 0                 | 0            | 0                 | 0                  | 0                          | $8.0 \times 10^7$     | $2.7 \times 10^7$ |

-169-

|                    | LIVP<br>Wt        | RVGL2<br>TK-      | RVGL5<br>HA-      | RVGL9<br>F3-      | RVGL20<br>TK-, F3- | RVGL21<br>TK-, F3-,<br>HA- | WR <sup>b</sup><br>Wt | RVGL23<br>TK-, WR |
|--------------------|-------------------|-------------------|-------------------|-------------------|--------------------|----------------------------|-----------------------|-------------------|
| Bladder            | $3.9 \times 10^3$ | 0                 | 0                 | 0                 | 0                  | 0                          | $2.8 \times 10^4$     | $1.2 \times 10^3$ |
| Liver              | $1.2 \times 10^4$ | 0                 | $1.7 \times 10^2$ | $5.2 \times 10^2$ | $1.7 \times 10^2$  | $1.0 \times 10^2$          | $4.0 \times 10^3$     | $4.8 \times 10^3$ |
| Heart              | $1.4 \times 10^2$ | 0                 | 0                 | 58.2              | $4.6 \times 10^2$  | 0                          | $6.3 \times 10^4$     | $2.2 \times 10^3$ |
| Serum <sup>c</sup> | 0                 | 0                 | 0                 | 0                 | 0                  | 0                          | $2.4 \times 10^3$     | 0                 |
| Tumor              | $8.6 \times 10^8$ | $1.0 \times 10^9$ | $2.5 \times 10^8$ | $1.1 \times 10^9$ | $5.6 \times 10^8$  | $1.0 \times 10^9$          | $2.9 \times 10^9$     | $6.6 \times 10^8$ |

About 10-fold more viral particles were recovered from breast tumor tissue compared to glioma tumor tissue. No virus particles were recovered from the brain tissue of mice injected with either wt LIVP or its mutants.  $7.2 \times 10^6$  and  $1.6 \times 10^4$  PFU/g was recovered from brain tissue of mice injected with wt WR and TK-virus of WR strain VV, respectively (Table). During the dissection of organs from euthanized mice, it was found that the ovaries from the mice being injected with wt WR and TK- of WR virus were drastically enlarged as compared to all other groups of mice. The analysis of viral recovery from ovaries demonstrated high titer of wt WR and TK- WR strain in ovaries, for example,  $8.0 \times 10^7$  and  $2.7 \times 10^7$  PFU/g, respectively. About  $1.6 \times 10^3$  PFU/g was recovered from the ovaries of the mice injected with wt LIVP virus, however no virus particles at all were recovered from either ovaries or from brain of mice injected with the mutants derived from LIVP strain (Table 1B).

#### 15 Presence of various VV in mice bearing a melanoma tumor

The tissue distribution of VV in the immunocompetent mice bearing melanoma tumors on foot pads also were studied. BL/6 mice on day 17 after B16F10 melanoma cell implantation were i.v. injected with the viruses at the dose of  $10^7$  PFU/mouse via the tail vein. All groups of mice were sacrificed on day 10 after virus injection due to huge tumor size in the PBS-injected control group. The results are set forth in Table 1C:

Table 1C. Viral recovery from C57BL/6 mice tissues in melanoma model.

|                      | LIVP<br>Wt        | RVGL2<br>TK-      | RVGL5<br>HA-      | RVGL9<br>F3-      | RVGL20<br>TK-, F3- | RVGL21<br>TK-, F3-,<br>HA- | WR <sup>b</sup><br>Wt | RVGL23<br>TK-, WR |
|----------------------|-------------------|-------------------|-------------------|-------------------|--------------------|----------------------------|-----------------------|-------------------|
| Tumor                | $5.4 \times 10^6$ | $3.9 \times 10^6$ | $3.7 \times 10^3$ | $9.5 \times 10^3$ | $2.5 \times 10^3$  | $2.4 \times 10^3$          | $9.9 \times 10^6$     | $2.2 \times 10^6$ |
| Tissues <sup>c</sup> | 0                 | 0                 | 0                 | 0                 | 0                  | 0                          | 0                     | 0                 |

-170-

<sup>a</sup> Mean of viral recovery PFU/g of tissue for 3-5 mice/group.

<sup>b</sup> Mice were sacrificed on day 7 after virus injection.

<sup>c</sup> PFU/20  $\mu$ l of serum

<sup>d</sup> Mice were sacrificed on day 9 after virus injection.

<sup>e</sup> No virus was recovered in all tested tissue.

No virus was recovered from kidneys, lung, spleen, brain, testes, bladder, liver, heart, and serum of the immunocompetent mice injected with the viruses. Virus was only recovered from the tumor tissue. About 10-fold virus particles were recovered from the tumors of mice injected with wt LIVP, TK- LIVP, wt WR, and TK- WR compared to other groups.

#### Example 5

**Reduction of human breast tumor implanted in nude mice by recombinant vaccinia viruses RVGL7, RVGL9 and RVGL21**

##### RVGL7 and RVGL9

Figure 1B shows a schematic representation of the recombinant vaccinia viruses used for these experiments. RVGL7 was prepared as described for the preparation of RVGL9. RVGL7 contains nucleic acid encoding EGFP and lacZ, and includes pE/L and p7.5 regulator regions inserted into the TK gene.

##### Luminescence and fluorescence images of tumors in a nude mouse

Human breast GI-101A cancer cells ( $5 \times 10^6$  cells/mouse) were subcutaneously implanted into the right thigh of the mice. Thirty days after cell implantation RVGL9, the *NotI* (F3)-interrupted virus expressing a fusion of *Renilla* luciferase and green fluorescence protein (RVGL9 = rVV-RG = rVVruc-gfp) was injected intravenously via tail vein at a dose of  $1 \times 10^7$  PFU/mouse. A fluorescence image of GFP and low-light image of luciferase expression were taken nine days after virus injection, i.e. 39 days post cell implantation showing dissemination of the virus.

##### Reduction of human breast tumor implanted into nude mice by vaccinia viruses RVGL7 or RVGL9

Human breast GI-101A cancer cells ( $5 \times 10^6$  cells/mouse) were subcutaneously implanted into the right thigh of the mice. Mice were injected i.v. with RVGL7=rVV-GFT=TK- or RVGL9-rVV-*ruc-gfp*=*NotI* (3) -interrupted viruses

-171-

( $1 \times 10^7$  PFU/mouse in 0.1 ml) and PBS control on day 30 after cell implantation. Images were taken on day 65 after GI-101A cell implantation and 35 days after virus or PBS injection. The results demonstrate drastic reduction of tumor volume in the mice injected with TK- or NotI (F3) -interrupted vaccinia viruses ~~6~~ compared with the tumor in the mice injected with PBS.

#### GFP in Human Breast Tumor after Viral Administration

Human breast GI-101A cancer cells ( $5 \times 10^6$  cells/mouse) were subcutaneously <sup>implanted the</sup> ~~implanted~~ into right thigh of the mice. Mice were injected i.v. with RVGL7=rVV-GFP=TK- or RVGL9=rVV-RG-rVV-ruc-gfp-NotI (F3) -interrupted viruses ( $1 \times 10^7$  PFU/mouse in 0.1 ml) on day 30 after cell implantation. The data demonstrate GFP expression in tumor area in the mice injected with TK- or NotI (F3) -interrupted vaccinia viruses. No GFP signals were observed in other parts of ~~the~~ <sup>the</sup> mice ~~body~~ <sup>body</sup>. The results also showed that expression of GFP can be visualized as early as 48 h after virus injection through <sup>the</sup> tail vein. On day 16 after virus injection very strong signals of GFP which correspond to a tumor volume of about 1300-1620  $\text{mm}^3$  for TK- or NotI (F3) -interrupted virus, respectively were observed. Reduced GFP signals were observed on day 25 (1218-1277  $\text{mm}^3$  for TK- or NotI (F3) -interrupted virus, respectively) and 32 (514-887  $\text{mm}^3$  for TK- or NotI (F3) -interrupted virus, respectively) due to reduction of tumor volume.

#### 20 Time course of Breast Tumor Volume over Time

GI-101A breast cancer cells were implanted subcutaneously into the right thigh of 4-5-week old female athymic (nu/nu) mice in the dose of  $5 \times 10^6$  cells/mouse. Thirty days after tumor implantation, when the tumor reached about 500  $\text{mm}^3$  in volume, a single dose ( $1 \times 10^7$  PFU/mouse in 0.1 ml) of RVGL7 = rVV-GFP = TK- or RVGL9 = rVV-RG = rVV-ruc-gfp = NotI (F3) -interrupted vaccinia viruses or PBS control was injected intravenously (via tail vein). Tumor dimensions were measured with vernier caliper twice a week and volumes were calculated as  $(L \times H \times W) / 2$ , where L, H and W represent the length, width, and height of the tumor, respectively and expressed in  $\text{mm}^3$ . The data demonstrate significant (60-80% on day 65) tumor reduction in the mice injected with TK-, NotI (F3) -interrupted

-172-

vaccinia viruses. In contrast, tumors grew very rapidly in the mice injected with PBS.

#### **Monitoring of tumor regression by light extinction.**

Subcutaneous GI-101A breast tumor reduction occurred in 100% of immunocompromised mice treated with a single i.v. injection of wt LIVP, single F3-, single TK-, and double F3-, TK-, mutants of LIVP strain. Some degree of toxicity was seen in the mice treated with above viruses. RVGL21 virus with the triple deletions TK, F3 and HA genes which showed no toxicity in nude mice; hence this virus was used for long-term studies. The difference in antitumor activity and survival between high and low doses of treatment using the triple mutant RVGL21 virus was not significant. GFP expression in tumor area in the mice injected with RVGL21 was monitored. No GFP signals were observed in other parts of mice body. Expression of GFP can be visualized as early as 48 h after virus injection through tail vein. On day 16 after virus injection we observed very strong signals of GFP, which correspond to tumor volume about 1300-1620 mm<sup>3</sup> and reduced GFP signals on days 25 (1218-1277 mm<sup>3</sup>) and 32 (514-887 mm<sup>3</sup>) due to reduction of tumor volume. Tumor volume reduction also was apparent by visual inspection of the mice.

#### **Example 6**

##### **20 : Reduction of vaccinia virus toxicity and virulence**

##### **Reduction of vaccinia virus pathogenicity by monitoring mouse body weight and survival**

The percentage of body weight change in athymic and immunocompetent mice bearing different s.c. tumors after i.v. administration of the viruses was examined. Injection of wt LIVP and wt WR and some mutants at the dose of 10<sup>7</sup> pfu/mouse via the tail vein led to a progressive vaccinia virus infection within a two week observation period. At one week after challenge, the mice showed typical blister formation on the tail and footpad. Later, weight loss, sometimes accompanied by swelling of the mouth region, in several cases led to death of the mice. In the case of wt WR strain of VV, mice started to die on day 7 after i.v.

-173-

injection of virus. While mice receiving the recombinant LIVP viruses gained weight or remained the same weight over the same time period.

#### Body weight in glioma model nude mice

Rat glioma C6 cells at the dose of  $5 \times 10^5/0.1$  ml/mouse were implanted s.c. into the right thigh of nude mice (5-6 old male mice) on day 0. Vaccinia viruses were injected i.v. (via tail vein) at the dose of  $1 \times 10^7$  PFU/0.1 ml/mouse on day 7. Animals were weighed twice a week. Gain/loss of body weight on day 14 post infection was calculated as the percentage:  $\text{body weight - tumor weight on day of virus injection (g) / body weight-tumor weight on day 14 (g) } \times 100\%$ . Injection of VGL (wild type vaccinia virus, strain LIVP) and RVGL5 (HindIII-N-interrupted) causes toxicity in nude mice: mice continue to lose the weight. Recombinant vaccinia viruses RVGL5 (HA-interrupted), RVGL7 (TK-interrupted), RVGL8 (NotI(F3) -interrupted), RVGL19 (double, TK- and NotI (F3) -interrupted) were less toxic in nude mice: after losing some body weight, 10 days post-infection, mice started to gain the body weight.

Nude mice with glioma that were injected with wild type WR strain of VV lost 31.9% of body weight on day 7 after virus injection. Mice injected with TK-virus of WR strain lost 22.4% of body weight on day 14 after virus injection compared to 1.5% in the group of mice injected with TK- virus of LIVP strain of VV. All mice injected with wild type LIVP strain survived for at least 14 days (the duration of the experiment). Mice without tumor injected with VGL (wt VV, strain LIVP) lost 11.23 % of body weight. Mice bearing tumor injected with VGL (wt VV) or with RVGL1 (HindIII-N-interrupted) lost 15.79% and 10.18% of body weight, respectively. Mice in the wt LIVP group lost 15.8% of body weight versus 9.4% in the PBS injected group. Tumor-bearing mice injected with RVGL2 (TK-), RVGL5 (HA-), RVGL7 (TK-), RVGL8 (F3-), RVGL9 (F3-), RVGL20 (TK-, F3-), RVGL21 (TK-, F3-, HA-) on day 14 after virus injection lost only 1.5%, 0.4%, 2.1%, 5.0%, 7.3%, 2.4%, and 3.2% of body weight, respectively. Tumor-bearing mice injected with virus carrying double gene interruption, RVGL19 (TK- and F3-) demonstrated 0.73% gain of body weight compared to the body weight on day 0.

-174-

Based on the results of body weight, a single interruption of HA, TK, F3 (NotI site) and double interruption of TK, F3 (NotI site) genes in vaccinia virus genome reduces virulence and toxicity of the vaccinia virus strain LIVP.

Injection of wt VV strain WR, however, was extremely toxic to nude mice, which died on day 7 after virus injection. Wild type and mutant VVs of strain LIVP were less toxic in nude mice. Although nude mice injected with various LIVP strains lost some body weight, after day 10-post infection mice started to gain the body weight.

#### **Body weight in breast tumor model athymic mice**

The body weight change of athymic mice with s.c. GI-101A human breast tumor after i.v. injection of vaccinia viruses was monitored. Mice injected with wt WR strain lost 25.6% of body weight and died due to virus toxicity. Although mice injected with wt LIVP virus survived for longer time, mice lost 26.4% of body weight. Mice injected with TK-WR strain lost 17.8% of body weight, while mice injected with TK- LIVP virus gained 1.9% of body weight. All mice injected with other mutants of LIVP strain were stable; no virus related toxicity was observed in these mice.

#### **Body weight in melanoma model immunocompetent mice**

The toxicity of the vaccinia viruses in immunocompetent C57BL/6 mice bearing mouse B16-F10 melanoma on their foot pad was studied. Although mice in all groups survived during the experiment, wt WR strain was more toxic in immunocompetent mice compared to wt LIVP and recombinant strains. Mice injected with wt WR strain lost about 11.4% of body weight on day 10 after i.v. injection of virus, while mice injected with wt LIVP strain and its double (RVGL20) and triple (RVGL21) mutants lost only 2.2%, 1.3%, and 0.6% of body weight, respectively, versus to 7.1% of body weight lost in PBS injected mice. Mice administered i.v. with RVGL2 (TK-), RVGL5 (HA-), RVGL9 (F3-), and RVGL23 (TK-WR strain) continued to gain weight over this same period.

#### **Long-term survival after viral infection for breast tumor-bearing mice**

-175-

To examine the effect of different mutations on long-term survival, mice bearing s.c. GI-101A human breast tumor received doses of  $10^7$  virus i.v., and were observed for survival after viral infection. The results showed that there are differences in survival depending upon the virus injected. Injection of the nude mice bearing s.c. breast tumor with wt WR strain (i.v.,  $1 \times 10^7$ /mouse) resulted in 100% mortality: four mice of five died on day 9 and one mouse died on day 11 after virus injection. Mice injected with strain L1VP survived for 35 days. Mice injected with a single mutated virus RVGL9 (F3-) developed the toxicity and 25% of mice died on day 34 after virus injection, however the deletion of F3 gene in L1VP strain prolonged the survival of mice up to 57 days. Mice injected with double mutant virus RVGL20 (F3-, TK-) began to die on day 34 after virus injection, but survived longer than F3- injected mice. The RVGL20 virus injected mice reached 50% survival point on day 65 and showed significantly longer survival time up to 116 days. The single mutant TK-virus of L1VP virus was less pathogenic than the single mutant F3- or double mutant F3-, TK- viruses; all mice were alive on day 80 after injection with TK- virus and 14.3% of the mice survived 130 days. All mice injected with the triple mutant TK-, F3-, and HA-virus (RVGL21) survived 130 days (duration of the experiment) and continued to live without any signs of virus toxicity compared to other groups of mice.

#### 20 Splenomegaly in various mice

##### Immunocompetent C57BL/6 mice

Several groups of the animals demonstrated enlargement of the spleen; therefore the relative spleen weight (RSW) was calculated. The results are shown in Table 2 as follows:

25 Table 2. Relative spleen weight (RSW) in mice with or without tumors.

| Groups         | Glioma model<br>nu/nu mice   | Breast cancer model<br>nu/nu mice | Melanoma model<br>C57BL/6 mice |
|----------------|------------------------------|-----------------------------------|--------------------------------|
| No tumor, PBS  | 43.6 $\pm$ 4.1 <sup>a</sup>  | 50.5 $\pm$ 11.2 <sup>d</sup>      | 30.1 $\pm$ 2.8 <sup>a</sup>    |
| No tumor, L1VP | 67.2 $\pm$ 11.9              | 48.0 $\pm$ 13.1                   | 68.1 $\pm$ 9.4                 |
| Tumor, PBS     | 92.4 $\pm$ 7.4 <sup>b</sup>  | 84.1 $\pm$ 14.6 <sup>e</sup>      | 106.0 $\pm$ 46.1 <sup>b</sup>  |
| L1VP           | 98.2 $\pm$ 28.2 <sup>c</sup> | 108.4 $\pm$ 39.4 <sup>f</sup>     | 148.4 $\pm$ 44.8 <sup>i</sup>  |
| RVGL2          | 96.0 $\pm$ 34.9              | 112.7 $\pm$ 15.6                  | 51.9 $\pm$ 6.6                 |

-176-

| Groups | Glioma model<br>nu/nu mice | Breast cancer model<br>nu/nu mice | Melanoma model<br>C57BL/6 mice |
|--------|----------------------------|-----------------------------------|--------------------------------|
| RVGL5  | 143.8 ± 20.5               | 169.6 ± 31.7                      | 61.6 ± 2.9                     |
| RVGL9  | 73.9 ± 10.5                | 151.8 ± 27.9                      | 63.3 ± 34.9                    |
| RVGL20 | 84.9 ± 6.6                 | 159.9 ± 22.7                      | 106.7 ± 36.0                   |
| RVGL21 | 114.4 ± 12.5               | 117.7 ± 15.3                      | 63.0 ± 24.6                    |
| WR     | 37.3 ± 3.5                 | 57.9 ± 10.9                       | 70.5 ± 1.8                     |
| RVGL23 | 46.9 ± 15.7                | 73.1 ± 19.3                       | 97.0 ± 43.9                    |

Mean ± SD for n=4-8 mice/group.

RSW = weight of spleen (g) × 10<sup>4</sup> / (animal body weight (g) - tumor weight (g)).

<sup>a</sup> p ≤ 0.02 vs. all groups, except no tumor LIVP, WR, RVGL23

<sup>b</sup> p ≤ 0.039 vs. no tumor PBS, no tumor LIVP, RVGL5, WR, RVGL23

<sup>c</sup> p ≤ 0.046 vs. all groups, except PBS, RVGL2, RVGL20, RVGL21

<sup>d</sup> p ≤ 0.006 vs. all groups except no tumor LIVP, PBS, WR, RVGL23

<sup>e</sup> p ≤ 0.048 vs. all groups, except no tumor PBS, LIVP, RVGL2, WR, RVGL23.

<sup>f</sup> p ≤ 0.045 vs. all groups, except PBS, RVGL2, RVGL21

<sup>g</sup> p ≤ 0.035 vs. PBS, LIVP, RVGL20, WR, RVGL23

<sup>h</sup> p ≤ 0.049 vs. all other groups, except no tumor LIVP, RVGL20, WR, RVGL23

<sup>i</sup> p ≤ 0.049 vs. all other groups.

As shown in the Table 2 above, some degree of splenomegaly was observed in mice. For immunocompetent C57BL/6 mice, a statistically significant difference (p < 0.035) was found in tumorous mice injected with PBS, LIVP, RVGL20, WR and RVGL23 compared to non-tumorous mice. In mice injected with wt VV strain LIVP spleen was enlarged greatly (p < 0.049) versus all other groups. In contrast, the smallest spleens were found in the mice without tumor.

#### Nude mice with a glioma tumor

In nude mice with or without s.c. glioma tumor, mice injected with wt WR or TK- of WR virus had the lowest RSW 37.3 or 46.9, respectively, which was similar to the RSW from the mice without tumor and injected with PBS (43.6). The largest RSW 143.8 and 114.4 was observed in RVGL5 (HA-) and RVGL21 (TK-, F3-, HA-) groups, respectively. No statistically significant difference was found among the groups of mice injected with wt LIVP, RVGL2, RVGL9, RVGL20 versus the PBS injected group.

-177-

**Nude mice with breast tumor**

The results of RSW in the immunocompromised mice bearing s.c human breast tumor indicate that all mice injected with wt LVP and its mutants have an enlarged spleen compared to the mice injected with wt WR or TK- WR viruses (p<0.045). The largest spleen was found in the mice injected with single HA-, single F3-, double F3-, TK- mutants of LVP strain.

**Other results using RVGL21 for injection**

Two mice #437 and #458 survived more then 190 days after RVGL21 injection ( $10^7$  and  $4 \times 10^5$ , respectively, i.v.) without any signs of diseases or virus related toxicities.

On day 30 after GI-101A cell implantation (tumor volume= $594.9 \text{ mm}^3$ ),  $10^7$  of RVGL21 was injected i.v. into mouse #437. On day 101 after virus injection (s.c. tumor size= $220.4 \text{ mm}^3$ ), metastasis (hard tissue) in chest area under the skin was observed. The size of the tumor was  $1223.6 \text{ mm}^3$ , which disappeared by day 148. The s.c. tumor did not disappear, it started to grow back, but the mouse remained metastasis-free.

Mouse #458 had a first s.c. tumor (GI-101A) on the right hind quarter. When the first tumor started to shrink (day 29 after RVGL21 virus injection, tumor size= $1924.3 \text{ mm}^3$ ), a second syngeneic tumor was implanted s.c. on the left hind quarter. The second tumor grew slowly, reached the size of  $1205.7 \text{ mm}^3$  and started to shrink. The mouse was free of <sup>the</sup> first tumor on day 127 post virus injection; the size of the second tumor was  $439.6 \text{ mm}^3$ . The tumor continued to shrink and the cells died. The body gradually absorbed remaining tumor tissues that were contributed by the host (such as the tumor vascular skeleton that was coming from the host). Since these remains are not considered foreign, the immune system doesn't destroy them. The tumor cells, on the other hand, were long gone and cleared by the immune system and the virus. Reduction of <sup>the</sup> second syngeneic tumor demonstrates that this mouse developed antibodies against the tumor cells. The antibodies resulted in the reduction of the second syngeneic tumor.

-178-

**EXAMPLE 7****Use of a Microorganism or Cell to Induce Autoimmunization of an Organism Against a Tumor**

This example shows that the method provided herein and in priority application EP 03 018 478.2 relating to "The production of a polypeptide, RNA or other compound in a tumor tissue" also can be used for the production of antibodies against the tumor tissue. These antibodies provide for autoimmunization of the organism bearing the tumor. Furthermore, these antibodies can be isolated and used for the treatment of tumors in other organisms.

Methods and uses of microorganisms, including cells, which can contain DNA encoding a desired polypeptide or RNA, to induce autoimmunization of an organism against a tumor are provided. Also provided are methods for the production of antibodies against a tumor by: (a) injecting a microorganism, such as a virus or cell, optionally containing a DNA sequence encoding a desired polypeptide or RNA, into an organism bearing a tumor and (b) isolating antibodies against the tumor.

This Example further demonstrates that administration of microorganisms, such as the triple mutant vaccinia virus strain provided herein, which accumulate in tumors, causing them to release tumor antigens for a sufficient time to permit production of antibodies by the host. This is exemplified by showing a reduction and elimination of xenogeneic GI-101A solid breast carcinoma tumors and their metastases in nu-/nu- mice (T cell deficient mice).

**Step#1:** Female nu-/nu- mice of 5 weeks age were chosen, and the GI-101A cells grown in RPMI1640 medium, supplemented with estrogen and progesterone. The confluence was reached, cells were harvested, washed with phosphate buffered saline. Cells ( $5 \times 10^6$  cells per mouse) were then injected subcutaneously into mice. The tumor growth was carefully monitored every two days.

**Step#2:** At two stages of tumor growth (at tumor size of  $400-600\text{mm}^3$ , and at tumor size of  $\sim 1700\text{mm}^3$ ), purified vaccinia viral particles (RVGL12) were delivered to each tumorous mice by intravenous injection through tail vein. The colony purified virus was amplified in CV-1 cell line and the intracellular viral particles were

-179-

purified by centrifugation in sucrose gradient. Two concentrations of virus ( $10^6$  pfu/100  $\mu$ l and  $10^7$  pfu/100  $\mu$ l resuspended in PBS solution) were injected. The viral replication was monitored externally by visualization of virus-mediated green fluorescence protein expression. The tumor development was monitored by tumor volume determination with a digital caliper.

Vaccinia viruses RVGL12+GCV(gancyclovir), and RVGL12 (RVGL12 is the same as RVGL7, except that the nucleic acid encoding gfp is replaced by herpes simplex virus thymidine kinase (HSV TK; see, SEQ ID Nos. 35 and 36) were injected 67 days after GI-101A cellular implantation. A second administration referred to as RVGL12a, was injected 30 days after cellular implantation.

**Step#3:** After viral administration, it was determined that first the tumors continued to grow to a size of  $\sim 900 \text{ mm}^3$  (from  $400\text{--}600 \text{ mm}^3$  at the time of viral injection), and to a size of  $\sim 2400 \text{ mm}^3$  (from  $1700 \text{ mm}^3$ ). Then the growth rate leveled off for approximately 6-8 days.

**Step#4:** Approximately 14 days after viral injection, the tumor volume started to decline rapidly. Forty days after viral application, all the treated animals showed more than 60% tumor regression. Sixty-five days after viral treatment and many of the animals had complete regression of tumors.

**Step#5:** Some of the animals were completely tumor-free for several weeks and their body weight returned to normal. RVGL-12+GCV treatment resulted in 86.3% reduction of tumor size (Day 52 after viral injection) from their peak volumes on Day 13, RVGL-12 treatment resulted in 84.5% reduction of tumor size (Day 52) from their peak volumes (Day 13). RVGL-12a treatment resulted in 98.3% reduction of tumor size (Day 89) from their peak volumes (Day 12). After PBS+GCV control treatment, the average volume of tumors were increased by 91.8% in 38 days

**Step#6:** The level of immune activation was determined. Sera were obtained from the animals with regressing tumors and the immune titer determined against a foreign protein (e.g. green fluorescent protein), vaccinia viral proteins, and GI-101A

-180-

cancer cell proteins were determined. The following antisera obtained from the following sources were used to analyze the following listed samples.

Samples:

- 1). Mouse cell lysate (control);
- 5 2). Purified and denatured vaccinia viral particles;
- 3). GI-101A tumor cell lysate;
- 4). Purified green fluorescent protein;
- 5). Purified luciferase protein;
- 6). Purified beta-galactosidase protein.

10 Antisera:

- a). Antiserum from nontumorous mouse;
- b). Antiserum from GI-101A tumorous mouse;
- c). Antiserum from GI-101A tumorous mouse 14 days after vaccinia i.v. injection;
- d). Antiserum from GI-101A tumorous mouse 65 days after vaccinia i.v. injection;
- 15 e). Antiserum from tumor-free mouse (after elimination of GI-101A tumor) 80 days after vaccinia i.v. injection.

The results showed that there was enormous tumor-specific vaccinia virus replication in the tumors, which led to tumor protein antigen and viral protein production in the tumors. In addition, the vaccinia virus did lyse the infected tumor cells thereby releasing tumor-cell-specific antigens. The continuous leakage of these  
20 antigens into the body led to a very high level of antibody titer (in approximately 7-14 days) against foreign cell proteins (tumor proteins), viral proteins, and the virus encoded engineered proteins in the mouse body. The newly synthesized antitumor antibodies and the enhanced macrophages, neutrophils counts were continuously  
25 delivered via the vasculature into the tumor and thereby providing for the recruitment of an activated immune system in the inside of the tumor. The active immune system then eliminated the tumor including the viral particles. This interconnected release of foreign antigens boosted antibody production and continuous return of the antibodies against the tumor-contained proteins function as

-181-

an autoimmunization vaccination system, initiated by vaccinia viral replication, followed by cell lyses, protein leakage and enhanced antibody production .

### EXAMPLE 8

#### 5 Production of $\beta$ -Galactosidase and Anti $\beta$ -Galactosidase via Vaccinia Virus Delivered lacZ in Tumor Bearing Mice

Thirty five athymic nu/nu mice (5 weeks old, 25g, male) were used to demonstrate the biodistribution and tumor targeting of vaccinia virus (strain LIVP) with different deletions in the genome. Mice were divided into 7 groups with 5 in each group as presented in Table 1

| Group | No. mice | Tumor implanted                | Virus Injected | Insertion locus        |
|-------|----------|--------------------------------|----------------|------------------------|
| 1     | 5        | None                           | VGL            | wtLIVP                 |
| 2     | 5        | C6, s.c. $5 \times 10^5$ cells | VGL            | wtLIVP                 |
| 3     | 5        | C6, s.c. $5 \times 10^5$ cells | RVGL1          | N-luc, lacZ            |
| 4     | 5        | C6, s.c. $5 \times 10^5$ cells | RVGL5          | HA- lacZ               |
| 5     | 5        | C6, s.c. $5 \times 10^5$ cells | RVGL7          | TK-egfp, lacZ          |
| 6     | 5        | C6, s.c. $5 \times 10^5$ cells | RVGL8          | NotI-lacZ              |
| 7     | 5        | C6, s.c. $5 \times 10^5$ cells | RVGL19         | TK-rTrf, lacZ, NotI-RG |

10

C6 gliomas were subcutaneously developed in Groups 2 to 7. Five days after tumor cell implantation ( $5 \times 10^5$  cells/mouse), each animal was treated with 0.1 ml of virus at a multiplicity of infection (MOI) of  $1 \times 10^7$  via tail vein injection. Two weeks after virus injection, all mice were sacrificed and blood samples were collected. Various organs and tumors also were taken from animals for virus titer and  $\beta$ -galactosidase analysis.

15

The  $\beta$ -galactosidase analysis was performed using the Galacto-Light Plus system (Applied Biosystems), a chemiluminescent reporter gene assay system for the detection of  $\beta$ -galactosidase, according to the manufacturer's instructions.

#### 20 $\beta$ -galactosidase Expression Measurements

In non-tumorous mice as well as in tumorous mice injected with wild type vaccinia virus (without reporter genes and without  $\beta$ -galactosidase gene) no  $\beta$ -galactosidase expression was detected in organs, blood and tumor samples. By contrast, in the tumors of mice infected with  $\beta$ -galactosidase expressing virus, high

-182-

levels of  $\beta$ -galactosidase was expressed.  $\beta$ -galactosidase also was detected in blood samples as shown in Table 2, but no virus <sup>was</sup> recovered from blood samples.

**Table 2. Production of  $\beta$  galactosidase by vaccinia virus in tumor and blood from tumor bearing mice (day 14 after virus injection)**

| Group | Virus Injected | $\beta$ -gal in tumor $\mu$ g/mg of total protein | $\beta$ -gal in serum $\mu$ g/ml of total protein | Est. total $\beta$ -gal/tumor ( $\mu$ g) | Est. total $\beta$ -gal/5ml blood ( $\mu$ g) |
|-------|----------------|---------------------------------------------------|---------------------------------------------------|------------------------------------------|----------------------------------------------|
| 3     | RVGL1          | $1.59 \pm 0.41$                                   | $1.38 \times 10^{-2} \pm 1.09 \times 10^{-2}$     | 489.84                                   | 4.00                                         |
| 4     | RVGL5          | $1.51 \pm 0.37$                                   | $1.16 \times 10^{-2} \pm 1.08 \times 10^{-2}$     | 330.21                                   | 3.62                                         |
| 5     | RVGL7          | $1.35 \pm 0.59$                                   | $0.95 \times 10^{-2} \pm 1.47 \times 10^{-2}$     | 616.60                                   | 1.83                                         |
| 6     | RVGL8          | $1.81 \pm 0.42$                                   | $0.86 \times 10^{-2} \pm 0.33 \times 10^{-2}$     | 962.36                                   | 2.38                                         |
| 7     | RVGL19         | $1.30 \pm 0.44$                                   | $0.26 \times 10^{-2} \pm 0.16 \times 10^{-2}$     | 463.75                                   | 0.60                                         |

#### Anti- $\beta$ -galactosidase antibody production

To determine whether the amount of  $\beta$ -galactosidase presented in mouse blood was sufficient to elicit antibody production, sera taken from two mice (mouse #116 from Group 5, and #119 from Group 6) were collected and tested for primary antibodies against  $\beta$ -galactosidase in Western analysis.  $\beta$ -galactosidase from *E. coli* (Roche, 567 779) was used as the antigen standard, and the mouse monoclonal anti  $\beta$ -galactosidase from *E. coli* (Sigma, G6282) was used as the antibody positive control. As additional sources of  $\beta$ -galactosidase, total protein was obtained from CV-1 cells 24 hours after infection with RVGL7 at MOI of 1 pfu/cell, and the tumor protein sample from mouse designated #143 (treated with RVGL7) was obtained.

The protein samples were prepared in triplicate, each set including a  $\beta$ -galactosidase antigen control, a cell lysate from RVGL7 infected CV-1 cells, and tumor lysate from mouse #143. All protein samples were separated by electrophoresis using a 10% polyacrylamide gel, and transferred to NitroBind nitrocellulose membrane (MSI) using a BioRad semidry blotting system. Immunoblotting was performed with either 1:3000 mouse monoclonal anti  $\beta$ -galactosidase, or 1:3000 mouse serum taken from either mouse #116 or #119, and 1:3000 Goat AntiMouse IgG-HRP (BioRad). An Amplified Opti-4CN Detection Kit (BioRad) was used for detection.

The results showed that sera taken from mouse #116 and #119 exhibited <sup>Similar</sup> ~~similar~~ levels of antibody when compared to a commercial mouse anti- $\beta$ -

-183-

galactosidase standard, and demonstrated that the tumor bearing mice #116 and #119 produced antibodies against  $\beta$ -galactosidase.

#### EXAMPLE 9

*Mammalian*

Mammalian cells for tumor therapy

- 5 As shown herein, certain bacteria, viruses, and mammalian cells (BVMC), when administered systemically, again <sup>enter</sup> and selectively replicate in tumors. Hence, systemically injected mammalian cells and certain bacterial <sup>anaerobic</sup> (anaerobic) bacteria, such as Salmonella, Clostridium sp., Vibrio, *E. coli* ) cells gain entry into solid tumors and replicate in tumor-bearing organisms. Genetically-labeled cells
- 10 can be used for tumor detection and therapy. In addition to gene expression in tumors through BVMC targeting, tumor-specific gene expression can be achieved by linking transgenes to tissue/tumor-specific promoters. To obtain tumor specific gene expression, a variety of systemic targeting schemes can be employed. These strategies include the use of tissue/tumor-specific promoters that allow the activation
- 15 of gene expression only in specific organs, such as prostate-specific promoter-directed viral gene expression; the use of extracellular matrix (*i.e.* collagen)-targeted viral vectors; and the use of antibody-directed viral vectors. Conditionally-replicating viruses have also been explored as tumor-specific delivery vehicles for marker genes or therapeutic genes, such as oncolytic adenovirus vector particles,
- 20 replication-selective HSV, vaccinia viruses and other such viruses.

- When light-emitting protein encoded BVMC are injected systemically into rodents, tumor-specific marker gene expression is achieved and is detected in real time based on light emission. Consequently, the locations of primary tumors and previously unknown metastases in animals are revealed *in vivo*. Hence diagnosis can
- 25 be coupled to therapy and to monitoring of therapy. The impaired lymphatic system in tumors may be responsible for the lack of clearance of bacteria from tumors by the host immunosurveillance after escaping the vascular system.

#### EXAMPLE 10

Tumor Development is inhibited following *S. pyogenes* <sup>pyogenes</sup> administration

-184-

This Example and following examples demonstrate the use of bacterial cells to colonize tumors, use of reporter in the cells to quantitate colonization; use of the colonized attenuated bacterial cells for tumor inhibition. Co-administration or sequential administration of bacteria and viruses. Administration of virus before bacteria increase tumor colonization by the bacteria. Administer bacteria that expresses an enzyme that will activate a prodrug, thereby targeting colonized cells.

#### Bacterial Strains

*Streptococcus pyogenes* M-type 1 T-type 1 (ATCC catalog no. 700294) was transformed with pDC123-luxF plasmid ) that contains the bacterial luciferase expression cassette (Lamberton GR, Pereau MJ, Illes K, Kelly IL, Chrisler J, Childers BJ, Oberg KC, Szalay AA. 2002. *Construction and characterization of a bioluminescent Streptococcus pyogenes*. Proceedings of the 12th International Symposium on Bioluminescence and Chemiluminescence, Case JF, Herring PJ, Robison BH, Haddock SHD, Kricka LJ, Stanley PE (eds). Chichester: Wiley, pp 85-88. Luciferase can be detected in the presence of exogenous decanal.

Transformed *S. pyogenes* were grown overnight in BH1 media in the presence of ~~in the presence of~~ 20 µg/ml of chloramphenicol at 37°C. After overnight growth, the bacteria were counted at OD<sub>600</sub> and bacteria were resuspended in BH1 media at the indicated density for injection.

#### 20 Tumor Development and Bacterial Injection

Twenty 5-week old mice were injected subcutaneously in the right lateral thigh. Each mouse was injected with  $5 \times 10^5$  C6 glioma cells transformed with pLEIN-derived retrovirus (Clontech; see also WO 03/14380). The subcutaneous tumors were developed for 7 days after implantation before bacterial injection.

25 For bacterial injection, the tumor-bearing mice were anesthetized with ~~isofluorane~~ <sup>isofluorane</sup> ~~isofluorene~~. The suspensions were injected intravenously with a 1-cc insulin syringe equipped with a 29 ½ -gauge needle through a surgically exposed femoral vein. After the injections, the incisions were sutured.

30 Tumor growth was monitored ~~or~~ twice a week following bacterial injection using a digital caliper. In addition, fluorescence imaging and photographic images

-185-

of the animals were taken at the end time points. The presence of luminescent bacteria was analyzed by intravenously injecting the animals with 30  $\mu$ l of decanal. Analysis of whole animals for bacterial luciferase activity, followed methods similar to Yu *et al.* (2004) *Nature Biotechnology* 22(3): 313-20. Briefly, anesthetized

5 animals were placed inside the dark box for photon counting (ARGUS 100 low light Imager, Hamamatsu). Photon collection was for 1 minute from ventral and dorsal sides of the animal and the images were recorded with Image Pro Plus 3.1 software (Media Cybernetics) and/or Lighttools<sup>®</sup> macroimaging system. A light image also was recorded. The luminescent images were superimposed on the light image to

10 localize the luminescent activity on the animal. Total intensity of photon emission in localized regions, e.g. in the tumor region, also was recorded. *S. pyogenes* was isolated from removed tumors and ground tissue was plated on LB-chloramphenicol (20  $\mu$ g/ml) plates. Luminescent bacteria were counted in the presence of decanal vapor.

## 15 Results

Four groups of mice were tested. Each group contained five mice.

| Group | <i>S. Pyrogenes Pyogenes</i> |
|-------|------------------------------|
| 1     | None                         |
| 2     | $1 \times 10^6$              |
| 3     | $1 \times 10^7$              |
| 4     | $5 \times 10^7$              |

Tumor volume was measured after 7 days of tumor development and the injection of *S. pyogenes*, through 21 days post-tumor development.

The control group of mice with no *S. pyogenes* had continuous and

20 accelerating tumor growth over the 2-week period. The mice injected with *S. pyogenes* had slower tumor growth. Groups 3 and 4 had the slowest tumor growth rates. Both groups maintained a slower linear rate throughout the monitoring period, whereas the control group, not injected with bacteria, exhibited tumor growth that accelerated at later time periods.

25 At all time points following bacterial injection, tumor volumes were smaller in Groups 3 and 4 mice than in the control mice (Group 1). At day 21, the average

-186-

tumor volume of the control group was approximately 2.5-3 fold greater than the average tumor volumes in Groups 3 and 4. Group 2, injected with the lowest titer of bacteria, also had a reduced tumor volume from the control group at the later time points, although the tumor volume was larger than Groups 3 and 4.

- 5 Bacterial colonization and tumor inhibition also is assayed in a fibrosarcoma model. HT1080 fibrosarcoma cells transformed with the pLEIN retrovirus are injected subcutaneously into the right lateral thigh of five week old nude male mice ( $5 \times 10^5$  cells/mouse). *S. pyrogenes* transformed with pDC123-luxF is injected into the femoral vein of the animals after 8 or 14 days of tumor growth (5 animals on  
10 each day). A group of 5 animals are not injected as serve as a control group. Tumor growth and luciferase activity is monitored at subsequent time points. *S. pyrogenes* is isolated from tumors and cultured on BH1 + chloramphenicol (20  $\mu$ g/ml) plates. Luminescent bacterial colonies are counted in the presence of decanal vapor.

#### Example 11

##### 15 **Vibrio Cholera localization to tumors**

##### **Plasmids and Bacterial Strains**

- Attenuated *Vibrio Cholerae*, strain Bengal 2 serotype 0139, M010 DattRS1, was transformed with pLITE201 which contains the luxCDABE cassette (Voisey *et al.* (1998) *Biotechniques* 24:56-58). The transformed strain is a light emitting strain  
20 due to the expression of the luciferase genes.

##### **Tumor Development and Bacterial Injection**

- Groups of nude mice ( $n > 20$ ) were implanted with C6 glioma tumors ( $500\text{mm}^3$ ) as described in the Examples herein.  $1 \times 10^8$  transformed bacteria (*V. Cholerae*) were suspended in 100  $\mu$ l of phosphate buffered saline (PBS). The  
25 bacterial suspension was injected into the right hind leg of each mouse. The animals were then monitored after injection under a low light imager as described in Example A.

In a separate experiment, for comparison, groups of nude mice ( $n > 20$ ) were implanted with C6 glioma tumors ( $500\text{mm}^3$ ) as described in the Examples herein.

-187-

These mice were injected with  $1 \times 10^8$  pfu/mouse of rVV-RUC-GFP virus (see Examples 1 and 4).

## Results

### Titer and luciferase activity

- 5 Mice from each of the two injected groups were sacrificed at time points after injection. Tumors were excised and homogenized. Bacterial and viral titers and luciferase activities were measured as described in the Examples herein.

- Both bacterial and viral titer increased following injection. The increase in bacterial growth over time was proportional to luciferase levels in the tumors. A log-log plot of bacterial titer versus luciferase activity in tumors in the mice injected with *V. cholera* demonstrated a linear relationship between bacterial titer and luciferase activity. The groups of mice injected with rVV-RUC-GFP virus, also demonstrated a linear relationship between virus titer and luciferase activity.

|                             | Time after <i>V. Cholera</i> /pLITE injection |                             |                            |                             |
|-----------------------------|-----------------------------------------------|-----------------------------|----------------------------|-----------------------------|
|                             | 4 hrs                                         | 8 hrs                       | 16 hrs                     | 32 hrs                      |
| Bacterial Titer (cfu/tumor) | $3.79 \times 10^4 \pm 2.93$                   | $3.14 \times 10^6 \pm 2.45$ | $1.08 \times 10^8 \pm 1.3$ | $5.97 \times 10^8 \pm 4.26$ |

15

|                         | Time after rVV-ruc-gfp virus injection |                             |                             |                             |
|-------------------------|----------------------------------------|-----------------------------|-----------------------------|-----------------------------|
|                         | 36 hrs                                 | Day 3                       | Day 5                       | Day 7                       |
| Viral Titer (pfu/tumor) | $3.26 \times 10^6 \pm 3.86$            | $7.22 \times 10^7 \pm 3.67$ | $1.17 \times 10^8 \pm 0.76$ | $3.77 \times 10^8 \pm 1.95$ |

The experiments demonstrated a linear relationship between titer and luciferase activity. Thus, luciferase activity of the injected bacteria and/or virus can be used a correlative measurement of titer.

### 20 Localization

Localization of *V. cholera* was performed as detailed in the Examples herein for virus. Briefly, organs and blood samples were isolated from animals euthanized

-188-

with CO<sub>2</sub> gas. The organs were ground and plated on agar plates with chloramphenicol drug selection for analysis of bacterial titer.

Bacterial titer was assayed in tumor, liver, testes, spleen, kidney, lung, heart, bladder and brain of the injected mice. Samples were taken from mice sacrificed at zero, and subsequent times up to 150 hours following *V.cholera* injection.

At the time point immediately following injection ( $t=0$ ), *V. cholera* was present in all samples, with the highest levels in the liver and spleen. By 50 hours post-injection, titer of *V.cholera* in all tissues had reduced with the exception of tumor tissue. In contrast, *V.cholera* titer had increased about 4 orders of magnitude as compared to time zero. This level increased slightly and then stayed constant throughout the remainder of the experiment. By 150 hours post-infection, titer in all samples except tumor had decreased. For example, the titer in liver had decreased by approximately 5 orders of magnitude from the time zero point. At the 150 hour point, the *V.cholera* titer in the tumor tissue was about 6 orders of magnitude greater than any other tissue sample.

#### Example 12

##### Co-administration and sequential administration of bacteria and virus

*V.Cholera*/pLITE (see Example B) and vaccinia virus VV-TK<sup>-</sup>-gfp-lacZ (see Example 4) were administered together or sequentially. Groups of nude mice with C6 glioma tumors were injected with bacteria and/or virus as shown in the Table below. Three male mice were injected per group. Bacteria and/or virus were injected on day 11 and day 16 following tumor implantation. Tumor growth, luciferase and GFP activity were monitored as described in the Examples herein.

| Group | Day 11 injection                             | Day 16 injection                                                                         |
|-------|----------------------------------------------|------------------------------------------------------------------------------------------|
| 1     | $1 \times 10^7$ VV-TK <sup>-</sup> -gfp-lacZ | $1 \times 10^7$ <i>V.Cholera</i> /pLITE                                                  |
| 2     | None                                         | $1 \times 10^7$ <i>V.Cholera</i> /pLITE                                                  |
| 3     | $1 \times 10^7$ <i>V.Cholera</i> /pLITE      | $1 \times 10^7$ VV-TK <sup>-</sup> -gfp-lacZ                                             |
| 4     | None                                         | $1 \times 10^7$ VV-TK <sup>-</sup> -gfp-lacZ                                             |
| 5     | None                                         | $1 \times 10^7$ VV-TK <sup>-</sup> -gfp-lacZ and $1 \times 10^7$ <i>V.Cholera</i> /pLITE |

-189-

## Results

On day 21 (21 days post tumor implantation) animals were sacrificed. Tumors were excised from each animal and ground. Viral titer was assayed on Groups 3, 4 and 5. Bacterial titer was assayed on Groups 1, 2 and 5. Titers (colony forming units and plaque forming units) were performed as previously described in the Examples.

A comparison of the bacterial titer in tumors Groups 1, 2 and 5 demonstrated that bacterial titer was highest in Group 1 that had been injected first with vaccinia virus at day 11, and followed by *V.cholera* injection on day 16. Co-injection of bacteria and virus at day 16 (Group 5) gave an intermediate bacterial titer. Group 2, injected only with *V.cholera* at day 16, had a lower bacterial titer in the tumor tissue than either of groups 1 or 5. Thus, tumors were more susceptible to bacterial colonization when first colonized by VV-TK<sup>-</sup>-gfp-lacZ virus.

A comparison of the viral titer in Groups 3, 4 and 5 demonstrated that Group 4, with only virus injection at day 16, had the highest viral titer followed by Groups 5 and 3. The viral titer of Group 5 was slightly higher than Group 3, but not apparently significantly different. One mouse in Group 4 had a viral <sup>titer</sup> ~~titer~~ that was an extreme outlier in comparison to the viral titer of the other 2 mice in Group 4. When the numbers were reassessed without this mouse, the general trend remained the same. The average viral titer in Group 4 was much closer to the viral titers of Groups 3 and 5. The data from the three groups in this analysis was not significantly different. Thus, pre-administration of bacteria followed by administration of virus did not significantly change the viral colonization of the tumor as compared with viral administration alone.

### Example 13

**Tumor Inhibition by Administering PNP-expressing bacteria and prodrug Plasmids** pSOD-DeoD contains the bacterial purine nucleoside phosphorylase gene (PNP) (Sorcher *et al.* (1994) GeneTher. 1(4):223-238), under the control of the constitutive SOD (superoxide dismutase) promoter. Plasmid pSOD-DeoD-lux,

-190-

contains the luxCDABE expression cassette (Voisey *et al.* (1998) *Biotechniques* 24:56-58) inserted into pSOD-DeoD.

PNP converts the non-toxic prodrug 6-methylpurine deoxyribose (6-MPDR) to 6-methyl purine which inhibits DNA replication, transcription and translation (Sorcher *et al.* (1994) *GeneTher.* 1(4):223-238).

#### Tumor Growth inhibition

Nude mice were injected with pLEIN retrovirus transformed C6 glioma cells. The pLEIN retrovirus expresses EGFP under the control of the viral promoter LTR (Clontech; see also WO 03/14380). *E. coli* DH5 $\alpha$  expressing the bacterial purine nucleoside phosphorylase gene was injected at day 8 following tumor implantation with or without prodrug (6-methylpurine deoxyribose (6-MPDR)). Tumor volume was monitored at subsequent time points (as performed in previous examples).

| Group | Administered                    |
|-------|---------------------------------|
| 1     | <i>E.coli</i> /PNP + prodrug    |
| 2     | <i>E.coli</i> /PNP              |
| 3     | <i>E.coli</i> control + prodrug |

Groups 2 and 3 exhibited equal tumor growth over time points from 8 to 21 days post tumor implantation. Group 1, which received both the *E.coli* expressing PNP and the prodrug exhibited ~20% reduction in tumor size as compared to the control Groups 2 and 3 at the end time points.

To further test bacterial colonization and prodrug effects on tumor growth, a human breast cancer model, GI-101A <sup>adenocarcinoma</sup> ~~adenocarcinoma~~ in nude mice, was chosen. GI-101A was derived from GI-101. GI-101 originated from a local first recurrence of an infiltrating duct <sup>adenocarcinoma</sup> ~~adenocarcinoma~~ (stage IIIa, T3N2MX) in a 57 year old female patient by researchers at Rumbaugh-Goodwin Institute for Cancer Research. In the subcutaneous xenograft nude mice model, the tumor consistently metastasizes to the lungs. The GI-101A is a slower growing tumor model as compared to the C6 glioma tumor model.

-191-

Fifteen 4 week old female nude mice are each injected <sup>subcutaneously</sup> ~~subcutaneously~~ in the right lateral thigh with GI-101A cells. Thirty days after tumor development, bacteria are injected. *Escherichia coli* DH5 $\alpha$  is transformed with pSOD-DeoD or pSOD-DeoD-lux. The bacteria are grown overnight in LB media in the presence of  
5 20  $\mu$ g/ml of chloramphenicol at 37°C. After overnight growth, the bacteria are counted at OD<sub>600</sub> and bacteria resuspended in BH1 media at the indicated density. The suspensions are injected intravenously with a 1-cc insulin syringe equipped with a 29 ½ -gauge needle into the animal through a surgically exposed vein or as otherwise indicated. After the injections, the incisions are sutured.

10 Prodrug is administered to groups of mice every four days following injection of bacteria. Tumor growth is monitored twice per week using a digital caliper. Luciferase imaging is performed as described in the Examples herein. At the end point, the animal are sacrificed and organs are assayed as described in Example B. Histological analyses are performed to determine the degree of tumor  
15 necrosis due to bacterial colonization and/or drug treatment.

Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

## CLAIMS:

1. A recombinant vaccinia virus, comprising a modified thymidine kinase (TK) gene and a modified HA gene and a modified F3 gene or interrupted F3 locus, wherein a modified gene is deleted or contains one or more truncations, mutations, insertions or deletions accompanied by at least a partial loss of function.
2. The recombinant vaccinia virus of claim 1, wherein (a) the F3 gene is inactivated or the F3 locus is interrupted by an insertion; and (b) the TK gene and HA gene are inactivated.
3. The recombinant vaccinia virus of claim 1 or 2, wherein at least the F3 gene or locus contains inserted heterologous nucleic acid therein.
4. The recombinant vaccinia virus of claim 2, wherein the F3 gene or locus, and the TK gene are inactivated by insertion of heterologous nucleic acid.
5. The recombinant vaccinia virus of any of claims 1 to 4, wherein the vaccinia virus is a Lister strain.
6. The recombinant virus of claim 5, where the strain is the LIVP strain.
7. The recombinant vaccinia virus of any of claims 1 to 6, wherein the modification of the F3 gene or locus is at the NotI site within the F3 gene or locus or at a corresponding gene or locus.
8. The recombinant vaccinia virus of claim 7, wherein the modification is at position 35 of the F3 gene or locus or at position 1475 inside of the HindIII F fragment of vaccinia virus DNA strain LIVP.
9. The recombinant vaccinia virus of any of claims 1 to 8, wherein the TK, HA and/or F3 gene or locus comprises an insertion of heterologous nucleic acid that encodes a protein.
10. The recombinant vaccinia virus of claim 9, wherein the heterologous nucleic acid comprises a

regulatory sequence operatively linked to the nucleic acid encoding the protein.

11. The recombinant vaccinia virus of claim 10, wherein the regulatory sequence comprises the vaccinia virus early/late promoter p7.5.

12. The recombinant vaccinia virus of claim 10 or 11, wherein the regulatory sequence comprises an early/late vaccinia pE/L promoter.

13. The recombinant vaccinia virus of any of claims 9 or 10, wherein the heterologous nucleic acid encodes a detectable protein or a protein capable of inducing a detectable signal.

14. A recombinant pox virus, comprising a modified TK and HA gene and a modified F3 gene or locus that corresponds to the F3 gene in vaccinia, wherein a modified gene or locus is deleted or contains one or more truncations, mutations, insertions or deletions accompanied by at least a partial loss of function.

15. An isolated host cell, comprising a recombinant vaccinia virus of any of claims 1 to 13.

16. A non-human tumor cell, comprising a recombinant virus of any of claims 1 to 14.

17. A pharmaceutical composition containing a recombinant virus of any of claims 1 to 14 in a pharmaceutically acceptable vehicle.

18. The pharmaceutical composition of claim 17 that is formulated for systemic administration.

19. The pharmaceutical composition of claim 17 that is formulated for intravenous administration or is formulated in a delivery vehicle.

20. A kit, comprising:  
a composition containing a pharmaceutical composition of any of claims 17-19; and  
a composition comprising a chemotherapeutic compound, wherein:  
the compositions are packaged as separate compositions or are provided as a single composition.

21. A combination, comprising:  
a composition containing a pharmaceutical composition of any of claims 17-19; and  
a composition comprising a chemotherapeutic compound, wherein:  
a combination is an association of two products;  
the compositions are separate compositions or are provided as a single composition.
22. The combination of claim 21, wherein the chemotherapeutic compound is selected from among alkylating agents,

antimetabolites, platinum coordination complexes, anthracenediones, substituted ureas, methylhydrazine derivatives, adrenocortical suppressants and anti-cancer polysaccharides.

23. The combination of claim 21, wherein the chemotherapeutic compound is selected from among gancyclovir, 5-fluorouracil, 6-methylpurine deoxyriboside, cephalosporindoxorubicin, 4-[(2-chloroethyl)(2-mesuloxyethyl)amino]benzoyl-L-glutamic acid, acetaminophen, indole-3-acetic acid, CB1954, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, bis-(2-chloroethyl)amino-4-hydroxyphenylaminomethanone 28, 1-chloromethyl-5-hydroxy-1, 2-dihydro-3H-benz[e]indole, epirubicin-glucoronide, 5'-deoxy-5-fluorouridine, cytosine arabinoside, and linamarin.

24. The combination of claim 21, wherein the compound and virus are packaged separately in two compositions.

25. The combination of claim 21, wherein the compound and virus are packaged as a single composition.

26. Use of the pharmaceutical composition of any claims 17-19 for formulation of a medicament for eliminating immunoprivileged cells or tissues.

27. The use of claim 26, wherein the immunoprivileged cells comprise tumor cells.

28. Use of a recombinant virus of any of claims 1 to 14 for the preparation of a pharmaceutical composition for gene therapy or vaccine therapy.

29. The use of claim 28, wherein the gene therapy is cancer gene therapy.

30. Use of a recombinant virus of any of claims 1 to 14 for formulation of a medicament for eliminating immunoprivileged cells or tissues, in an animal, wherein:

the virus accumulates in the immunoprivileged cells;

the virus does not accumulate to toxic levels in organs and tissues that do not comprise immunoprivileged cells or tissues.

31. Use of the combination of claims 21-25 for the formulation of a medicament for the treatment of cancer.

32. A method for production of a polypeptide or RNA or a product compound, comprising:

isolating the polypeptide or RNA or compound from tumor tissue that has been harvested from a tumor-bearing animal, wherein:

the animal is a non-human animal that has been administered a microorganism containing nucleic acid encoding the polypeptide or RNA or a pathway for producing the product compound;

the microorganism had accumulated in immunoprivileged cells; and

the microorganism did not accumulate to toxic levels in organs and tissues that do not comprise immunoprivileged cells or tissues, wherein the microorganism is a recombinant virus of any of claims 1-14.

33. The method of claim 32, wherein the vaccinia virus is an LIVP strain.

34. A method for simultaneously producing a polypeptide, RNA molecule or cellular compound and an antibody that specifically reacts with the polypeptide, RNA molecule or compound, comprising:

isolating the antibody from serum that has been obtained from a tumor-bearing animal, wherein the animal is a non-human animal that has been administered a microorganism, wherein the microorganism expressed or produced the compound, polypeptide or RNA molecule, and wherein the microorganism is a recombinant virus of any of claims 1-14.

35. The method of claim 34, further comprising the step of  
isolating the polypeptide, RNA molecule or cellular compound from tumor tissue that has been harvested from the animal.

36. The method of claim 34 or 35, wherein:  
the microorganism accumulates in the immunoprivileged cells; and  
the microorganism does not accumulate to toxic levels in organs and tissues that do not comprise immunoprivileged cells or tissues.
37. The method of any of claims 32-36, wherein the microorganism comprises a DNA molecule that encodes a reporter gene construct.
38. The method of claim 37, wherein the reporter gene construct encodes a detectable protein or a protein that induces or produces a detectable signal.
39. The method of claim 38, wherein the protein is a luciferase or a fluorescent protein.
40. The method of claim 37, wherein the reporter gene construct encodes a bioluminescence generating system and optionally encodes a fluorescent protein.

41. The method of any of claims 32-40, wherein the virus is a LIVP strain.
42. The method of any of claims 32-41, wherein the tumor is a solid tumor.
43. Use of at least two microorganisms for formulation of a medicament for elimination of immunoprivileged cells or tissues in an animal, wherein:  
the microorganisms accumulate in the immunoprivileged cells, whereby the animal is autoimmunized against the immunoprivileged cells or tissues; and  
at least one of the microorganisms is a recombinant virus of any of claims 1-14.
44. The use of claim 43, wherein the immunoprivileged cells or tissues comprises tumor cells.
45. A combination, comprising at least two microorganisms formulated for administration to an animal for elimination of immunoprivileged cells or tissues,

wherein one of the microorganisms is a recombinant virus of any of claims 1-14.

46. The combination of claim 45, wherein the immunoprivileged cells or tissues comprises tumor cells.

47. The combination of claim 45, wherein the microorganisms are formulated in separate compositions.

48. A kit comprising the combination of any of claims 45-47, wherein each microorganism is contained in a composition formulated and packaged for single dosage administration.

49. A method for the production of antibodies against products encoded by heterologous nucleic acid in immunoprivileged tissues or cells, comprising:

isolating antibodies against a protein or RNA from the blood or serum that has been obtained from an animal containing immunoprivileged tissues or cells, wherein the animal is a non-human animal that has been administered a microorganism containing nucleic acid encoding a selected protein or RNA and wherein the microorganism is a recombinant virus of any of claims 1-14.

50. The method of claim 49, wherein the immunoprivileged tissues or cells comprise tumor cells.

51. The method of claim 49 or 50, wherein the products produced in the immunoprivileged cells comprise tumor antigens.

- 52. The method of any of claims 49-51, wherein the virus is a Lister strain.
- 53. The method of claim 52, wherein the virus is an LVP strain.
- 54. The method of claim 52 or 53, wherein the virus contains an insertion in the F3 gene or a locus corresponding to the F3 locus of LVP (SEQ ID No. 34).

55. A vaccine, comprising a recombinant virus of any of claims 1-14.
56. Use of the virus of any of claims 1-14 for formulation of a vaccine for vaccination against smallpox.

## SEQUENCE LISTING

<110> Genenlux  
Szalay, Aladar A.  
Timiryasova, Tatyana  
Yu, Yong A.  
Zhang, Qian

<120> Microorganisms for Therapy

<130> 17248-002wo1 (4802PC)

<140> Unassigned  
<141>

<150> EP03013826.7  
<151> 2003-06-18

<150> EP03018478.2  
<151> 2003-08-14

<150> EP03024283.8  
<151> 2003-10-22

<160> 36

<170> FastSEQ for Windows Version 4.0

<210> 1  
<211> 148  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> LIVP F3

<400> 1  
aatatagcaa cagtagttct tgctctctct tgattctagc atcctcttca ttattttctt 60  
ctacgtacat aaacatgtcc aatacgttag acaacacacc gacgatggcg gccgtacag 120  
acacgaatat gactaaaccg atgaccat 148

<210> 2  
<211> 49  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Translation LIVP F3

<400> 2  
Met Val Ile Gly Leu Val Ile Phe Val Ser Val Ala Ala Ala Ile Val  
1 5 10 15  
Gly Val Leu Ser Asn Val Leu Asp Met Phe Met Tyr Val Glu Glu Asn  
20 25 30  
Asn Glu Glu Asp Ala Arg Ile Lys Glu Glu Gln Glu Leu Leu Leu Leu

Tyr 35

40

45

<210> 3  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Forward primer

<400> 3  
gggaattcctt atacatcctg ttctatc

27

<210> 4  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Reverse primer

<400> 4  
ccaagcttat gaggagtatt gcggggctac

30

<210> 5  
<211> 7252  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> psc65

<300>  
<308> GenBank No. AX003206  
<309> 2000-08-24

<400> 5  
agcttttgcg atcaataaat ggatcacaaac cagtatctct taacgatggt ctctgcagat 60  
gatgattcat tttttaagta ttggctagt caagatgatg aaatcttcat tatctgatat 120  
attgcaaatac actcaatata tagactttct gttattatta ttgatccaat caaaaaataa 180  
attagaagcc gtgggtcatt gttatgaatc tctttcagag gaatacagac aattgacaaa 240  
attcacagac tttcaagatt ttaaaaaact gtttaacaag gtccctattg ttacagatgg 300  
aagggtcaaa ottaataaag gatatttggt cgactttgtg attagtttga tgcgattcaa 360  
aaaagaatcc tctctagcta ccaccgcaat agatcctgtt agatacatag atoctcgtcg 420  
caatatcgca ttttctaacg tgatggatat attaaagtcg aataaagtga acaataatta 480  
attcttttatt gtcacatga acggcggaca tattcagttg ataatcggcc ccattgtttc 540  
aggtaaaagt acagaattaa ttagacgagt tagacgttat caaatagctc aatataaatg 600  
cgtgactata aaatattcta acgataatag atacggaacg ggactatgga cgcattgataa 660  
gaataatttt gaagcattgg aagcaactaa actatgtgat ctcttggaat caattacaga 720  
tttctccgtg ataggtatcg atgaaggaca gttctttcca gacattgttg aattagatcg 780  
ataaaaaatta attaattacc cgggtaccag gcttagatct gtcgacttcg agcttattta 840  
tattccaaaa aaaaaaata aaatttcaat ttttaagctt tcactaatcc caaaccacc 900

cgcttttttat agtaagtttt tcaccataa ataataaata caataattaa tttctcgtaa 960  
aagtagaaaa tatattctaa tttattgcac ggtaagggaag tagatcataa ctcgagcatg 1020  
ggagatcccc tegttttaca acgtcgtgac tgggaaaacc ctggcggttac ccaacttaat 1080  
cgcccttgca caccatcccc tttcgccagc tggcgtaata gcgaagaggc ccgcacccgat 1140  
cgcccttccc aacagttgcg cagcctgaat ggcgaaatggc gctttgcctg gtttccggca 1200  
ccagaagcgg tggcggaag ctggcgtggag tgcgatcttc ctgaggccga tactgtcgtc 1260  
gtcccttcaa actggcagat gcacgggttac gatcgcccca tctacaccaa cgtaacctat 1320  
cccattacgg tcaatccgcc gtttgttccc acggagaatc cgacgggttg ttactcgtc 1380  
acatttaagt ttgatgaaag ctggctacag gaaggccaga cggaattat ttttgatggc 1440  
gttaactcgg cgtttcatct gtggtgcaac gggcgctggg tcggttacgg ccaggacagt 1500  
cgtttgccgt ctgaatttga cctgagcgca tttttacggc ccggagaaaa ccgcctcggc 1560  
gtgatgggtg tgcgttgagg tgacggcagt tatctggaag atcaggatat gtggcggtatg 1620  
agcggcattt tccgtgacgt ctggttgcgt cataaaccca ctacacaaat cagcgatttc 1680  
catggttgcca ctgcttttaa tgatgatttc agccgcgtg tactggaggc tgaagttcag 1740  
atgtgcggcg agttgcgtga ctacctacgg gtaacagttt ctttatggca ggggtgaaacg 1800  
caggtcgcca ggggcacccg gcctttcggc ggtgaaatta tcgatgagcg tgggtggtat 1860  
gocgatcgcg tcacactacg tctcaacgct gaaaacccga aactgtggag cgccgaaatc 1920  
ccgaatctct atcgtgcggg ggttgaactg cacaccgccc acggcacgct gattgaagca 1980  
gaagcctcgg atgtcgggtt ccgogagggt cggttgaaa atgggtctgt gctgctgaac 2040  
ggcaagccgt tgcgtattcg aggcgttaac cgtcacgagc atcatcctct gcatggtcag 2100  
gtcatggatg agcagacgat ggtgcaggat atcctgctga tgaagcagaa caactttaac 2160  
ggcgtgcgct gtttcgatta tccgaacct ccgctgtggt acacgctgtg cgaccgctac 2220  
ggcctgtatg tgggtgagga agccaatatt gaaacccacg gcatgggtgc aatgaatcgt 2280  
ctgaccgatg atccgcgtg gctaccggcg atgagcgaac gcgtaacgcg aatggtgcag 2340  
cgcgatcgta atcacccgag tgtgatcacc tggctcgtgg ggaatgaatc aggccacggc 2400  
gctaatacag acgcgctgta tcgctggatc aaatctgtcg atccttcccg ccgggtgcag 2460  
tatgaaggcg gcggagccga caccacggcc accgatatta tttgcccgat gtacgcgcgc 2520  
gtggatgaag accagccctt ccgggtgtg ccgaaatggt ccatcaaaaa atggctttcg 2580  
ctacctggag agacgcgccc gctgatcctt tgcgaatacg cccacgcgat gggtaacagt 2640  
cttgccgggt tgcgtaaata ctggcaggcg tttcgtcagt atccccgtt acaggcgggc 2700  
ttcgtctggg actggtgga tcagtcgctg attaaatatg atgaaaacgg caaccggtg 2760  
tcggcttacg gcgggtgatt tggcgatcag ccgaacgac gccagttctg tatgaacggt 2820  
ctggtctttg ccgaccgcac gccgcattca gcgtgacgg aagcaaaaca ccagcagcag 2880  
tttttccagt tccgtttatc cgggcaaac atcgaaatga ccagcgaata cctgttccgt 2940  
catagcgata acgagctcct gcaactggat gtggcgctgg atggttaagg gctggcaagc 3000  
ggtgaagtgc ctctggatgt cgtccacaa ggtaaacagt tgattgaact gcctgaacta 3060  
ccgcagccgg agagcgcgg gcaactctgg ctacacgtac gcgtagtga accgaacgcg 3120  
accgcatggt cagaagccgg gcacatcagc gcctggcagc agtggcgtct ggccgaaaac 3180  
ctcagtgtga cgtccccgc cgcgtcccac gccatccgc atctgaccac cagcgaatg 3240  
gatttttgca tcgagctggg taataagcgt tggcaattta accgccagtc aggttttctt 3300  
tcacagatgt ggattggcga taaaaaaca ctgctgacgc cgtgcgcga tcagttcacc 3360  
cgtgcaccgc tggataacga cattggcgta agtgaagcga ccgcattga ccctaaccgc 3420  
tgggtcgaac gctggaaggc ggcgggccc ataccaggccg aagcagcgtt gttgcagtgc 3480  
acggcagata cacttgctga tgcgggtgct attacgaacc ctacgcgtg gcagcatcag 3540  
gggaaaacct tatttatcag ccggaaaacc taccggattg atggtagtgg tcaaatggcg 3600  
attaccgttg atgttgaaat ggcgagcgat acaccgcac cggcgcggat tggcctgaac 3660  
tgccagctgg cgcaggtagc agagcgggta aactggctcg gattagggcc gcaagaaaac 3720  
tatcccgacc gecttactgc cgcctgtttt gaccgctggg atctgcatt gtcagacatg 3780  
tataccccgt acgtcttccc gagcgaaaac ggtctgcgt gcgggacgcg cgaattgaat 3840  
tatggcccac accagtggcg cggcgacttc cagttcaaca tcagccgcta cagtcaacag 3900  
caactgatgg aaaccagcca tcgccatctg ctgcacgccc aagaaggcac atggctgaat 3960  
atcgacgggt tccatatggg gattggtggc gacgactcct ggagcccgtc agtatcggcg 4020  
gaattcagct gagcgcgggt cgtaccatt accagttggg ctggtgtcaa aaataataat 4080  
aacggggcag gggggtacct tctgtgagc tatggcaaac gaaggaaaa tagttatagt 4140  
agccgcactc gatgggacat ttcaacgtaa accggttaat aatattttga atcttattcc 4200

attatctgaa atggtggtgaa aactaactgc tgtgtgtatg aaatgcttta aggaggcttc 4260  
cttttctaaa cgattgggtg aggaaaccga gatagaaata ataggaggta atgatatgta 4320  
tcaatcgggtg tgtagaaagt gttacatcga ctcataatat tatatttttt atctaaaaaa 4380  
ctaaaaataa acattgatta aattttaata taataactta aaatggatgt tgtgtcgtta 4440  
gataaacctg ttatgtattt tgaggaaatt gataatgagt tagattacga accagaaagt 4500  
gcaaagtagg tcgcaaaaaa actgccgtat caaggacagt taaaactatt actaggagaa 4560  
ttattttttc ttagtaagtt acagcgacac ggtatattag atggtgccac cgtagtgtat 4620  
ataggatctg ctcccggtag acatatacgt tatttgagag atcattttcta taatttagga 4680  
gtgatcatca aatggatgct aattgacggc cgccatcatg atcctatttt aaatggattg 4740  
cgtgatgtga ctctagtgc tcggttcgtt tctgatgtga atctacgatc catcaaaaaa 4800  
caactgcac cttctaagat tattttaatt tctgatgtga gatccaaacg aggaggaaat 4860  
gaacctagta cggcggattt actaagtaat tacgctctac aaaatgtcat gattagtatt 4920  
ttaaaccccg tggcgtctag tcttaaatgg agatgcccg ttccagatca atggatcaag 4980  
gacttttata tcccacacgg taataaaatg ttacaacctt ttgctccttc atattcagct 5040  
gaaatgagat tattaagtat ttataccggt gagaacatga gactgaactc ggccgcgttg 5100  
ctggcgtttt tccataggct ccgccccct gacgagcatc acaaaaaatc acgctcaagt 5160  
cagaggtggc gaaacccgac aggactataa agataccagg cgtttcccc tggaaagctcc 5220  
ctcgtgcgct ctctgttcc gaccctgcc cttaccggat acctgtccgc etttctccct 5280  
tcgggaagcg tggcgtttt tcaatgctca cgctgtagg atctcagttc ggtgtaggtc 5340  
gttcgctcca agctgggctg tgtgcacgaa ccccgcttc agcccgaccg ctgcgcctta 5400  
tccggttaact atcgtcttga gtcacacccg gtaagacacg acttatcgcc actggcagca 5460  
gccactggta acaggattag cagagcgagg tatgtaggcg gtgctacaga gttcttgaag 5520  
tggtggccta actacggcta cactagaagg acagtatttg gtatctgcgc tctgtgaag 5580  
ccagttacct tcggaaaaag agttggtagc tcttgatccg gcaaacaaac caccgctgg 5640  
agcggtggtt ttttgtttg caagcagcag attacgcgca gaaaaaaagg atctcaagaa 5700  
gatcctttga tctttctac ggggtctgac gctcagtgga acgaaaactc acgtaagg 5760  
attttggtca tgagattatc aaaaaggatc ttcacctaga tctttttaa ttaaaaaatga 5820  
agttttaaat caatctaaag tatatatgag taaacttgg ctgacagtta ccaatgctta 5880  
atcagtggag cacctatctc agcgatctgt ctatttctgt catccatagt tgcctgactc 5940  
cccgtcgtgt agataactac gatacgggag ggcttaccat ctggccccag tgctgcaatg 6000  
ataccgcgag acccacgctc accggctcca gatttatcag caataaacca gccagccgga 6060  
agggccgagc gcagaagtgg tccctgcaact ttatccgct ccattccagt tattaattgt 6120  
tgccgggaag ctagagtaag tagttcgcca gttaatagtt tgcgcaacgt tgttgccatt 6180  
gctgcaggca tcgtggtgct acgctcgctg tttggtatgg cttcattcag ctccggttcc 6240  
caacgatcaa ggcgagttac atgatcccc atgttggtgca aaaaagcgg tagctcttc 6300  
ggtcctccga tcgttgtagc aagtaagtgg gccgcagtgt tatcactcat ggttatggca 6360  
gcactgcata attctcttac tgtcatgcca tccgtaagat gcttttctgt gactggtgag 6420  
tactcaacca agtcattctg agaatagtgt atgcggcgac cgagttgctc ttgcccggcg 6480  
tcaacacggg ataataccgc gccacatagc agaactttaa aagtgtcat cattggaaaa 6540  
cgttcttcgg ggcgaaaact ctcaaggatc ttaccgctgt tgagatccag ttcgatgtaa 6600  
cccactcgtg cacccaactg atcttcagca tcttttactt tcaccagcgt ttctgggtga 6660  
gcaaaaacag gaaggcaaaa tgccgcaaaa aagggaataa gggcgacacg gaaatgttga 6720  
atactcatac tcttctctt tcaatattat tgaagcattt atcagggtta ttgtctcatg 6780  
agcgataca tatttgaatg tatttagaaa aataaacaat taggggttcc gcgcacattt 6840  
cccgaaaag tgccacctga cgtctaagaa accattatta tcatgacatt aacctataaa 6900  
aataggcgta tcacgaggcc ctttcgtctt cgaataaata cctgtgacgg aagatcactt 6960  
cgcagaataa ataaatcctg gtgtccctgt tgataccggg aagccctggg ccaacttttg 7020  
gcgaaaatga gacgttgatc ggcacgtaag aggttccaac ttccaccata atgaaataag 7080  
atcactaccg ggcgtatttt ttgagttatc gagattttca ggagctaagg aagctaaaat 7140  
ggagaaaaaa atcactggat ataccaccgt tgatatatcc caatggcatc gtaagaaca 7200  
ttttgaggca tttcagtcag ttgctcaatg tacctataac cagaccgttc ag 7252

&lt;210&gt; 6

&lt;211&gt; 28

&lt;212&gt; DNA

<213> Artificial Sequence

<220>

<223> Primer pUC28 I

<400> 6

aattcagatc tccatggatc gatgagct

28

<210> 7

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer pUC28 II

<400> 7

catcgatcca tggagatctg

20

<210> 8

<211> 1665

<212> DNA

<213> Artificial Sequence

<220>

<223> Renilla luciferase-Aequorea GFP fusion gene

<400> 8

atgacttoga aagtttatga tccagaacaa aggaaacgga tgataactgg tccgcagtgg 60  
tgggccagat gtaaacaaat gaatgttctt gattcattta ttaattatta tgattcagaa 120  
aaacatgcag aaaatgctgt tattttttta catggtaacg cggectcttc ttatttatgg 180  
cgacatggtg tgccacatat tgagccagta gcgcggtgta ttataccaga tcttattggg 240  
atgggcaaat caggcaaatc tggtaatggt tcttataggt tacttgatca ttacaaatat 300  
cttactgcat gggttgaact tcttaattta ccaaagaaga tcatttttgt cggecatgat 360  
tggggtgctt gtttggcatt tcattatagc tatgagcatc aagataagat caaagcaata 420  
gttcacgctg aaagtgtagt agatgtgatt gaatcatggg atgaatggcc tgatattgaa 480  
gaagatattg cgttgatcaa atctgaagaa ggagaaaaaa tggttttgga gaataacttc 540  
ttcgtggaaa coactgttgc atcaaaaatc atgagaaagt tagaaccaga agaatttgca 600  
gcatactctg aaccattcaa agacaaagggt gaagttcgtc gtccaacatt atcatggcct 660  
cgtgaaatcc cgttagtaaa aggtggtaaa cctgacgttg tacaaattgt taggaattat 720  
aatgcttatac tacgtgcaag tgatgattta ccaaaaatgt ttattgaatc ggatccagga 780  
ttcttttoca atgctattgt tgaaggcgcc aagaagtttc ctaatactga atttgtcaaa 840  
gtaaaagggtc ttcatttttc gcaagaagat gcacctgatg aaatgggaaa atatatcaaa 900  
tcgttcgttg agcaggttct caaaaatgaa caagcggccg caccgcatac gagtaaagga 960  
gaagaacttt tcaactggagt tgtcccaatt cttggtgaat tagatgggtga tgttaatggg 1020  
cacaaatttt ctgtcagtgg agagggtgaa ggtgatgcaa catacggaaa acttaccctt 1080  
aaattttatt gcactactgg aaaactacct gttccatggc caacacttgt cactactttc 1140  
tcttatgggtg ttcaatgctt ttcaagatac ccagatcata tgaaacagca tgactttttc 1200  
aagagtgcc a gcccgaagg ttatgtacag gaaagaacta tatttttcaa agatgacggg 1260  
aactacaaga cactgctga agtcaagttt gaagggtgata cccttggttaa tagaatcgag 1320  
ttaaaaaggta ttgattttta agaagatgga aacattcttg gacacaaatt ggaatacaac 1380  
tataactcac acaatgtata catcatggca gacaaacaaa agaattggaat caaagttaac 1440  
ttcaaaatta gacacaacat tgaagatgga agcgttcaac tagcagacca ttatcaacaa 1500  
aatactccaa ttggcgatgg ccctgtcctt ttaccagaca accattacct gtccacacaa 1560  
tctgcccctt cgaaagatcc caacgaaaag agagaccaca tggtccttct tgagtttcta 1620

acagctgctg ggattacaca tggcatggat gaactataca aataa

1665

&lt;210&gt; 9

&lt;211&gt; 11096

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; pLacGus Plasmid

&lt;400&gt; 9

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| aagcttgcat  | gcctgcagca  | attccccgagg | ctgtagccga  | cgatggtgcg  | ccaggagagt  | 60   |
| tggttgattca | ttgtttgcct  | cctgctgctg  | gtttttcacc  | gaagttcatg  | ccagtcacgc  | 120  |
| gtttttgcag  | cagaaaagcc  | gccgacttcg  | gtttgcggtc  | gcgagtgaa   | atccctttct  | 180  |
| tgttaccgcc  | aacgcgcaat  | atgccttgcg  | aggtcgcaaa  | atcggcgaaa  | ttccatacct  | 240  |
| gttcaccgac  | gacggcgctg  | acgcgatcaa  | agacgcgggtg | atacatatcc  | agccatgcac  | 300  |
| actgatactc  | ttcactccac  | atgtcgggtg  | acattgagtg  | cagcccggtc  | aacgtatcca  | 360  |
| cgccgtattc  | ggtgatgata  | atcggctgat  | gcagttttctc | ctgccaggcg  | agaagttctt  | 420  |
| tttccagtac  | cttctctgcc  | gtttccaaat  | cgccgctttg  | gacataccat  | ccgtaataac  | 480  |
| ggttcaggca  | cagcacatca  | aagagatcgc  | tgatgggtatc | ggtgtgagcg  | tcgcagaaca  | 540  |
| ttacattgac  | gcagggtgatc | ggacgcgctg  | ggtcgagttt  | acgcgttgct  | tccgccagtg  | 600  |
| gcgcgaaata  | ttcccgtgca  | ccttgccggac | gggtatccgg  | ttcggttgga  | atactccaca  | 660  |
| tcaccacgct  | tgggtggttt  | ttgtcacgcg  | ctatcagctc  | tttaatcgcc  | tgtaagtgcg  | 720  |
| cttgctgagt  | ttcccgttg   | actgcctctt  | cgctgtacag  | ttctttcggc  | ttgttgcccg  | 780  |
| cttcgaaacc  | aatgcctaaa  | gagaggttaa  | agccgacagc  | agcagtttca  | tcaatcacca  | 840  |
| cgatgccatg  | ttcatctgcc  | cagtcgagca  | tctcttcagc  | gtaagggtaa  | tgcgaggtag  | 900  |
| ggtaggagtt  | ggccccaatc  | cagtcacatta | atgcgtggtc  | gtgcaccatc  | agcacgttat  | 960  |
| cgaatccttt  | gccacgcaag  | tcgcgatctt  | catgacgacc  | aaagccagta  | aagtagaacg  | 1020 |
| gtttgtgggt  | aatcaggaac  | tgttcgccct  | tcactgccac  | tgaccggatg  | ccgacgcgaa  | 1080 |
| gcgggtagat  | atcacactct  | gtctggcttt  | tggctgtgac  | gcacagttca  | tagagataac  | 1140 |
| cttcaccogg  | ttgccagagg  | tgccgattca  | ccacttgcaa  | agtcocgcta  | gtgccttgct  | 1200 |
| cagttgcaac  | caectgttga  | tcgcgatcac  | caggttcaac  | gctgacatca  | ccattggcca  | 1260 |
| ccacctgcca  | gtcaacagac  | gcgtgggttac | agtccttgcc  | gacatgcgtc  | accacgggtga | 1320 |
| tatcgtccac  | ccagggtgtc  | ggcgtgggtg  | agagcattac  | gctgcgatgg  | attccggcat  | 1380 |
| agttaaagaa  | atcatggaag  | taagactgct  | ttttcttgcc  | gttttcgtcg  | gtaatcacca  | 1440 |
| ttcccgccgg  | gatagctctg  | cagttcagtt  | cgttgttcac  | acaaacgggtg | atacgtacac  | 1500 |
| ttttcccgcc  | aataacatac  | ggcgtgacat  | cggcttcaaa  | tggcgtatag  | ccgcctgat   | 1560 |
| gtcccatcac  | ttcctgatta  | ttgacccaca  | ctttgccgta  | atgagtgacc  | gcacgaaac   | 1620 |
| gcagcacgat  | acgctggcct  | gcccacacct  | tcggtataaa  | gacttcgcgc  | tgataccaga  | 1680 |
| cgttgcccg   | ataattacga  | atatctgcat  | cggcgaaactg | atcggttaaaa | ctgcctggca  | 1740 |
| cagcaattgc  | ccggctttct  | tgtaacgcgc  | tttcccacca  | acgtgatca   | attccacagt  | 1800 |
| tttcgcgatc  | cagactgaat  | gcccacaggc  | cgtcgagttt  | tttgatttca  | cgggttgggg  | 1860 |
| tttctacagg  | acgtaacatt  | ctagacatta  | tagttttttc  | tccttgacgt  | taaagtatag  | 1920 |
| aggtatatta  | acaatttttt  | gttgatactt  | ttattacatt  | tgaataagaa  | gtaatacaaa  | 1980 |
| ccgaaaatgt  | tgaaagtatt  | agttaaagtg  | gttatgcagt  | ttttgcattt  | atatatctgt  | 2040 |
| taatagatca  | aaaatcatcg  | gttcgctgat  | taattacccc  | agaaataagg  | ctaaaaaact  | 2100 |
| aatcgcatca  | tcacccctcg  | agctatcacc  | gcaagggtata | aatatctaac  | accgtgcgtg  | 2160 |
| ttgactattt  | tacctctggc  | ggtgataatg  | ctcgaggtaa  | gattagatat  | ggatagtgtat | 2220 |
| atggatatgt  | atatgggtgt  | aatgccatgt  | aatatgatta  | ttaaacttct  | ttgcgtccat  | 2280 |
| ccaaaaaaaa  | agtaagaatt  | tttgaaaatt  | caatataaat  | gacagctcag  | ttacaaaagt  | 2340 |
| aaagtacttc  | taaaattgtt  | ttggttacag  | gtgggtgctg  | atacattggt  | tcacacactg  | 2400 |
| tggtagagct  | aattgagaat  | ggatatgact  | gtgtgtgtgc  | tgataacctg  | tcgaatagat  | 2460 |
| cgacctgaag  | tctaggtccc  | tatttatatt  | tttatagtta  | tgttagtatt  | aagaacgtta  | 2520 |
| tttatatttc  | aaatttttct  | tttttttctg  | tacagacggc  | tgtaacgaatt | tcgacctcga  | 2580 |
| cgggccgggt  | ttacaaaatca | gtaagcaggt  | cagtgcgtag  | gccatggccg  | gagtggtcca  | 2640 |

cagtcggtgg tccggcagta caatggattt ccttacgcga aatacgggca gacatggcct 2700  
gcccggttat tattattttt gacaccagac caactggtaa tggtagcgac cggcgctcag 2760  
ctggaattcc gccgatactg acgggctcca ggagtcgctg ccaccaatcc ccatatggaa 2820  
accgctcgata ttcagccatg tgccttcttc cgcgtgcagc agatggcgat ggctggtttc 2880  
catcagttgc tgttgactgt agcggtgat gttgaactgg aagtcgccgc gccactgggtg 2940  
tgggccataa ttcaattcgc gcgtcccgca gcgcagaccg ttttcgctcg ggaagacgta 3000  
cggggtatac atgtctgaca atggcagatc ccagcgggtca aaacaggcgg cagtaaggcg 3060  
gtcgggtag ttttcttgcg gccctaatec gagccagttt acccgctctg ctacctgcgc 3120  
cagctggcgag ttcaggccaa tccgcgcgg atgcggtgta tcgctcgcca cttcaacatc 3180  
aacggtaatc gccatttgac cactaccatc aatccggtag gttttccggc tgataaataa 3240  
ggttttcccc tgatgctgcc acgctgagc ggtcgtaatc agcaccgcat cagcaagtg 3300  
atctgccgtg cactgcaaca acgctgcttc ggctggtaa tggcccgccg ccttccagcg 3360  
ttcgaccag gcgttagggc caatgcgggt cgettcactt acgccaatgt cgttatccag 3420  
cgggtgcacgg gtgaactgat cgcgcagcgg cgtcagcagt tgttttttat cgccaatcca 3480  
catctgtgaa agaaagcctg actggcggtt aaattgcca cgttattac ccagctcgat 3540  
gcaaaaatcc atttcgctgg tggtcagatg cgggatggcg tgggacgcgg cggggagcgt 3600  
cacactgagg ttttcgcgca gacgccaactg ctgccaggcg ctgatgtgcc cggcttctga 3660  
ccatgcgggtc gcgttcgggt gcactacgcg tactgtgagc cagagttgcc cggcgctctc 3720  
cggctgcgggt agttcaggca gttcaatcaa ctgtttacct tgtggagcga catccagagg 3780  
cacttcaccg cttgccagcg gcttaccatc cagcgccacc atccagtgca ggagctcgtt 3840  
atcgctatga cggaacaggt attcgctgggt cacttcgatg gtttgcccg ataaaacggaa 3900  
ctggaaaaac tgcgtgctgggt gttttgcttc cgtcagcgt ggatgcggcg tgcggtcggc 3960  
aaagaccaga ccggttcatac agaactggcg atcgttcggc gtatcgcca aatcacgcc 4020  
gtaagcgac cacgggttgc cgttttcate atatttaac agcgactgat ccaccagtc 4080  
ccagacgaag ccgcctgtga aacggggata ctgacgaac gcctgccagt atttagcgaa 4140  
accgccaaga ctgttaccga tcgctggggc gtattcgcaa aggatcagcg ggcgcgtctc 4200  
tccaggtagc gaaagccatt ttttgatgga ccatttcggc acagccggga agggctgggtc 4260  
ttcatccacg cgcgcgtaca tcgggcaaat aatateggtg gccgtgggtg cggctccgcc 4320  
gccttcatac tgcacpgggc gggaaggatc gacagatttg atccagcgat acagcgcgtc 4380  
gtgattagcg ccgtggcctg attcattccc atcgaccag atgatcacac tcgggtgatt 4440  
acgatcgcgc tgcacattc gcgttacgcg ttcgctcatc gccggtagcc agcgcggatc 4500  
atcggtcaga cgttccattg gcaccatgcc gtgggtttca atattggctt catccaccac 4560  
atacagggcg tagcggtcgc acagcgtgta ccacagcgga tgggtcggat aatgcgaaca 4620  
ggcacggcg ttaaagtgt tctgcttcat cagcaggata tctgcaacca tegtctgctc 4680  
atccatgacc tgaccatgca gaggatgatg ctggtgacgg ttaacgcctc gaatcagcaa 4740  
cggcttgccg ttcagcagca gcagaccatt ttcaatccgc acctcgcgga aaccgacatc 4800  
gcaggttct gcttcaatca gcgtgcgtc ggcggtgtgc agttcaacca ccgcacgata 4860  
gagattcggg atttcgggcg tccacagttt cgggttttcg acgttcagac gtagtgtgac 4920  
gcgatcggca taaccaccac gctcatcgat aatttcaccg ccgaaaggcg cgggtgcgct 4980  
ggcgacctgc gtttcaacct gccataaaga aactgttacc cgtaggtagt caccgaactc 5040  
gccgcacatc tgaacttcag cctccagtac agcggcgctg aaatcatcat taaagcgagt 5100  
ggcaacatgg aaatcgctga tttgtgtagt cgggttatgc agdaacgaga cgtcacggaa 5160  
aatgcgcgtc atccgccaca tatcctgatc ttccagataa ctgcgctcac tccagcgag 5220  
caccatcacc gcgaggcggt tttctccggc gcgtaaaaat gcgctcaggt caaattcaga 5280  
cggcaaacga ctgtcctggc cgtaaccgac ccagcgcccg ttgcaccaca gatgaaacgc 5340  
cgagttaacg ccatacaaaa taattcgctg ctggccttcc tgtagccagc tttcatcaac 5400  
attaaatgtg agcgagtaac aaccgcgtcg attctccgtg ggaacaaacg gcggattgac 5460  
cgtaatggga taggtcacgt tgggtgtagat gggcgcatcg taaccgtgca tctgccagtt 5520  
tgaggggacg acgacagtat cggcctcagg aagatcgac tccagccagc tttccggcac 5580  
cgcttctgggt gccggaacc aggcaaacgc ccattcgcca ttcaggctgc gcaactgttg 5640  
ggaaggcgga tcggtgcggg cctcttcgct attacgccag ctggcgaaag ggggatgtgc 5700  
tgcaaggcga ttaagtccgg aaacctgtcg tgccagctgc attaatgaat cggccaacgc 5760  
gcggggagag gcggtttgcg tattgggcgc caggggtggt tttcttttca ccagtggagc 5820  
gggcaacagc caagctccgg atccgggctt ggccaagctt ggaattccgc acttttcggc 5880  
caatggtctt ggtaattcct ttgcgctaga attgaactca ggtacaatca cttcttctga 5940

atgagattta gtcattatag ttttttctcc ttgacgttaa agtatagagg tatattaaca 6000  
atTTTTgtt gatactttta ttacatttga ataagaagta atacaaaccg aaaatgttga 6060  
aagtattagt taaagtgggt atgcagtttt tgcattttata tatctgttaa tagatcaaaa 6120  
atcatcgctt cgctgattaa ttaccccaga aataaggcta aaaaactaat cgcattatca 6180  
tcccctcgac gtactgtaca tataaccact ggTTTTatat acagcagtac tgtacatata 6240  
accactgggt ttatatacag cagtcgacgt actgtacata taaccactgg ttttatatac 6300  
agcagtactg gacatataac cactggTTTT atatacagca gtcgaggtaa gattagatat 6360  
ggatatgtat atggatatgt atatggtggt aatgccatgt aatatgatta ttaaacttct 6420  
ttgctcccat ccaaaaaaaa agtaagaatt tttgaaaatt caataataat gacagctcag 6480  
ttacaaagtg aaagtacttc taaaattggt ttggttacag gtgggtgctgg atacattgggt 6540  
tcacacactg tggtagagct aattgagaat ggatatgact gtgttggtgc tgataacctg 6600  
tcgaattcca agctcggtac cccgagctcg gatcccccta agaaaccatt attatcatga 6660  
cattaacctt taaaaatagg cgtatcacga ggcccttctg tctcgcgctt ttcggtgatg 6720  
acggtgaaaa cctctgacac atgcagctcc cggagacggt cacagcttgt ctgtaagcgg 6780  
atgccgggag cagacaagcc cgtcagggcg cgtcagcggg tgttgccggg tgtcggggct 6840  
ggcttaacta tgcggcatca gagcagattg tactgagagt gcaccataac gcattttaagc 6900  
ataaacacgc actatgccgt tcttctcatg tatatatata tacaggcaac acgcagatat 6960  
agggtgcgacg tgaacagtga gctgtatgtg cgcagctcgc gttgcatttt cggagcgctg 7020  
cgttttcgga aacgctttga agttcctatt ccgaagtcc tattctctag ctgaaaagta 7080  
taggaacttc agagcgcttt tgaaaaccaa aagcgctctg aagacgcact ttcaaaaaac 7140  
caaaaacgca cgggactgta acgagctact aaaatattgc gaataccgct tccacaaaca 7200  
ttgctcaaaa gtatctcttt gctatatatc tctgtgctat atccctatat aacctacca 7260  
tccacctttc gctccttgaa cttgcatcta aactcgacct ctacattttt tatgtttatc 7320  
tctagtatta ctcttttagac aaaaaaattg tagtaagaac tattcataga gtgaatcgaa 7380  
aacaatacga aaatgtaac atttctata cgtagtatat agagacaaaa tagaagaac 7440  
cgttcataat tttctgacca atgaagaatc atcaacgcta tcactttctg ttcaaaagt 7500  
atgcgcaatc cacatcggtg tagaatataa tcggggatgc ctttatcttg aaaaaatgca 7560  
cccgcagctt cgctagtaat cagtaaacgc ggggaagtga gtcaggcttt ttttatggaa 7620  
gagaaaaatg acaccaaagt agccttcttc taaccttaac ggacctacag tgcaaaaagt 7680  
tatcaagaga ctgcattata gagcgcacaa aggagaaaaa aagtaatcta agatgctttg 7740  
ttagaaaaat agcgtctctg ggatgcattt ttgtagaaca aaaaagaagt atagattctt 7800  
tgttggttaa atagcgtctc cgcgttgcat ttctgttctg taaaaatgca gctcagattc 7860  
tttgtttgaa aaattagcgc tctcgcgttg catttttgtt ttacaaaaat gaagcacaga 7920  
ttcttcgttg gtaaaatagc gctttcgcgt ttctgttctg ttctgtaaaa atgcagctca 7980  
gattctttgt ttgaaaaatt agcgtctctg cgttgcatct ttgttctaca aaatgaagca 8040  
cagatgcttc gttgcttccg tgtggaagaa cgattacaac aggtgttgtc ctctgaggac 8100  
ataaaataca caccgagatt catcaactca ttgctggagt tagcatatct acaattcaga 8160  
agaactcgtc aagaaggcga tagaaggcga tgcgctgcga atcgggagcg gcgataccgt 8220  
aaagcacgag gaagcgggca gcccttcgc cgcgaagctc ttcagcaata tcacgggtag 8280  
ccaacgctat gtcctgatag cggctcgcga caccagcgc gccacagtcg atgaatccag 8340  
aaaagcggcc attttccacc atgatattcg gcaagcaggc atcgccatgg gtcacgacga 8400  
gatcctcgcc gtcgggcatg ctgccttgga gcctggcgaa cagttcggct ggcgcgagcc 8460  
cctgatgtc ttctgtccaga tcatcctgat cgacaagacc ggcttccatc cgagtacgtg 8520  
ctcgctcgat gcgatgtttc gcttggtggt cgaatgggca ggtagccgga tcaagcgtat 8580  
gcagccgccc cattgcatca gccatgatgg atactttctc ggcaggagca aggtgagatg 8640  
acaggagatc ctgccccggc acttcgccc aatagcagca gtcccttccc gcttcagtga 8700  
caacgtcgag cacagctgcg caaggaacgc cgtcgtggc cagccacgat agccgcgctg 8760  
cctcgtcttg cagttcattc agggcaccgg acaggtcggg cttgacaaaa agaaccgggc 8820  
gcccctgccc tgacagccgg aacacggcgg catcagagca gccgattgtc tgttgtgccc 8880  
agtcatagcc gaatagctc tccaccaag cggccggaga acctgctgc aatccatctt 8940  
gttcaatcat gcgaaacgat cctcatcctg ttctttgatc agagcttgat cccctgcgcc 9000  
atcagatcct tggcggcaag aaagccatcc agtttacttt gcagggttcc ccaaccttac 9060  
cagagggcgc ccagctggc aattccggtt cgcttgctgt ccataaaacc gccagctcta 9120  
gctatcgcca tgtaagccca ctgcaagcta cctgctttct ctttgcgctt gcgttttccc 9180  
ttgtccagat agcccagtag ctgacattca tccgggggtc gcaccgtttc tgcggactgg 9240

```

ctttctacgt gaaaaggatc taggtgaaga tcctttttga taatctcatg accaaaatcc 9300
cttaacgtga gttttcgttc cactgagcgt cagaccccggt agaaaagatc aaaggatctt 9360
cttgagatcc tttttttctg cgcgtaatct gctgcttgca aacaaaaaaa ccaccgctac 9420
cagcgggtgg ttgtttgccc gatcaagagc taccaactct ttttccgaag gtaactggct 9480
tcagcagagc gcagatacca aatactgttc ttctagtgtg gccgtagtta ggccaccact 9540
tcaagaactc tgtagcaccg cctacatacc tcgctctgct aatcctgtta ccagtggctg 9600
ctgccagtggt cgataagtcg tgtcttaccc ggttggactc aagacgatag ttaccggata 9660
aggcgcagcg gtcggggtga acgggggggt cgtgcacaca gccagccttg gagcgaacga 9720
cctacaccga actgagatac ctacagcgtg agctatgaga aagcgccacg cttcccgaag 9780
ggagaaaagg ggacagggtat ccggtaagcg gcagggtcgg aacaggagag cgcacgaggg 9840
agcttccagg gggaaaacgc tgggtatctt atagtcctgt cgggtttcgc cacctctgac 9900
ttgagcgtcg atttttgtga tgcctgtcag gggggcggag cctatggaaa aacgccagca 9960
acgcggcctt tttacggttc ctggcctttt gctggccttt tgctcacatg atataattca 10020
attgaagctc taatttgtga gtttagtata catgcattta cttataatac agtttttttag 10080
ttttgctggc cgcctcttct caaatatgct tcccagcctg cttttctgta acgttcaccc 10140
tctaccttag catcccttcc ctttgcaaat agtctcttcc caacaataat aatgtcagat 10200
cctgtagaga ccacatcac caccggttcta tactgttgac ccaatgcgtc tccctgtgca 10260
tctaaaccca caccgggtgt cataatcaac caatcgtaac cttcatctgt tccaccatg 10320
tctctttgag caataaagcc gataacaaaa tctttgtcgc tcttcgcaat gtcaacagta 10380
cccttagtat attctccagt agatagggag cccttgcatg acaattctgc taacatcaaa 10440
aggcctctag gttcctttgt tacttcttct gccgcctgct tcaaaccgct aacaatacct 10500
gggcccacca caccgtgtgc attcgtaatg tctgcccatt ctgctattct gtatacacc 10560
gcagagtact gcaatttgac tgtattacca atgtcagcaa attttctgtc ttcgaagagt 10620
aaaaaattgt acttggcgga taatgccttt agcggcttaa ctgtgcctc catggaaaaa 10680
tcagtcaaga tatccacatg tgtttttagt aaacaaattt tgggacctaa tgcttcaact 10740
aactccagta attccttggt ggtacgaaca tccaatgaag cacacaagtt tgtttgcttt 10800
tcgtgcatga tattaaatag cttggcagca acaggactag gatgagtagc agcacgttcc 10860
ttatatgtag ctttcgacat gatttatctt cgtttcctgc aggtttttgt tctgtgcagt 10920
tgggttaaga atactgggca atttcagtgt tcttcaacac tacatatgcg tatatatacc 10980
aatctaagtc tgtgctcctt ccttcgttct tcctctgtgt cggagattac cgaatcaaaa 11040
aaatttcaag gaaaccgaaa tcaaaaaaaa gaataaaaaa aaaatgatga attgaa 11096

```

&lt;210&gt; 10

&lt;211&gt; 150

&lt;212&gt; DNA

&lt;213&gt; Vaccinia Virus/LIVP

&lt;300&gt;

&lt;308&gt; GenBank No. M57977

&lt;309&gt; 2000-04-14

&lt;400&gt; 10

```

atgggtcatcg gtttagtcat attcgtgtct gtggcgcccg ccacgtcggg tgtgtgtct 60
aacgtattgg acatgcttat gtacgtagaa gaaaataatg aagaggatgc tagaatcaag 120
gaggagcaag aactactggt gctatattga

```

&lt;210&gt; 11

&lt;211&gt; 49

&lt;212&gt; PRT

&lt;213&gt; Vaccinia Virus/LIVP

&lt;300&gt;

&lt;308&gt; GenBank No. AAA48282

&lt;309&gt; 2000-04-14

10/74

<400> 11  
Met Val Ile Gly Leu Val Ile Phe Val Ser Val Ala Ala Ala Ile Val  
1 5 10 15  
Gly Val Leu Ser Asn Val Leu Asp Met Leu Met Tyr Val Glu Glu Asn  
20 25 30  
Asn Glu Glu Asp Ala Arg Ile Lys Glu Glu Gln Glu Leu Leu Leu Leu  
35 40 45  
Tyr

<210> 12  
<211> 150  
<212> DNA  
<213> Vaccinia Virus/WR

<300>  
<308> GenBank No. AY243312  
<309> 2003-04-10

<400> 12  
tcaatatagc aacagtagtt cttgctcctc cttgattcta gcaccccttt cattattttc 60  
ttctacgtac ataagcatgt ccaatacgtt agacaacaca ccgacgatgg cggccgccac 120  
agacacgaat atgactagac cgatgaccat 150

<210> 13  
<211> 49  
<212> PRT  
<213> Vaccinia Virus/WR

<400> 13  
Met Val Ile Gly Leu Val Ile Phe Val Ser Val Ala Ala Ala Ile Val  
1 5 10 15  
Gly Val Leu Ser Asn Val Leu Asp Met Leu Met Tyr Val Glu Glu Asn  
20 25 30  
Asn Glu Glu Asp Ala Arg Ile Lys Glu Glu Gln Glu Leu Leu Leu Leu  
35 40 45  
Tyr

<210> 14  
<211> 150  
<212> DNA  
<213> Vaccinia Virus/Ankara

<300>  
<308> GenBank No. U94848.1  
<309> 2003-04-14

<400> 14  
tcaatatagc aacagtagtt cttgctcctc cttgattcta gcaccccttt cattattttc 60  
ttctacgtac ataaacatgt ccaatacgtt agacaacaca ccgacgatgg cggccgccac 120  
agacacgaat atgactaaac cgatgaccat 150

11/74

<210> 15  
<211> 49  
<212> PRT  
<213> Vaccinia Virus/Ankara

<400> 15  
Met Val Ile Gly Leu Val Ile Phe Val Ser Val Ala Ala Ala Ile Val  
1 5 10 15  
Gly Val Leu Ser Asn Val Leu Asp Met Phe Met Tyr Val Glu Glu Asn  
20 25 30  
Asn Glu Glu Asp Ala Arg Ile Lys Glu Glu Gln Glu Leu Leu Leu  
35 40 45  
Tyr

<210> 16  
<211> 146  
<212> DNA  
<213> Vaccinia Virus/Tian Tan

<300>  
<308> GenBank No. AF095689  
<309> 2000-02-14

<400> 16  
caatatagca acagtagttc ttgctcctcc ttgattctag catcctcttc attattttct 60  
tctaegtaca taaacatgtc caatacgtta gacaacacac cgacgatggc cgccacagac 120  
acgaatatga ctagaccgat gaccat 146

<210> 17  
<211> 48  
<212> PRT  
<213> Vaccinia Virus/Tian Tan

<400> 17  
Met Val Ile Gly Leu Val Ile Phe Val Ser Val Ala Ala Ile Val Gly  
1 5 10 15  
Val Leu Ser Asn Val Leu Asp Met Phe Met Tyr Val Glu Glu Asn Asn  
20 25 30  
Glu Glu Asp Ala Arg Ile Lys Glu Glu Gln Glu Leu Leu Leu Tyr  
35 40 45

<210> 18  
<211> 150  
<212> DNA  
<213> Vaccinia Virus/Acambis 3000 MVA

<300>  
<308> GenBank No. AY603355  
<309> 2004-05-15

<400> 18

12/74

tcaatatagc aacagtagtt cttgctcctc cttgattcta gcacccctctt cattattttc 60  
ttctacgtac ataaacatgt ccaatacgtt agacaacaca ccgacgatgg cggccgccac 120  
agacacgaat atgactaaac cgatgaccat 150

&lt;210&gt; 19

&lt;211&gt; 49

&lt;212&gt; PRT

&lt;213&gt; Vaccinia Virus/Acambis 3000 MVA

&lt;400&gt; 19

Met Val Ile Gly Leu Val Ile Phe Val Ser Val Ala Ala Ala Ile Val

1

5

10

15

Gly Val Leu Ser Asn Val Leu Asp Met Phe Met Tyr Val Glu Glu Asn

20

25

30

Asn Glu Glu Asp Ala Arg Ile Lys Glu Glu Gln Glu Leu Leu Leu

35

40

45

Tyr

&lt;210&gt; 20

&lt;211&gt; 150

&lt;212&gt; DNA

&lt;213&gt; Vaccinia Virus/Copenhagen

&lt;300&gt;

&lt;308&gt; GenBank No. M35027.1

&lt;309&gt; 1993-08-03

&lt;400&gt; 20

tcaatatagc aacagtagtt cttgctcctc cttgattcta gcacccctctt cattattttc 60

ttctacgtac ataaacatgt ccaatacgtt agacaacaca ccgacgatgg cggccgccac 120

agacacgaat atgactagac cgatgaccat 150

&lt;210&gt; 21

&lt;211&gt; 49

&lt;212&gt; PRT

&lt;213&gt; Vaccinia Virus/Copenhagen

&lt;400&gt; 21

Met Val Ile Gly Leu Val Ile Phe Val Ser Val Ala Ala Ala Ile Val

1

5

10

15

Gly Val Leu Ser Asn Val Leu Asp Met Phe Met Tyr Val Glu Glu Asn

20

25

30

Asn Glu Glu Asp Ala Arg Ile Lys Glu Glu Gln Glu Leu Leu Leu

35

40

45

Tyr

&lt;210&gt; 22

&lt;211&gt; 150

&lt;212&gt; DNA

&lt;213&gt; Cowpox Virus

&lt;300&gt;

&lt;308&gt; GenBank No. X94355.2

&lt;309&gt; 2003-05-09

&lt;400&gt; 22

```
tcaatatagc aacagtagtt cttgctcttc cttgattcta gcacccctctt cattattttc 60
ttctacgtac ataagcatgt ccaatacgtt agacaacaca ccgacgatgg cggccgccac 120
agacacgaat atgactagac cgatgaccat                                     150
```

&lt;210&gt; 23

&lt;211&gt; 49

&lt;212&gt; PRT

&lt;213&gt; Cowpox Virus

&lt;400&gt; 23

```
Met Val Ile Gly Leu Val Ile Phe Val Ser Val Ala Ala Ala Ile Val
  1             5             10             15
Gly Val Leu Ser Asn Val Leu Asp Met Leu Met Tyr Val Glu Glu Asn
          20             25             30
Asn Glu Glu Asp Ala Arg Ile Lys Glu Glu Gln Glu Leu Leu Leu
      35             40             45
Tyr
```

&lt;210&gt; 24

&lt;211&gt; 150

&lt;212&gt; DNA

&lt;213&gt; Rabbitpox Virus

&lt;300&gt;

&lt;308&gt; GenBank No. AY484669

&lt;309&gt; 2004-03-30

&lt;400&gt; 24

```
tcaatatagc aacagtagtt cttgctcttc cttgattcta gcacccctctt cattattttc 60
ttctacgtac ataagcatgt ccaatacgtt agacaacaca ccgacgatgg cggccgccac 120
agacacgaat atgactagac cgatgaccat                                     150
```

&lt;210&gt; 25

&lt;211&gt; 49

&lt;212&gt; PRT

&lt;213&gt; Rabbitpox Virus

&lt;400&gt; 25

```
Met Val Ile Gly Leu Val Ile Phe Val Ser Val Ala Ala Ala Ile Val
  1             5             10             15
Gly Val Leu Ser Asn Val Leu Asp Met Leu Met Tyr Val Glu Glu Asn
          20             25             30
Asn Glu Glu Asp Ala Arg Ile Lys Glu Glu Gln Glu Leu Leu Leu
      35             40             45
Tyr
```

<210> 26  
<211> 150  
<212> DNA  
<213> Camelpox Virus/CMS

<300>  
<308> GenBank No. AY009089  
<309> 2002-07-30

<400> 26  
tcaatatagc aacagtagtt cttgctcctc ctttaattcta gcattcttctt cattatttttc 60  
ttctacatac ataagcatgt ccaatacgtt agacaacaca ccgacgatgg cggccgccac 120  
agacacgaat atgactagac cgatgaccat 150

<210> 27  
<211> 49  
<212> PRT  
<213> Camelpox Virus/CMS

<400> 27  
Met Val Ile Gly Leu Val Ile Phe Val Ser Val Ala Ala Ala Ile Val  
1 5 10 15  
Gly Val Leu Ser Asn Val Leu Asp Met Leu Met Tyr Val Glu Glu Asn  
20 25 30  
Asn Glu Glu Asp Ala Arg Ile Lys Glu Glu Gln Glu Leu Leu Leu Leu  
35 40 45  
Tyr

<210> 28  
<211> 150  
<212> DNA  
<213> Ectromelia Virus/Moscow

<300>  
<308> GenBank No. AF012825  
<309> 2002-08-06

<400> 28  
tcaatatagc aacaacagtt cttgctcctc cttgattcta gcattcttctt cattatttttc 60  
ttctacgtac ataagcatgt ccaatacgtt agacaacaca ccgacaatgg cggccgccac 120  
agacacgaat atgactagac cgaggaccat 150

<210> 29  
<211> 49  
<212> PRT  
<213> Ectromelia Virus/Moscow

<400> 29  
Met Val Leu Gly Leu Val Ile Phe Val Ser Val Ala Ala Ala Ile Val  
1 5 10 15  
Gly Val Leu Ser Asn Val Leu Asp Met Leu Met Tyr Val Glu Glu Asn  
20 25 30  
Asn Glu Glu Asp Ala Arg Ile Lys Glu Glu Gln Glu Leu Leu Leu Leu

Tyr 35

40

45

<210> 30  
<211> 146  
<212> DNA  
<213> Monkeypox Virus/Zaire

<300>  
<308> GenBank No. AF380138  
<309> 2001-12-13

<400> 30  
tatagcaaca gtaattcttg ctctctcttg atttttagcat cctcttcatt attttcttct 60  
acgtacataa gcatgtccaa tacgttagac aacacaccga cgatggtggc cgccacagac 120  
acgaatatga ctagaccgat gaccat 146

<210> 31  
<211> 48  
<212> PRT  
<213> Monkeypox Virus/Zaire

<400> 31  
Met Val Ile Gly Leu Val Ile Phe Val Ser Val Ala Ala Thr Ile Val  
1 5 10 15  
Gly Val Leu Ser Asn Val Leu Asp Met Leu Met Tyr Val Glu Glu Asn  
20 25 30  
Asn Glu Glu Asp Ala Lys Ile Lys Glu Glu Gln Glu Leu Leu Leu Leu  
35 40 45

<210> 32  
<211> 150  
<212> DNA  
<213> Variola Virus

<300>  
<308> GenBank No. X69198.1  
<309> 1996-12-13

<400> 32  
tcaatatagc aacagtagtt ctgtctctc ottaattcta gcatcttctt cattattttc 60  
ttctacatac ataagcatct ccaatacgtt agacagcaca ccgatgatgg cggecgccac 120  
agacacgaat atgactagac tgatgaccat 150

<210> 33  
<211> 49  
<212> PRT  
<213> Variola Virus

<400> 33  
Met Val Ile Ser Leu Val Ile Phe Val Ser Val Ala Ala Ala Ile Ile  
1 5 10 15

Gly Val Leu Ser Asn Val Leu Glu Met Leu Met Tyr Val Glu Glu Asn  
 20 25 30  
 Asn Glu Glu Asp Ala Arg Ile Lys Glu Glu Gln Glu Leu Leu Leu Leu  
 35 40 45  
 Tyr

<210> 34  
 <211> 186854  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> LIPV Complete Genome

<400> 34  
 ttccactatc tgtggtacga acgggtttcat cttctttgat gccatcaccc agatgttcta 60  
 taaacttggg atcctcgctc gatttcatat cctttgccaa ccaatacata tagctaaact 120  
 caggcatatg ttccacacat cctgaacaat gaaattctcc agaagatgtt acaatgtcta 180  
 gatttggaca tttgggttca accgcgttaa catatgagtg aacacaccca tacatgaaag 240  
 cgatgagaaa taggattctc atcttgccaa aatatcacta gaaaaaattt atttatcaat 300  
 tttaaaggta taaaaaatac ttattgttgc tcgaatatat tgtatttgat ggtatacggg 360  
 agattagaaa tgtaggtatt atcatcaact gattctatgg ttttatgtat tctatcatgt 420  
 ttcactattg cgttggaaat aatatcatat gcttccacat atattttatt ttgttttaac 480  
 tcataatact cacgtaattc tggattattg gcatactcat gaataatttt agctccatga 540  
 tcagtaataa ttaatgagaa catagtatta ccacctacca ttattttttt catctcattc 600  
 aattcttaat tgcaaaagatc tatataatca ttatagcgtt gacttatgga ctctggaatc 660  
 ttagacgatg tacagtcac tataatcatg gcataatttaa tacattgttt tatagcatag 720  
 tcgttatcta cgatgttaga tatttctctc aatgaatcaa tcacacaatc taatgtaggt 780  
 ttatgacata atagcatttt cagcagttca atgtttttag attcgttgat ggcaatggct 840  
 atacatgtat atcgtttatt tgatctaatt ttgacatctg aaccggattc tagcagtaaa 900  
 gatactagag attgtttatt atatctaaca gccttgtgaa gaagtgtttc tctcgtttg 960  
 tcaatcatgt taatgtcttt aagataaggt aggc aaatgt ttatagtact aagaattggg 1020  
 caagcataag acatgtcaca aagacccttt tttgtatgta taagtgtaaa aattataaca 1080  
 tccatagttg gatctacata ggtgtccaat cgggattctc ccatcatcga gataattgat 1140  
 ggcattctcc ttcctttttt agtagatatt tcatcgtgta agaatacaata ttaatatctc 1200  
 taaagtatcc gtgtatagcc tctttattta ccacagctcc atattccact agagggatat 1260  
 cgccgaatgt catatactca attagtatat gttggaggac atccgagttc attgttttca 1320  
 atatcaaaga gatgggttcc ttatcatttc tccatagtgg tacaatacta cacattattc 1380  
 cgtgcggctt tccattttcc aaaaacaatt tgaccaaaac taaatctaca tctttattgt 1440  
 atctataatc actattttaga taatcagcca taattcctcg agtgcaacat gttagatcgt 1500  
 ctatatatga ataagcagtg ttatctattc ctttcattaa caatttaacg atgtctatat 1560  
 ctatatgaga tgacttaata taatattgaa gagctgtaca atagttttta tctataaaaag 1620  
 acggcttgat tccgtgatta attagacatt taacaacttc cggacgcaca tatgctctcg 1680  
 tatccgactc tgaatacaga tgagagatga tatacagatg caatacggta ccgcaatttc 1740  
 gtatgtgata atcatcatac gcgtatcagt actcgtcttc ataaagaaca ctgcagccat 1800  
 tttctatgaa caaatcaata attttagaaa caggatcatt gtcattacat aattttctat 1860  
 aactgaacga tgggtttcac atttaacact caagtcaaat ccatgttcta ccaacacctt 1920  
 tatcaagtca acgtctacat ttttggattt catatagctg aatatattaa agttatttat 1980  
 gttgctaaat ccagtggctt ctagttagagc catcgctata tctttattaa ctttaacatg 2040  
 tctactatct gtgtattctt ctaatggggg aagctgtctc caatttttgc gtaatggatt 2100  
 agtgccactg tctagtagta gtttgacgac ctgcacatta ttacaatgct cattaaaaag 2160  
 gtatgcgtgt aaagcattat tcttgaattg gttcctggta tcattaggat ctctgtcttt 2220  
 caacatctgt ttaagttcat caagagccac ctctctattt tccaaatagt caaacatttt 2280

gactgaatga gctactgtga actctatata cccacacaa taatgtcatt aaatatcatg 2340  
 tcaaaaactt gtacaattat taataaaaaat aatttagtgt ttaaatttta ccagttocag 2400  
 attttacacc tccgttaata cctccattaa cccactgga cgatcctcct cccacacattc 2460  
 caccgccacc agatgtataa gttttagatc ctttattact accatcatgt ccatggataa 2520  
 agacactcca catgccgcca ctacccctt tagaagacat attaataaga ctttaaggaca 2580  
 agtttaacaa taaaattaat cagcagtagc ctactacca cctacactat tatatgatta 2640  
 tagttcttat ttttacagta ccttgactaa agtttctagt cacaagagca atactaccaa 2700  
 cctacactat tatatgatta tagttcttat ttttatagga acgcgtacga gaaaatcaaa 2760  
 tgtctaattt ctaacggtag tgttgataaa cgattgttat ccgcgggatac ctctctatc 2820  
 atgtcgtcta ttttcttact ttgttctatt aacttattag cattatata ttttgatta 2880  
 taaaacttat attgcttatt agcccaatct gtaaatatcg gattattaac atatcgtttc 2940  
 tttgtaggtt tatttaacat gtacatcact gtaagcatgt ccttaccatt tattttaatt 3000  
 tgacgcatac ccgcaatttc ttttccgcag tccggttataa attctatata tgatggatac 3060  
 atgctacatg tgtacttata atcgactaat atgaagtact tgatacatat tttcagtaac 3120  
 gatttattat taccacctat gaataagtac ctgtgatcgt ctaggtaatc aactgttttc 3180  
 ttaatacatt cgtagggttg taatttactc agaataattt ccaatatctt aatatataat 3240  
 tctgctattt ctgggatata tttatctgcc agtataacac aaatagtaac acatgtaaac 3300  
 ccatattttg ttattatatt aatgtctgcg ccattatcta ttaaccattc tactaggctg 3360  
 acactatgcg actcaatata atgataaagt ataactacac catgtttatc tattttgttt 3420  
 atatcatcaa tatacggctt acaaagtttt agtatcgata acacatecaa ctacgcata 3480  
 gagaaggtag ggaataatgg cataatattt attagggtat cateattgtc attatctaca 3540  
 actaagtttc cattttttaa aatatactcg acaactttag gatctctatt gccaaatttt 3600  
 tgaaaatatt tatttatatg cttaaatcta tataatgtag ctcttccatc aatcacatc 3660  
 ttaataacat tgatgtatac tgtatgataa gatacatatt ctaacaatag atcttgtata 3720  
 gaattctgat atcttttaag aattgtggat attaggatat tattacataa actattacac 3780  
 aattctaaaa tataaaacgt atcacggtcg aataatagtt gatcaactat ataattatcg 3840  
 attttgtgat ttttcttctt aaactgttta cgtaaatagt tagatagaat attcattagt 3900  
 tcatgaccac tatagttact atcgaataac gcgtcaataa tttcccgttt aatatcgcat 3960  
 ttgtcaagat aataatagag tgtggtatgt tcacgataag tataataacg catctctttt 4020  
 tegtgtgaaa ttaaatagtt tattacgtcc aaagatgtag cataaccatc ttgtgacctt 4080  
 gtaataatat aataatagag aactgtttta cccattctat catcataatc agtgggtgtg 4140  
 tctaatcgt aatcgtctaa ttcacatccc caattataat attcaccagc acgtcctaac 4200  
 tgttctattt tgatcttgta tccatactgt atgttgctac atgtagggat tcttttatcc 4260  
 aataatagtt taaacacatc tacattggga tttgatgttg tagcgtattt ttctacaata 4320  
 ttaataccat ttttgatact atttatttct ataccttccg aaattagtaa tttcaataag 4380  
 tctatattga tgttatoaga acatagatat tcgaatatat caaaatcatt gatattttta 4440  
 tagtcgactg acgacaataa caaaatcaca acatcgtttt tgatattatt attttcttg 4500  
 gtaacgtatg cctttaatgg agtttcacca tcatactcat ataattgatt tgcaccactt 4560  
 tctatcaatg attgtgcact gctggcatcg atgttaaatg ttttacaact atcatagagt 4620  
 atcttatcgt taaccatgat tggttgttga tgcatacgca ttttttgggt tctttcattt 4680  
 cagttatgta tggatttagc acgtttggga agcatgagct catatgattt cagtactgta 4740  
 gtgtcagtag tattagtttc aataagatca atctctagat ctatagaatc aaaacacgat 4800  
 aggtcagaag ataatagaata tctgtaggct tcttgttgta ctgtaacttc tggttttgtt 4860  
 agatgggttc atcgtgcttt aacgtcaatg gtacaaattt tatectcgtt ttgtgtatca 4920  
 tattcgtccc tactataaaa ttgtatatcc agattatcat gcgatgtgta tacgctaacc 4980  
 gtatcaataa acggagcaca ccatttagtc ataaccgtaa tccaaaaatt tttaaagtat 5040  
 atcttaacga aagaagtgtt gtcattgtct acggtgtatg gtaactagatc ctcataagtg 5100  
 tatafatcta gagtaattgt taatttatta aatggttgat aatatggatc ctctgacaa 5160  
 tttccgaaga tggaaataag acataaacac gcaataaatc taattgcgga catgggttact 5220  
 ccttaaaaaa atacgaataa tcaccttggt tatttagtaa gtgtcattta aactataact 5280  
 catattaatc catggactca taatctctat acgggattaa cggatgttct atatacgggg 5340  
 atgagtagtt ctcttcttta actttatact ttttactaat catatttaga ctgatgtatg 5400  
 ggtaatagtg tttgaagagc tcgttctcat catcagaata aatcaatata tctgtttttt 5460  
 tgttatacag atgtattaca gcctcatata ttacgttaata gaacgtgtca tctacettat 5520  
 taactttcac cgcatagttg tttgcaataa cggttaatcc tttgacctcg tctgatttccg 5580

```

accaatctgg gcgtataatg aatctaaact ttaattgott gtaatcattc gaaataattt 5640
ttagtttgca tccgtagtta tcccctttat gtaactgtaa atttctcaac gcgatattctc 5700
cattaataat gatgtcgaat tegtgtgta taccataact gaatggatga acgaataccg 5760
acggcgtaa tagtaattta ctttttcac tttacatatt gggtagtagt tttactatca 5820
taagtttata aattccacaa gotactatgg aataagccaa ccatcttagt ataccacaca 5880
tgtcttaaag tttatttaatt aattacatgt tgttttatat atatcgctac gaatttaaag 5940
agaaatttag ttaggaagaa aaattatcta tctacatcat cactctctctg tattctacga 6000
tagagtgcga cttaagatg cgacagatcc gtgtcatcaa atatatactc cattaaaatg 6060
attattccgg cagcgaactt gatattggat atatcacaa ctttggttaat atctacgaca 6120
atagacagca gtcccatggg tccataaaca gtgagtttat ctttctttga agagatattt 6180
tgtagagatc ttataaaact gtcgaatgac atcgcattta tatctttagc taaatcgat 6240
atgttaccat cgtaatatct aaccgcgtct atcttaaacg tttccatcgc tttaaagacg 6300
tttccgatag atggtctcat ttcacagtc atactgagcc aacaaatata atcgtgtata 6360
acatctttga tagaatcaga ctctaaagaa aacgaatcgg ctttattata cgcattcatg 6420
ataaacttaa tgaaaaatgt ttttcgttgt ttaagttgga tgaatagtat gtcttaataa 6480
ttgttattat ttcattaatt aatatttagt aacgagtaca ctctataaaa acgagaatga 6540
cataactaat cataactagt tatcaaagt tctaggagcc gtaattttca tatggtatag 6600
atcctgtaag cattgtctgt attctggagc tatcttctct atcgcattag tgagttcaga 6660
atatgttata aatttaaatc gaataacgaa cataacttta gtaaagtcgt ctatattaac 6720
tcttttattt tctagccatc gtaataccat gtttaagata gtatattctc tagttactac 6780
gatctcatcg ttgtctagaa tatcacatac tgaatctaca tccaatttta gaaattgggc 6840
tgtgttacat atctcttcta tattattggt gatgtattgt cgtagaaaac tattacgtag 6900
accattttct ttataaaacg aatatatagt actccaatta tctttaccga tatatttgca 6960
cacataatcc attctctcaa tcaactacatc ttttaagattt tegtgtttaa gatatttggc 7020
taaactatat aattctatta gatcatcaac agaatcagta tatatttttc tagatccaaa 7080
gacgaactct ttggcgctct ctataatatt cccagaaaag atattttcgt gtttttagttt 7140
atcgagatct gatctgttca tatacgccat tactattcat aattgagatt gatgtaatac 7200
aaataaaaat ccattttcat ttttaacca aactattcat aattgagatt gatgtaatac 7260
tttggtactt tgaacgtaaa gacagtacac ggatccgtat ctccaacaag cactagtaa 7320
tcaaatttgg tgtgtttaa cttegcaata ttcattcaatt tagatagaaa cttatactca 7380
tcatctgttt taggaatcca tgtattatta ccactttcca acttatcatt atcccaggct 7440
atgtttcgtc catcatcggt gcgcagagtg aataattctt ttgtattcgg tagttcaaat 7500
atatgatcca tgcatagatc ggcaaagcta ttgtagatgt gatttttctt aaatctaata 7560
taaaactcgt ttactagcaa acactttcct gattttatga ccaagacaca tatggtttct 7620
aaatctatca agtgggtggg atccatagtt atgacgcagt aacatatatt attacattct 7680
tgactgtcgc taatatctaa atatttattg ttatcgtatt ggattctgca tatagattggc 7740
ttgtatgtca aagatataga acacataacc aatttatagt cgcgctttac attctcgaat 7800
ctaaagttaa gagattttag aaacattata tcttcggatg atgttatcac tgtttctgga 7860
gtaggatata ttaaagtctt tacagatttc gtccgattca aataaatcac taaataatat 7920
cccacattat catctgttag agtagtatca ttaaactctat tatattttat gaaagatata 7980
tcaactgtca cctctatatt tegtacattt ttaaactggt tgtataatat ctctctgata 8040
caatcagata tatctattgt gtcggtagac gataccgta catttgaatt aatgggtgtc 8100
cattttacaa cttttaacaa gttgaccaat tcatttctaa tagtatcaaa ctctccatga 8160
ttaaatattt taatagtatc catttttatat cactacggac acaaagtagc tgacataaac 8220
cattgtataa tttttatggt ttatgtttat tagcgtacac attttggag ttccggtctc 8280
catgtatttc ctggagagca agtagatgat gaggaaccag atagtttata tccgtacttg 8340
cacttaaagt ctacattgtc gttgtatgag tatgatcttt taaacccgct agacaagtat 8400
cgttttgata ttgtaggatg tggacattta acaatctgac acgtgggtgg atcggaccat 8460
tctcctctg aacacaggac actagagtta ccaatcaacg aatatccact attgcaacta 8520
taagttacaa cgctccatc ggtataaaaa tctcgtatc cgttatgtct tccgttgat 8580
atagatggag gggattggca tttaacagat tcacaaatag gtgcctcggg attccatacc 8640
atagatccag tagatcctaa ttcacaatac gatttagatt cacegatcaa ctgatattccg 8700
ctattacaag agtacgttat actagagcca aagtctactc caccaatatc aagttggcca 8760
ttatcgatat ctcgaggcga tgggcatctc cgtttaatac attgattaaa gagtgtccat 8820
ccagtacctg tacatttagc atatataggt cccatttttt gctttctgta tccaggtaga 8880

```

catagatatt ctatagtgtc tcctatgttg taattagcat tagcatcagt ctccacacta 8940  
ttcttaaatt tcatattaat gggtcgtgac ggaatagtag agcatgatag aacgcaccc 9000  
attcccaaca atgtcaggaa cgtcacgctc tccaccttca tatttattta tccgtaaaaa 9060  
tggtatcctg gacatcgtac aaataataaa aagcccatat atgttcgcta ttgtagaaat 9120  
tggttttcac agttgctcaa aaacgatggc agtgacttat gagttacgtt acactttgga 9180  
gtctcatctt tagtaaacat atcataatat tcatattac gagttgacat atcgaacaaa 9240  
ttccaagtat ttgatttttg ataataattog tattttgcat ctgctataat taagatataa 9300  
tcaccgcaag aacacacgaa catcttttct acatgggttaa agtacatgta caattctatc 9360  
catttgctt ccttaactat atatttgtat agataattac gagtctcgtg agtaattcca 9420  
gtaattacat agatgtcgcc gtctactct acagcataaa ctatactatg atgtctaggc 9480  
atgggagact tttttatcca acgattttta gtgaaacatt coacatcgtt taatactaca 9540  
tatttctcat acgtggtata aactccaccc attacatata tatcatcgtt tacgaatacc 9600  
gacgcgctg aatatctagg agtaattaag tttggaagtc ttatccattt cgaagtgcgc 9660  
tggttcaaatt attctgccac accggttgaa atagaaaatt ctaactctcc tattacatat 9720  
aactttccat cgtaaacaca agtactaact tctgatttta acgacgacat attagtaacc 9780  
gttttccatt ttttcgtttt aagatctacc cgcgatacgg aataaacatg tctattgtta 9840  
atcatgcgc caataatgta tagacaatta tgtaaaacat ttgcattata gaattgtcta 9900  
tctgtattac cgactatcgt ccaatattct gttctaggag agtaatgggt tattgtggat 9960  
atataatcag agtttttaat gactactata ttatgtttta taccatttcg tgcactggc 10020  
ttttagatt tggatatagt taatcccaac aatgatatag cattgcgcgt agtattagtc 10080  
ataaacttg gatgtaaaat gttgatgata tctacatcgt ttggattttt atgtatccac 10140  
tttaataata tcatagctgt aacatctcca tgatttacgt taacgtcttc gtgggataag 10200  
atagttgtca gttcatcctt tgataatttt ccaaattctg gatcggatgt caccgcagta 10260  
atattgttga ttatttctga catcgacgca ttatatagtt ttttaattcc atatctttta 10320  
gaaaagttaa acatccttat acaatttgtg aaattaatat tatgaatcat agtttttaca 10380  
catagatcta ctacaggcgg aacatcaatt attatggcag caactagtat cattcttaca 10440  
ttgtttatgg tgatgtttat cttcttcag ccatatagtt ctaatagcga ttcaaacgcg 10500  
tgatagttta taccattcaa tataatoget tcatccttta gatgggtgac ctgaatgcgt 10560  
ttaaaaaaat taccgagga cgccgtaata atttcttat tcatctgtat aatttcccca 10620  
ttgatagaaa atattacgct tccattctt aaagtactat aagtaattat agtataatgt 10680  
aaacgtttat atattcaata tttttataaa aatcattttg acattaattc ctttttaaat 10740  
ttcgtctat catctataga aacgtattct atgaatttat aaaatgcttt tacgtgtcct 10800  
atcgtaggcg atagaaccgc taaaaagcct atcgaatttc taaaaagaa tctgttatat 10860  
ggtagtagga gagtataaaa cattaaatgt cgtacttat taaagtattc agtagccaat 10920  
cctaactctt tcgaataact attaatggct cttgtctgt acgaatctat ttttttgaac 10980  
aacggacctg gtggtatctt ttgttctatg tatctaaaat aatgtctgac tagatccgtt 11040  
agtttaatat ccgcagtcct cttgtctaga atggcaaatc taactgcggg tttaggcttt 11100  
agtttagttt ctatatctac atctatgtct ttatctaaca ccaaaaatat aatagctaatt 11160  
attttattac aatcatccgg atattcttct acgatctcac taactaatgt ttctttgggt 11220  
atactagtat agtcactatc ggacaaaata agaaaatcag atgatcgatg aataatacat 11280  
ttaaattcat catctgtaag atttttgaga tgtctcatta gaattattat aggggttagta 11340  
ctcattatca ttcggcagct attacttatt ttattatttt tcaccatata gatcaatcat 11400  
tagatcatca aaatatgttt caatcatcct aaagagtatg gtaaatgact ctteccatct 11460  
aatttctgaa cgttcaccaa tgtctctagc cactttggca ctaatagcga tcattcgttt 11520  
agcgtcttct atattattaa ctgggtgatt caatctatct agcaatggac cgtcggacag 11580  
cgtcattctc atgttcttaa tcaatgtaca tacatcgccg tcatctacca attcatocaa 11640  
caacataagc tttttaaaat catcattata ataggtttga tegtgtgcat ttctccaaag 11700  
aatatatcta aaagtagag tootcatgct tagttaacaa ctatttttta tgttaaataa 11760  
attagtagac cgctatgttt aatacttatt catatttttag tttttaggat tgagaatcaa 11820  
tacaanaatt aatgcacat taatttttag aatacttagt ttcacgtag tcaatgaaac 11880  
atttgaactc atcgtaagg acgttctcgt acaggacgta actataaacc ggtttatatt 11940  
tggtcaagat agatacaaat ccgataaact tttttacgaa ttctacggga tccactttaa 12000  
aagtgtcata ccgggttctt tttatttttt taaacagatc aatgggtgtga tgttgattag 12060  
gtcttttacg aatttgatat agaatacgt ttacatatcc tccataatgg tcaatgcgca 12120  
ttgttcgta tgcataaat cctttaatta tatgacactg tgtattattt agttcactct 12180

|             |             |             |             |             |             |       |
|-------------|-------------|-------------|-------------|-------------|-------------|-------|
| tgttcattgt  | taggaatcta  | tccaaaatgg  | caattatact  | agaactatag  | gtgcgttgta  | 12240 |
| tacacatatt  | gatgtgtctg  | ttatatacaat | caatgctact  | accttcgggt  | aaaattgtag  | 12300 |
| catcatatac  | cattttctagt | acttttaggtt | cattattatc  | cattgcagag  | gacgtcatga  | 12360 |
| tcgaatcata  | aaaaaatata  | ttatttttat  | gttattttgt  | taaaaataat  | catcgaatac  | 12420 |
| ttcgttaagat | actccttcat  | gaacataatc  | agttacaaaa  | cgttttatatg | aagtaaagta  | 12480 |
| tctacgattt  | ttacaaaagt  | ccggatgcat  | aagtacaaag  | tacgcgataa  | acggaataat  | 12540 |
| aatagattta  | tctagtctat  | ctttttctat  | agctttcata  | gttagatata  | tggtctcaga  | 12600 |
| agtaggatta  | tgtaacatca  | gottcgataa  | aatgactggg  | ttatttagtc  | ttacacattc  | 12660 |
| gctcatacat  | gtatgaccgt  | taactacaga  | gtctacacta  | aaatgattga  | acaatagata  | 12720 |
| gtctaccatt  | gtttcgtatt  | cagatagtac  | agcgtagtac  | atggcatctt  | cacaaaattat | 12780 |
| atcattgtct  | aatagatatt  | tgacgcactc  | tatggatccc  | acttcaacag  | ccatcttaaa  | 12840 |
| atcggtagaa  | tcatattgct  | ttcctttatc  | attaataatt  | tctagaacat  | catctctatc  | 12900 |
| ataaaagata  | caaatattaa  | ctgtttgac   | cgtaataaca  | ttgctagtcg  | atagcaattt  | 12960 |
| gttaataaga  | tgcgctgggc  | tcaatgtctt  | aataagaagt  | gtaagaggac  | tatctccgaa  | 13020 |
| tttgttttgt  | ttattaacat  | ccgttgatgg  | aagtaaaaga  | tctataatgt  | ctacattctt  | 13080 |
| gactgtttta  | gagcatacaa  | tatggagagg  | tgtatttcca  | tcatgatctg  | gttttgaggg  | 13140 |
| actaattcct  | agtttcatca  | tccatgagat  | tgtagaagct  | tttggattgt  | ctgacataag  | 13200 |
| atgtctatga  | atatgatttt  | tgccaaaatt  | atccactatc  | ctggcttcga  | atccgatgga  | 13260 |
| cattattttt  | ttaaacactc  | tttctgaagg  | atctgtacac  | gccacaacg   | gaccacatcc  | 13320 |
| ttcttcatca  | accgagttgt  | taatcttggc  | tccatactgt  | accaataaat  | ttattctctc  | 13380 |
| tatgacttca  | tcatctgttc  | ccgagagata  | atatagaggc  | gttttatgct  | gtttatcaca  | 13440 |
| cgcgtttgga  | tctgcgcgct  | gcgtcagcag  | catcgcgact  | attctattat  | tattaatttt  | 13500 |
| agaagctata  | tgcaatggat  | aatttccatc  | atcatccgtc  | tcatttgag   | agtatcctct  | 13560 |
| atgaagaagt  | tcttcgacaa  | atcgttcatc  | tagtccctta  | attccacaat  | acgatgtag   | 13620 |
| aatgtgataa  | ttatttccag  | aaggttcgat  | agcttgtagc  | atattcctaa  | atacatctaa  | 13680 |
| atttttacta  | ttatatattg  | cataaagaga  | tagataatac  | tcggccgaca  | taatgttgtc  | 13740 |
| cattgtagta  | taaaaattaa  | tattttctatt | tctgtatatt  | tgcaacaatt  | tactctctat  | 13800 |
| aacaaatata  | ataacttagt  | tcttttatgt  | caagaaggca  | ctggtttagt  | tcatctataa  | 13860 |
| atgtcacgcc  | ataactacca  | cgcatgccat  | actcagaatt  | atgataaaga  | tatttatcct  | 13920 |
| tggggtgtag  | gtaatgggga  | ttaatctttg  | ttggatcagt  | ctctaagtta  | acacatgtca  | 13980 |
| cacatgatcc  | atttatagtt  | atatcacacg  | atgatgattt  | atgaattgat  | tccggaagat  | 14040 |
| cgctatcgta  | ttttgtgggt  | ccacaattca  | tttccataca  | tgttattgtc  | acactaatat  | 14100 |
| tatgatgaac  | tttatctagc  | cgctgagtg   | ttaacaacag  | aacagatagt  | ttattatctt  | 14160 |
| taccaacacc  | ctcagccgct  | gccacaaatc  | tctgatccgt  | atccatgatg  | gtcatgttta  | 14220 |
| tttctagtc   | gtatccagtc  | aacactatgt  | tagcatttct  | gtcgatatag  | ctttcactca  | 14280 |
| tatgacactc  | accaataata  | gtagaattaa  | tgtcgttaatt | tacaccaata  | gtgagttcgg  | 14340 |
| cggcaaaagta | ccaataccgg  | taatcttgtc  | gaggaggaca  | tatagtattc  | ttgtattcta  | 14400 |
| ccgaataccc  | gagagatgcg  | atacaaaaga  | gcaagactaa  | tttgtaaacc  | atcttactca  | 14460 |
| aaatatgtaa  | caatagtacg  | atgcaatgag  | taagacaata  | ggaaatctat  | cttatataca  | 14520 |
| cataattatt  | ctatcaattt  | taccaattag  | ttagtgtaat  | gttaacaaaa  | atgtgggaga  | 14580 |
| atctaattag  | tttttcttta  | cacaattgac  | gtacatgagt  | ctgagttcct  | tgtttttgct  | 14640 |
| aattatttca  | tccaatttat  | tattcttgac  | gatatcgaga  | tcttttgtat  | aggagtcaaa  | 14700 |
| cttgatttca  | acatgctttt  | ctataatcat  | tttagctatt  | tcggcatcat  | ccaatagtac  | 14760 |
| attttccaga  | ttagcagaat  | agatattaat  | gtcgtatttg  | aacagagcct  | gtaacatctc  | 14820 |
| aatgtcttta  | ttatctatag  | ccaatttaat  | gtccggaatg  | aagagaaggg  | aattattggg  | 14880 |
| gtttgtcgac  | gtcatatagt  | cgagcaagag  | aatcatcata  | tccacgtgtc  | cattttttat  | 14940 |
| agtgtatgta  | atacaactaa  | ggagaatagc  | cagatcaaaa  | gtagatggta  | tctctgaaag  | 15000 |
| aaagtaggaa  | acaatactta  | catcattaag  | catgacggca  | tgataaaatg  | aagttttcca  | 15060 |
| tccagttttc  | ccatagaaca  | tcagtctcca  | atttttctta  | acaaacagtt  | ttaccgtttg  | 15120 |
| catgtttacca | ctatcaaccg  | cataatacaa  | tgcggtgttt  | cccttgtcat  | caaattgtga  | 15180 |
| atcatccagt  | ccactgaata  | gcaaaaatctt | tactattttg  | gtatcttcca  | atgtgggtgc  | 15240 |
| ctgatgtaat  | ggaaattcat  | tctctagaag  | atttttcaat  | gtccacagct  | tcaacaacgt  | 15300 |
| acatactaga  | cgcacgttat  | tatcagctat  | tgcataatc   | aaggcactat  | gtccatggac  | 15360 |
| atccgcctta  | aatgtatctt  | tactagagag  | aaagcttttc  | agctgcttag  | acttccaagt  | 15420 |
| attaattcgt  | gacagatcca  | tgtctgaaac  | gagacgctaa  | ttagtgtata  | ttttttcatt  | 15480 |

ttttataatt ttgtcatatt gcaccagaat taataatatt ttttaatagat ctgattagta 15540  
gatacatggc tatcgcaaaa caacatatac acattttaata aaaataatat ttattaagaa 15600  
aattcagatt tcacgtaccc atcaatataa ataaaaaat gatttccttac accgtaccca 15660  
tattaaggag attccacctt acccataaac aatataaat cagtaatatc atgtctgatg 15720  
atgaacacaa atgggtgtatt aaattccagt ttttcaggag atgatctcgc cgtagctacc 15780  
ataatagtag atgcctctgc tacagtccct tgttcgtcga catctatctt tgcattctga 15840  
aacattttat aaatatataa tgggtcccta gtcataatgt taaacgacgc attatctgga 15900  
ttaaacatac taggagccat catttcggct atcgacttaa tatccctctt attttcgata 15960  
gaaaatttag ggagtttaag attgtacact ttattcccta attgagacga ccaatagctt 16020  
aattttgcag ccgtgataga atctgtgaaa tgggtcatat tatcacctat tgcagggtac 16080  
atactaatat tagcatcctt atacggaagg cgtaccatgt catattcttt gtcategatt 16140  
gtgattgtat ttcccttgcaa tttagtaact acgttcacatc tgggaaccgt tttcgtacgg 16200  
tacttattag taaaactagc attgctgtgt ttagtatat caaacggata ttgccatata 16260  
cctttaaaat ataatagtag aatgattgcc catagagtag tattgtcgag catattagaa 16320  
tctactacat tagacatacc ggatctacgt tctactatag aattaatttt attaacggca 16380  
tctcgtctaa agtttaactt atataggccg aatctatgat attgttgata atacgacggg 16440  
ttaatgcaca cagtattatc tacgaaactt tgataagtta gatcagtgta cgtatattta 16500  
gatgttttca gcttagctaa tectgatatt aattctgtaa atgctggacc cagatctctt 16560  
tttctcaaat ccatagcttt caataattct attctagtag tacctgatgc aggcaatagc 16620  
gacataaaca tagaaaacga ataaccacac ggtgagaaga caatattatc atcttgata 16680  
tttttatagc ctactatacc ggcatttgta aatccttgta gacgataggc ggacgctgaa 16740  
cacgctaacc atagatcaaa taacgcaatc atgattttat ggtattaata attaacctta 16800  
tttttatgtt cgggtataaaa aaattattga tgtctacaca tctttttgta attgacatct 16860  
atatactctt ttgtataatc aactctaact actttaactt ttacagtttt cctaccagt 16920  
ttatccctat attcaacata tctatccata tgcattctaa cactctctgc caagatagct 16980  
tcagagttag gatagtcaaa aagataaata tatagagcat aatcattctc gtatactctg 17040  
ccctttatta catcacccgc attgggcaac gaataacaaa atgcaagcat cttgttaacg 17100  
ggctcgtaaa ttgggataaa aattatgttt ttattgtctt atatctattt tattcaagag 17160  
aatattcagg aatttctttt tccggttgta tctcgtcgca gtatatatca tttgtacatt 17220  
gtttcatatt ttttaatagt ttacaccttt tagtaggact agtatcgtac aattcatagc 17280  
tgtattttga attccaatca cgcataaaaa tatcttccaa ttgttgacga agacctaatc 17340  
catcatccgg tgtaatatata atagatgtct cacatgtatc cgtaaagtaa tttcctgtcc 17400  
aatttgaggt acctatatag gccgttttat cggttaccat atatttgga tggtttacc 17460  
tagaatacgg aatgggagga tcagcatctg gtacaataaa tagctttact tctatattta 17520  
tgtttttaga ttttagcata gcgatagatc ttaaaaagtt tctcatgata aacgaagatc 17580  
gttgccagca actaatcaat agcttaacgg atactgtctt gtctatagcg gatcttcta 17640  
attcatcttc tatataaggc caaaacaaaa ttttaccgc cttogaataa ataatagga 17700  
taaagttcat aacagatata taaacgaatt tactcgcatt tctaatacat gacaataaag 17760  
cggttaaatc attggttctt tccatagtag atagttgttg cgggtgcagaa gcaataaata 17820  
cagagtgtgg aacgcgcgtt acgttaatac taagaggatg atctgtatta taatagcag 17880  
gataaaaagt tttccaatta tatggttagat tgttaactcc aagataaccg tatacctcaa 17940  
aaatttgagt gagatccgct gccagttcc tattattgaa gatcgcaata cccaattcct 18000  
tgacctgagt tagtgatctc caatccatgt tagcgttcc taaataaata tgtgtattat 18060  
cagatatcca aaattttgta tgaagaactc ctctaggat atttgtaata tctatgtatc 18120  
gtacttcaac tccggccatt tgtagtcttt caacatcctt taatggtttg ttagatttat 18180  
taacggctac tctaactcgt actcctcttt tgggtaattg tacaatctcg tttaatatta 18240  
tcgtgccgaa attcgtaacc acttcacccg ataaactcca ataaaagat gatatatcta 18300  
gtgtttttgt ggtattggat agaatttccc tccacatgtt aaatgtagac aaatatactt 18360  
tatcaaattg catacctata ggaatagttt ctgtaatcac tgcgattgta ttatccggat 18420  
tcattttatt tgttaaaaga ataactctat atcaactcac tctattaaaa atccaagttt 18480  
ctatttcttt catgactgat ttttaactt cctccgtttc cttatgaaga tgatgtttgg 18540  
caccttcata aatttttatt tctctattac aatttgcatt ttgcatgaaa taatatgcac 18600  
ctaaaacatc gctaattctt ttgtttgttc cctggagtag gagagtcggg ggggtgttaa 18660  
tcttggaat tatttttcta acctgtgttg tagccttcaa gacctgacta gcaaatccag 18720  
ccttaatttt ttcattgattg actaatgggt cgtatttgta tttataaact ttatccatat 18780

ctctagatagc tgattctgga catagctttc cgactggcgc atttgggtgtg atgggttccca 18840  
taagtttggc agctagcaga ttcagtcttg aaacagcatc tgcattaact agaggagaca 18900  
ttagaatcat tgcgtgaaac aagtttggat tatcgtaaga ggctagctcc catggaatga 18960  
cccaataagt agatttaata gttaccacgt gctgtaccaaa agtcatcaat catcattttt 19020  
tcaccattac ttcttccatg tccaatatga tcatgtgaga atactaaaat tcccaacgat 19080  
gatatgtttt cagctagttc gtcataacgt ccagaatgtt taccagctcc atgacttatg 19140  
aataactaatg ccttaggata tgaatatagg ttccaatatt tacaatatat gtaateattg 19200  
tccagattga acatacagtt tgcactcatg attcacgtta tataactatc aatattaaca 19260  
gttcgtttga tgatcatatt atttttatgt tttattgata attgtaaaaa catacaatta 19320  
aatcaatata gaggaaggag acggctactg tcttttgtaa gatagtcagc gcgactaaat 19380  
tagattatga ggatgctgtt ttttactttg tggatgatga taaaatatgt agtcgcgact 19440  
ccatcatcga tctaatagat gaatatatta cgtggagaaa tcatgttata gtgtttaaca 19500  
aagatattac cagttgtgga agactgtaca aggaattgat gaagttcgat gatgtcgcta 19560  
tacggtacta tggattgatg aaaattaatg agattgtcga agctatgagc gaaggagacc 19620  
actacatcaa ttttacaaaa gtccatgacg aggaaggttt attcgctacc ataggaatat 19680  
gtgctaaaat cactgaacat tggggataca aaaagatttc agaactctaga ttccaatcat 19740  
tgggaaacat tacagatttg atgaccgacg ataataataa catcttgata ctttttctag 19800  
aaaaaaaatt gaattgatga tataggggtc ttcataacgc ataattatta cgtttagcatt 19860  
ctatatccgt gttaaaaaaa attatccat catgtatttg agagttttat atgtagcaaa 19920  
catgatagct gtgatgccaa taagcttttag atattcacgc gtgctagtgt tagggatgg 19980  
attatctggt ggtgaaatgt ccgttatata atctacaaaa caatcatcgc atatagtatg 20040  
cgatagtaga gtaaacattt ttatagtttt tactggatc atacatcgtc tacccaattc 20100  
ggttataaat gaaattgtcg ccaatcttac acccaacccc ttgttatcca ttagcatagt 20160  
attaacttcg ttattttatgt cataaactgt aaatgatttt gtagatgcca tatcatatc 20220  
gatattcatg tccctattat aatcattact aactttatca caatatatgt tgataatc 20280  
tatatatgat ctagtctttg tgggcaactg tctatacaag tctgtctaaac gttgtttact 20340  
catatagtat cgaacagcca tcattacatg gtcccgttcc gttgatagat aatcgagat 20400  
gttagtgagc ttgtcaaatc tatataccat attttctgga agtggatata catagtcgtg 20460  
atcaacatta ttgctagcct catcttetat atcctgtact ataccattat ctatatcatc 20520  
tacataatct atgatattat tacacataaa catcgacaac atactattgt ttattatcta 20580  
agtcctgttg atccaaaccc ttgatctcct ctatttgtac tatctagaga ttgtacttct 20640  
tccagttctg gataatatat acgttgatag attagctgag ctattctatc tccagtattt 20700  
acattaaacg tacattttcc attattaata agaatgactc ctatgtttcc cctataatct 20760  
tcgtctatta caccacctcc tatatcaatg ccttttagtg acagaccaga cctaggagct 20820  
attctaccat agcagaactt aggcattggac atactaatat ctgtcttaac taactgtctt 20880  
tctctggag ggatagtata atcgtaagcg ctatacaaat catatccggc agcaccggc 20940  
gattgcctag taggagattt agctctgtta gtttctctaa caaatctaac tgggtgagtta 21000  
atattcatgt tgaacataaa actaatattt tatttcaaaa ttatttacca tcccatatat 21060  
tccatgaata agtgtgatga ttgtacactt ctatagtatc tatatacgat ccacgataaa 21120  
atctcctat caatagcagt ttattatcca ctatgatcaa ttctggatta tccctcggat 21180  
aaataggatc atctatcaga gtccatgtat tgctggattc acaataaaaat tccgcatttc 21240  
taccaccaa gaataacctt ctaccgaaca ctaacgcgca tgatttataa tgaggataat 21300  
aagtggatgg tccaaactgc cactgatcat gattgggtag caaatattct gtagttgtat 21360  
cagtttcaga atgtcctccc attacgtata taacattgtt tatggatgcc actgctggat 21420  
tacatctagg tttcagaaga ctggcctat taaccaagc agcatccccg tggaaaccaac 21480  
gctcaacaga tgtgggattt ggtagacctc ctactacgta taatttattg ttagcgggta 21540  
tcccgttagc atacagtctg gggctattca tggagggaat tggaaatcaa ttgtttgata 21600  
tataatttac cgctatagca ttgttatgta tttcattgtt catccatcca ccgatgagat 21660  
atactacttc tccaacatga gtacttgtac acatatggaa tatatctata atttgatcca 21720  
tgttcatagg atactctatg aatggatact tgtatgattt gcgtgggtgt ttatcacaat 21780  
gaaatatttt ggtacagtct agtatccatt ttacattatt tatacctctg ggagaaagat 21840  
aatttgacct gattacattt ttgataagga gtgacagatt tccctaattta tttcttctg 21900  
ttatatacca cttaatgaca aaatcaacta cataatcctc atctggaaca tttagttcat 21960  
cgctttctag aataagtttc atagatagat aatcaaaaat gtctatgatg tcatcttcca 22020  
gttccaaaaa gtgtttggca ataaagtttt tagtatgaca taagagattg gatagtcctg 22080

attctataacc catcatgtaa cactcgacac aatattcctt tctaaaatct cgtaagataa 22140  
agtttataca agtgtagatg ataaattcta cagaggttaa tatagaagca cgtaataaat 22200  
tgacgacgtt atgactatct atatatacct ttccagtata cgagtaaata actatagaag 22260  
ttaaactgtg aatgtcaagg tctagacaaa ccctcgtaac tggatcctta tttttcgtgt 22320  
atttttgacg taaatgtgtg cgaaagtaag gagataactt tttcaatata gtagaattga 22380  
ctattatatt gcctcctatg gcatcaataa ttgttttgaa tttcttagtc atagacaatg 22440  
ctaataatatt cttacagtac acagtattga caaatatcgg catttatgtt tctttaaaag 22500  
tcaacatcta gagaaaaatg attatctttt tgagacataa ctcccathtt ttggtattca 22560  
cccacacgtt tttcgaaaaa attagttttt ccttccaatg atatatthtt catgaaatca 22620  
aacggattgg taacattata aattttttta aatcccaatt cagaaatcaa tctatccggt 22680  
acgaattcta tatatgtttt catcatttca caattcattc ctataagttt aactggaaga 22740  
gccgcagtaa gaaattcctt ttcaatggat actgcatctg ttataataga tctaaccggt 22800  
tcttcaactg gtggatgcaa taaatgttta aacatcaaac atgcgaaatc gcagtgacga 22860  
ccctcgtctc tactaattag ttctgttgaa aacgtgagtc cgggcattag gccacgcttt 22920  
ttaagccaaa atattggaagc gaatgatccg gaaaagaaga ttccttctac tgcagcaaat 22980  
gcaataagtc tctctccata accggcgctg tcatgtatcc acttttgagc ccaatcggtc 23040  
ttctttttta cacaaggcat cgtttctatg gcattaaaga gatagttttt ttcattacta 23100  
tctttaacat aagtatcgat caaaagacta tacatttccg aatgaatgtt ttcaatggc 23160  
atctgaaatc cgtagaaaca tctagcctcg gtaatctgta cttctgtaca aaatcgttcc 23220  
gccaaatttt cattcactat tccgtcactg gctgcaaaaa acgccaatac atgttttata 23280  
aaatattttt cgtctgggtg tagttttatt caatcattga tatctttaga tatatctact 23340  
tcttccactg tccaaaatga tgctctgccc tttttataca tgttccagat gtcataat 23400  
tggattggga aaataacaaa tctatttggg tttgttgcaa ggaatgggtc catacataa 23460  
ttaacaataa caataaattt tttttcagtt atctatatgc ctgtacttgg attttttgta 23520  
catcgatata gccgcaatca ctacaataat tacaagtatt attgatagca ttgttattag 23580  
tactatcata attaaattat ctacattcat ggggtgctgaa taatcgttat tatcatcatt 23640  
atcattttgt aattgtgaca tcatactaga taaatcgttt gcgagattgt tgtgggaagc 23700  
gggcatggag gatgcattat cattattatt taacgccttc catttggatt cacaatgtt 23760  
acgcacattc aacattttat ggaaactata attttgtgaa aaacaaatac aagaaaactc 23820  
gtcattcggtc aaatttttaa cगतatgtaa ccगतatgaa gtcgagctaa tttctaaccg 23880  
tagcagctct gttggatagc ggttccaga tatatatctt ttcagttccc ctacgtatct 23940  
ataatcatct gtaggaaatg gaagatatct ccatttatct actgttecta atatcatatg 24000  
tggtgggtgta gtagaaccat taagcgcgaa agatgttatt tgcgcatgta ttttaacttc 24060  
gcaataattt ctgggttagat aacgcactct accagtcaag tcaatgatat tagcctttac 24120  
agatatattc atagtagtcg taacgatgac tccatctttt agatgcgata ctcccttgta 24180  
tgtaccagaa tottctgacc tcaaacctga tatattttaa caagttaatg agatattaac 24240  
gcgttttatg aatgatgata tataaccaga agttttatcc tgggtggcta gcgctataac 24300  
cttatcatta taataccaac tagtgtgatt aatatgtgac acgtcagttg gggtagaaat 24360  
atgtacatta tctgtacgt cgtattcgat acatccgcat acagccaaca aatataaaat 24420  
gacaaatact ctaacgcctg tctgaccat cttgatgcgg tttataaat gttttgattt 24480  
caatttattg taaaaaaaga ttctggtttt tactgttcga tattctcatt gottatattt 24540  
tcatctatca tctccacaca gtcaaatccg tgggttagcat gcacctcatc aaccggtaaa 24600  
agactatcgg actcttctat cattataact ctagaatatt taatttgggtc attattaatc 24660  
aagtcaatta tcttattttt aacaaacgtg agtattttac tcatthttta taaaaacttt 24720  
tagaaatata cagactctat cgtgtgtcta tatcttcttt ttatatccaa tgtatttatg 24780  
tctgattttt ctccatttat catatataat ggtccaaatt ctacacgtgc ttoggattca 24840  
tccagatcat taaggttctt ataattgtaa catccttctc ttccctcttc tacatcttcc 24900  
ttcttattct tattcttagc gtcacagaat ctaccacagc aggatcccat gacgagcgtc 24960  
atattaaact aatccatttt caattataat atatgattag taatgaccat taaaataaaa 25020  
aatattcttc ataaccggca agaaagtga aagttcacat tgaactatg tcagtagtat 25080  
acatcatgaa atgatgatat atatatactc tattttgggtg gaggattata tgatataatt 25140  
cgtggataat catthtttaag acacatttct ttattcgtaa atctthttcac gttaaatgag 25200  
tgtccatatt ttgcaatttc ttcatatgat ggggggtgac gtggacgagg ctgctctgt 25260  
tcttggtgtg gtcgcgact cgtgtgtctg cgttttagtc cgtccattat cgcgattgct 25320  
tagatggagt actattttat acctgtgaat taaatttttt tattaattaa acgtataaaa 25380

acgttcogta tctgtatttta agagccagat ttcgtctaata agaacaata gctacagtaa 25440  
aaataactag aataattgct acaccacta gaaaccacgg atcgtaatac ggcaatcggg 25500  
tttcgataat aggtggaacg tatattttat ttaaggactt aacaattgtc tgtaaaccac 25560  
aatttgcttc cgcggatcct gtatttaacta tctgtaaaag catatgttga ccgggcgagg 25620  
ccgaacattc tccgatatct aatttctgtat tatctataat attattaacc tccgcatacg 25680  
cattacagtt cttttctagc ttggataccg cactagggtac atcgctctaga tctattccta 25740  
tttccctcagc gatagctctt ctatcccttt ccggaagcaa tgaaatcact tcaataaatg 25800  
attcaacat gagtgtgaaa ctaagtcgag aattactcat gcatttggtta gttattcgga 25860  
gcgcgcaatt tttaaactgt cctataacct ctccatatg aatagcacia gtgacattag 25920  
tagggataga atgttgagct aatttttgta aataactatc tataaaaaga ttatacaaag 25980  
ttttaaactc tttagtttcc gccatttctc cagtctgaga aaatgtctct cataataaat 26040  
ttttccaaga aactaattgg gtgaagaatg gaaaccttta atctatattt atcacagtct 26100  
gttttggtac acatgatgaa ttcttccaat gccgtactaa attcgatctc tttttcgatt 26160  
tctggatag tttttaataa agtatgaaca aagaaatgga aatcgtaata ccagttatgt 26220  
ttaactttga aattgttttt tattttcttg ttaatgattc cagccacttg ggaaggtca 26280  
aagtcttta atgcccattt aatacgttca ttaaaaacaa actttttatc ctttagatga 26340  
attattattg gttcattgga atcaaaaagt aagatattat cgggtttaag atctgcgtgt 26400  
aaaaagttgt cgcaacaggg tagttcgtag attttaatgt ataacagagg catctgtaaa 26460  
aagataaact ttatgtattg taccaaagat ttaaatccta atttgatagc taactcggta 26520  
tctactttat ctgcccgaata cagtgcctag ggaaaaatta taatgtttcc tctttcatat 26580  
tcgtagttag ttctcttttc atgttcgaaa aagtgaaca tgcgggttaa atagttttata 26640  
acattaatat tactgttaat aactgccgga taaaagtggg atagtaattt cacgaatttg 26700  
atactgtcct ttctctcgtt aaacgccttt aaaaaaactt tagaagaata tctcaatgag 26760  
agttcctgac gttccatagt ttgtatcaat aatgacaca tatgaagaac acgtttatac 26820  
agagtatgta aaaatgttaa tttatagttt aatcccattg ccacgcaca cagcgttaac 26880  
tttttttcat ctccctttag attgttgat agaaatttggt gtaactgtga ctccgcgtga 26940  
gtttccatgg gactatataa ttttgtggcc tgaatacaa attttactac atagttatct 27000  
atcttaaaga ctataccata tctcctctgta gatattgtat aaaaatcgtc gtttatagga 27060  
taaaatcgtt tatccttttg ttggaaaaag gatgaattaa tgtaatcatt ctctctctatc 27120  
tttagtagtg tttccttatt aaaattctta aaataattta acaatctaac tgacggagcc 27180  
caattttggt gtaaatctaa ttgggacatt atattgttaa aatacaaaac gtctcctaac 27240  
ataacagtat ctgataatct atggggagac atccattgat attcagggga tgaatcattg 27300  
gcaacacca tttattgtac aaaaagcccc aatttacaac cgaaagtcca ggtttgatag 27360  
agacaaacaa ttaactattt tgtctctgtt ttaaacacct ccacagtttt taatttcttt 27420  
agtaatgaaa ttattcaca tatcagtatc ttctttatct accagagatt ttactaactt 27480  
gataaccttg gctgtctcat tcaatagggt agtaatatit gtatgtgtga tattgatctc 27540  
tttttgaatt gtttctttta gaagtgatcc tttgatgggt ccagcatagc aattacaata 27600  
atgcagaaac tcggttaaca tgcaggaatt atagtaagcc aattccaatt gttgcctgtg 27660  
ttgtattaga gtgtcaatat gagcaatggt gtccctgctt ttctctgata gaatgcgagc 27720  
agcgattttg gcgttatcat ttgacgatat ttctggaatg acgaatcctg tttctactaa 27780  
ctttttggta ggacaaagtg aaacaatcaa gaagatagct tctcctccta tttgtggaag 27840  
aaattgaact cctctagatg atctactgac gatagtatct ccttgacaga tattggaccg 27900  
aattacagaa gtacctggaa tgtaaagccc tgaaccccc tcatTTTTTA agcagattgt 27960  
tgccgtaaat cctgcactat gcccaagata gagagctcct ttgggtgaatc catctctatg 28020  
tttcagttta accaagaaac agtcagctgg tctaaaattt ccatctctat ctaatacagc 28080  
atctaacttg atgtcaggaa ctatgaccgg tttaatgtta tatgtaacat tgagtaaatc 28140  
cttaagttca taatcatcac tgtcatcagt tatgtacgat ccaaacaatg tttctaccgg 28200  
catagtggat acgaagatgc tatccatcag aatgtttccc tgattagtat tttctatata 28260  
gctattcttc tttaaacgat ttccaaatc agtaactatg ttcatttttt taggagtagg 28320  
acgcctagcc agtatggaag aggtttttct agtcctctc ttcaacatct ttgatctcga 28380  
tggaatgcaa aaccccatag tgaaacaacc aacgataaaa ataactattgt ttttactttt 28440  
ttataatttt accatctgac tcatggattc attaatatct ttataagagc tactaacgta 28500  
taattcttta taactgaact gagatatata caccggatct atgggtttcc taattgagta 28560  
aatgaatgct cggcaataac taatggcaaa tgtatagaac aacgaaatta tactagagtt 28620  
gttaaagtta atattttcta tgagctgttc caataaatta tttgttggtga ctgcgttcaa 28680

gtcataaatc atcttgatac tatccagtaa accgttttta agttctggaa tattatcatc 28740  
ccattgtaaa gcccctaatt cgactatcga atatcctgct ctgatagcag tttcaatc 28800  
gacggacgtc aatactgtaa taaaggtggt agtattgtca tcatcgtgat aaactacggg 28860  
aatatggtcg ttagtaggta cggtaacttt acacaacgcg atataaact ttcctttgt 28920  
accattttta acgtagttgg gacgtcctgc agggattgtt tttgaagaaa tgatacgag 28980  
aacagatttg atacgatatt tgttggttc ctgattattc actataatat aatctagaca 29040  
gatagatgat tcgataaata gagaaggtat atcgttggtta ggataatata tccccattcc 29100  
agtattctcg gatactctat tgatgacact agttaagaac atgtcttcta ttctagaaaa 29160  
cgaaaacatc ctacatggac tcattaaaac ttctaacgct cctgattgtg tctcgaatgc 29220  
ctcgtacaag gatttcaagg atgccataga ttctttgacc aacgatttag aattgcgttt 29280  
agcatctgat ttttttatta aatcgaatgg tcggctctct ggtttgctac cccaatgata 29340  
acaatagtct tgtaaagata aaccgcaaga aaattttata gcatccatcc aaataaccct 29400  
agcaccatcg gatgatatta atgtattatt atagattttc catccacaat tattggggcca 29460  
gtatactggt agcaacggta tatcgaatag attactcatg taacctacta gaatgatagt 29520  
tcgtgtacta gtcataatat ctttaatcca atctaaaaaa tttaaaatta gattttttac 29580  
actgttaaag ttaacaaaag tattaccggg gtacgtggat atcatatatg gcattgggtcc 29640  
attatcagta atagctccat aaactgatac ggcgatgggt tttatatgtg ttgatctaa 29700  
cgaggaagaa attcgcgcgc acaattcatc tctagatatg tatttaatat caaacggtaa 29760  
cacatcaatt tcgggacgcg tatatgtttc taaattttta atccaaatat aatgatgacc 29820  
tatatgcctt attatcatac tgtcaactat agtacacctt gggaacttac gatacatctg 29880  
tttctataaa tcgttaaatt ttacaaatct ataactgtct aaaccttttg acgacagcca 29940  
ttcattaatt tctgatatgg aatctgtatt ctcgataccg tatcgttcta aagccagtgc 30000  
tatatctccc tgttcgtggg aacgctttcg tataatatcg atcaacggat aatctgaagt 30060  
ttttggagaa taatatgact catgatctat ttctgccata aacaatctag acataggaat 30120  
tggaggcgat gatcttaatt ttgtgcaatg agtcgtcaat cctataactt ctaatcttgt 30180  
aatattcatc atcgacataa tactatctat gttatcatcg tatattagta taccatgacc 30240  
ttcttcattt cgtgccaaaa tgatatacag tcttaaatag ttacgcaata tctcaatagt 30300  
ttcataattg ttagctgttt tcatcaagggt ttgtatcctg tttacatga tggcggttcta 30360  
taacgtctct attttctatt ttttaatttt taaattttta acgatttact gtggctagat 30420  
acccaatctc tctcaaatat ttttttagcc tcgcttacaa gctgtttatc tatactatta 30480  
aaactgacga atccgtgatt ttggtaatgg gttccgtcga aatttgccga agtgatatga 30540  
acatatctgt cgtcgactat caacaatttt gtattattct gaatagtga aaccttcaca 30600  
gatagatcat tttgaacaca caacgcactt agacttttg cggttgccat agaataacg 30660  
tcgttcttat cccaattacc aactagaagt ctgatcttaa ctctctatt aatggetgct 30720  
tctataatgg agttgtaaat gtcgggcca tagtagctat taccgtcgac acgtgtagt 30780  
ggaaactatg ccaaagtgtt aatatctata ctagtcttag ctgacctgag tttatcaata 30840  
actacatcgg tatctagatc tctagaatat cccaatagggt gttccggaga atcagtaaaag 30900  
aacactccac ctataggatt cttaatatga tacgcagtgc taactggcaa acaacaagcc 30960  
gcagagcata aattcaacca tgaatttttt gcgctattaa aggttttaaa agtatcaaat 31020  
cttctacgaa gatctgtggc cagcggggga taatcagaat atacacctaa cgttttaac 31080  
gtatgtatag atcctccagt aaatgacgcg ttctctacat aacatctttc atcatctgac 31140  
acccaaaaac aaccgagtag tagtccaca ttattttttt tatctatatt aacggttata 31200  
aaattttatat cggggcagtg actttgtagc tctcccagat ttcttttccc tctttcatct 31260  
agcaaaaacta ttattttta ccttttttca gatgcctctt ttagttttatc aaaaaataagc 31320  
gctcccctag tcgtactcag aggtattaca caaaaagatg ctatgtatat atatttctta 31380  
gctagagtga taatttcgtt aaaacattca aatgttggtta aatgatcgga tctaaaatcc 31440  
atattttctg ttagtgtttc taccagccta cattttgctc cgcaggtac cgatgcaaat 31500  
ggccacattt agttaacata aaaacttata catcctgttc tatcaacgat tctagaatat 31560  
catcggctat atcgctaaaa ttttcatcaa agtcgacatc acaacctaac tcagtcaata 31620  
tattaagaag ttccatgatg tcatcttctt ctatttctat atccgtatcc attgtagatt 31680  
gttgaccgat tatcgagttt aaatcattac taatactcaa tcttcagaa tacaatctgt 31740  
gtttcattgt aaatttatag gcggtgtatt taagttggta gattttcaat tatgtattaa 31800  
tatagcaaca gtagttcttg ctctccttg attctagcat cctcttctt attttcttct 31860  
acgtacataa acatgtccaa tacgttagac aacacaccga cgatggcggc cgctacagac 31920  
acgaatatga ctaaacccgat gaccatttaa aaacctctc cttagctttca cttaaactgt 31980

atcgatcatt cttttagcac atgtataata taaaaacatt attctatttc gaatttaggc 32040  
ttccaaaaat ttttcatccg taaaccgata ataatatata tagacttggt aatagtcgga 32100  
ataaatagat taatgcttaa actatcatca tctccacgat tagagataca atatttacat 32160  
tctttttgct gtttcgaaac tttatcaata cacgttaata caaaccagg aaggagatat 32220  
tgaaactgag gctgttgaaa atgaaacggt gaatacaata attcagataa tgtaaaaatca 32280  
tgattccgta ttctgatgat attagaactg ctaatggatg tccgatggtat gtatctagga 32340  
gtatctattt taacaaagca tcgatttgct aatatacaat tatccttttg attaattggt 32400  
attttattca tttctttaa aggtttcata tttatcaatt cttctacatt aaaaatttcc 32460  
atttttaatt tatgtagccc cgcaatactc ctcatctacgt ttcatttttt gtctataata 32520  
tccattttgt tcatctcggt acatagatta tccaattgag aagcgcattt agtagttttg 32580  
tacattttta gtttattgac gaatcgctga aaactagtta tagttaacat tttattattt 32640  
gataccctga tattaatacc cctgccggtta ctattattta taactgatgt aatccacgta 32700  
acattggaat taactatcga tagtaatgca tccgacgctt caaaattgtc tattataaac 32760  
tcaccgataa tttttttatt gcatgttttc atattcatta ggattatcaa atctttaatc 32820  
tttttacgat tgtatgcgtt gatattacaa gacgtcattc taaaagacgg aggatctcca 32880  
tcaaatgccg gacaatcacg tacaagtagt atggaaatag gttttgttct attgcgcac 32940  
atagatttat atagaacacc cgtagaaata ctaatttggt ttactctata aaatacta 33000  
gcatctattt catcgttttg tataacgtct tccaagtgt caaattccaa atttttttca 33060  
ttgatagtag caaattcttc tatctcttta actacttgca tagataggta attacagtga 33120  
tgccctacat cgtttttttg aaactgaata gatgcgtcta gaagcgatgc tacgctagtc 33180  
acaatcacca ctttcatatt tagaatatat atatgtaaaa atatagtaga atttcatttt 33240  
gtttttttct atgctataaa tgaattctca ttttgcattc gctcatactc cgttttatat 33300  
taataccaaa gaaggaagat atctggttct aaaagccgtt aaagtatgcg atgttagaac 33360  
tgtagaatgc gaaggaagta aagcttcctg cgtactcaaa gtagataaac cctcatcgcc 33420  
cgcgtgtgag agaagacctt cgtcccgtc cagatgcgag agaatagaata accctggaaa 33480  
acaagtccg tttatgagga cggacatgct acaaaatatg ttccgcggcta atccgcgataa 33540  
tgtagcttct agacttttgt cctaaaatac tattatatcc ttttcgatat taataaatcc 33600  
gtgtcgtcca ggttttttat ctctttcagt atgtgaatag ataggtattt tatctctatt 33660  
catcatcgaa ttttaagagat ccgataaaaca ttgtttgfat tctccagatg tcagcatctg 33720  
atacaacaat atatgtgcac ataaacctct ggcacttatt tcatgtacct tccccttate 33780  
actaaggaga atagtatttg agaaatatgt atacatgata ttatcatgaa ttagatatac 33840  
agaatttgta acactctcga aatcacacga tgtgtcggcg ttaagatcta atatatcact 33900  
cgataacaca ttttcatcta gatcacctag acatttttta aagctaaaat agtctttagt 33960  
agtaacagta actatgcat tattttcatc gatgatacat ttcacggca tattattacg 34020  
cttaccatca aagactatac catgtgtata tctaactgat tctagcatgg ttgccatacg 34080  
cgcattaaac ttttcaggat ctttgatag atcttccaat ctatctattt gagaaaacat 34140  
ttttatcatg ttcaatagtt gaaacgtcgg atccactata tagatattat ctataaagat 34200  
tttaggaact acgttcatgg tatcctggcg aatattaaaa ctatcaatga tatgattatc 34260  
gttttcatct tttatcacca tatagtttct aagatatggg attttactta atataatatt 34320  
atttcccgty ataaatttta ttagaaaggc caaatctata agaaaagtcc tagaattagt 34380  
ctgaagaata tctatatcgc cgtatagtat atttggatta attagatata gagaatatga 34440  
tccgtaacat atacaacttt tattatggcg tctaagatat tcttccatca acttattaac 34500  
atttttgact aggggaagata cattatgacg tccattact tttgccttgt ctattactgc 34560  
gacgttcata gaatttagca tatctcttgc caattcttcc attgatgta cattataaga 34620  
aatttttagat gaaattacat ttggagcttt aatagtaaga actcctaata tgtccgtgta 34680  
tgtggtcact aatacagatt gtagttctat aatcgtaaat aatttaccta tatttatagt 34740  
ttgagtctgt ttgaaaaagt agctaagtat acgatctttt atttctgatg cagatgtatt 34800  
aacatcgga aaaaatcttt ttttattctt ttttactaaa gatacaata tgtctttgtt 34860  
aaaaacagtt attttctgaa tatttctagc ttgtaatttt aacatatgat attcgttcac 34920  
actagggtact ctgcctaaat aggtttctat aatctttaat gtaatatag gaaaagtatt 34980  
ctgatcagga ttctatttca ttttgaggat ttaaaactct gattattgtc taatatgggtc 35040  
tctacgcaaa ctttttcaca gacgataga gtttttgata actcgttttt cttaagaaat 35100  
ataaaactac tgtctccaga gctcgtcta tcttttattt tatctaattc gatacaaaact 35160  
cctgatactg gttcagaaag taattcatta attttcagtc ctttatagaa gatattta 35220  
atagataata caaaatcttc agtttttgat atcgatctga ttgatcctag aactagatat 35280

attaataacg tgcctcattag gcagtttatg gcagcttgat aattagatat agtatattcc 35340  
agttcataatt tattagatac cgcattgccc agattttgat attctatgaa ttccctctgaa 35400  
aataaatcca aaataactag acattctatt ttttgtggat tagtgactc tcttccctct 35460  
atcatgttca ctactggtgt ccacgatgat aaatatctag agggaaatata atatagtcca 35520  
taggatgcca atctagcaat gtcgaataac tgtaatttta ttcttcgctc ttccattatga 35580  
attgattctt gaggtataaa cctaaccaca attatattat tagacttttc gtatgtaatg 35640  
tctttcatgt tataagtttt taatcctgga atagaatcta ttttaatgag gcttttaaac 35700  
gcagagttct ccaacgagtc aaagcataat actctgttgt ttttcttata tacgatgtta 35760  
cgattttctt ctttgaatgg aatagggtttt tgaattagtt tataattaca acataataga 35820  
taaggaagtg tgcaaatagt acgcggaaaa aacataatag ctccctgtt ttcattccatg 35880  
gttttaagta aatgatcact ggcttcttta gtcaatggat attcgaacat taacogtttc 35940  
atcatcattg gacagaatcc atattttetta atgtaaagag tgatcaaatc attgtgttta 36000  
ttgtaccatc ttgttgtaaa tgtgtattcg gttatcggat ctgctccttt ttctattaaa 36060  
gtatcgatgt caatctcgtc taagaattca catatatoga catatttcat ttgtatacac 36120  
ataaccatta ctacgttaga atgtatagga agagatgtaa cgggaacagg gttgtgtgat 36180  
tcgcaacta ttctaataca taattcttct gtttaaacgt cttgcacgta atctattata 36240  
gatgccaaga tatctatata attattttgt aagatgatgt taactatgtg atctatataa 36300  
gtagtgtaat aattcatgta ttttgatata tgttccaaact ctgtctttgt gatgtctagt 36360  
ttcgtaatat ctatagcatc ctcaaaaaat atattcgcac atattcccaa gtcttcagtt 36420  
ctatcttcta aaaaatcttc aacgtatgga atataataat ctattttacc tcttctgata 36480  
tcattaatga tatagttttt gacactatct tctgtcaatt gattcttatt cactatatct 36540  
aagaaacgga tagcgtccct aggcgaact actggcatta atatctctat tatagcttct 36600  
ggacataaatt catctattat accagaatta atgggaacta ttccgtatct atctaacata 36660  
gttttaagaa agtcagaatc taagacttga tgttcatata ttgggtcata catgaaatga 36720  
tctctattga tgatagtgaac tatttcatc tctgaaaatt ggtaactcat tctatataatg 36780  
ctttccttgt tgatgaagga tagaatatac tcaatagaat ttgtaccaac aaactgttct 36840  
cttatgaatc gtatatcatc atctgaaata atcatgtaag gcatacattt aacaattaga 36900  
gacttgtctc ctgttatcaa tatactatct ttgtgataat ttatgtgtga ggcaaatgtg 36960  
tccacgttct ttaattttgt tatagtagat atcaaatcca atggagctac agttcttggc 37020  
ttaaacagat atagtttttc tggacaacat tctacaacat tattataaag gactttgggt 37080  
aaataagtggt gatgaaatcc tattttaatt aatgcatag ccttgtctc gtgcagatat 37140  
ccaaacgctt ttgtgatagt atggcattca ttgtctagaa acgctctacg aatatctgtg 37200  
acagatatca tcttttagaga atatactagt cgcgttaata gtactacaat ttgtattttt 37260  
taatctatct caataaaaaa attaatatgt atgattcaat gtataactaa actactaact 37320  
gttattgata actagaatca gaatctaata atgacgtacc caagaagttt atctactgcc 37380  
aatttagctg cattattttt agcatctcgt ttagattttc catctgcctt atcgaatact 37440  
cttccgtcga tatctacaca ggcataaaat gtaggagagt tactaggccc aactgattca 37500  
atacgaaaag accaatctct cttagtattt tggcagtaact cathtaatac ggtgacaggg 37560  
ttagcatctt tccaatcaat aattttttta gccggaataa catcatcaaa agacttatga 37620  
tcctctctca ttgatttttc gcgggataca tcatctatta tgacgtcagc cataacatca 37680  
gcacccggtc tatccgctc cgttgtcata aaccaacgag gaggaatata gteggagctg 37740  
tacaccatag cactacgttg aagatcgtac agagctttat taacttctcg cttctccata 37800  
ttaagttgtc tagtttagttg tgcagcagta gtccttcga ttccaatggt ttttaatagcc 37860  
tcacacacaa tctctcggtt agaacgttcg tcgatataga ttttagacat ttttagagag 37920  
aactaacaca accagcaata aaactgaacc tactttatca tttttttatt catcatctc 37980  
tgggtggtcg tcgttccat caaatgtagc tctgattaac cegtcatcta taggtgatgc 38040  
tggttctgga gattctggag gagatggatt attatctgga agaactctcg ttatttctct 38100  
gttttcatgt atcgattgag ttgtaacatt aagattgcga aatgctctaa atttgggagg 38160  
cttaagtggt tgttgcaat ctctacacgc gtgtctaact agtggagggt cgtcagctgc 38220  
tctagtttga atcatcatcg gcgtagtatt cctactttta cagttaggac acggtgtatt 38280  
gtatttctcg tcgagaacgt taaaataatc gttgtaactc acatccttta ttttatctat 38340  
attgtattct actcctttct taatgcattt tacaacgaat aagagatagc gaagaaattc 38400  
tttttcgggt ccgctagtag ccttaatcat atcaataggt gttttatatt ccaaatttgt 38460  
ggcaatagac ggtttatttc tatacगत tagttgttctg gaatccttgc agtattctat 38520  
accaatatta ttctttgatt cgaatttagt ttcttcgata ttagattttg tattacctat 38580

attcttggatg tagtactttg atgatttttc catggcccat tctattaagt cttccaagtt 38640  
ggcatcatcc acatattgtg atagtaattc tcggatatca gtagcggcta ccgccattga 38700  
tgtttgttca ttggatgagt aactactaat gtatacattt tccatttata acatttattg 38760  
attaactttg ttcatTTtata ttttttcatt attatgttga tattaacaaa agtgaatata 38820  
tatatatgtt aataattgta ttgtgggttat acggctacaa ttttataatg agtgaaagtc 38880  
agtgtccgat gatcaatgac gatagcttta ctctgaaaag aaagtatcaa atcgatagtg 38940  
cggagtcaac aataaaaaatg gataagaaga ggataaaagt tcagaataga gccaaaatgg 39000  
taaaagaaat aaatcagaca ataagagcag cacaaactca ttacgagaca ttgaaactag 39060  
gatacataaa atttaagaga atgattagga ctactactct agaagatata gcaccatcta 39120  
ttccaaataa tcagaaaact tataaactat tctcggacat ttcagccatc ggcaaagcat 39180  
cacagaatcc gagtaagatg gtatatgctc tgctgtctta catgtttccc aatttgtttg 39240  
gagatgatca tagattcatt cgttatagaa tgcattccaat gagtaaaaatc aaacacaaga 39300  
tcttctctcc tttcaaaactt aatcttatta gaattattag ggaagaaaga ttctataata 39360  
atgaatgcag atctaataaa tggaaaataa ttggaacaca agttgataaa atgttgatag 39420  
ctgaatctga taaatataca atagatgcaa ggtataacct aaaacccatg tatagaatca 39480  
agggagaatc tgaagaagat accctcttca tcaaacagat ggtagaacaa tgtgtgacat 39540  
cccaggaatt ggtggaaaaa gtgttgaaga tactgttttag agatttgttc aagagtggag 39600  
aatacaaagc gtacagatac gatgatgatg tagaaaatgg atttattgga ttggatacac 39660  
taaaattaaa cattgttcat gatatagttg aaccatgtat gcctgttcgt aggccagtgg 39720  
ctaagatact gtgtaaagaa atggtaaata aatactttga gaatccgcta catattattg 39780  
gtaagaatct tcaagagtgc attgactttg ttagtgaata ggcatttcat ctttctccaa 39840  
tactaattca aattgttaaa ttaataatgg atagtataaa tagtaaaaaa aattattaga 39900  
ataagagtgt agtatcatag ataactctct tctataaaaa tggattttat tctagaaaag 39960  
tatcttatat acacagtaga aaataatata gatttttttaa aggatgatac attaaagtaa 40020  
gtaaacaaat ttacctcoa tcatgtacta gctctcaagt atctagttag caattttcct 40080  
caacacgta ttactaagga tgtattagct aataccaatt tttttgtttt catacatatg 40140  
gtacgatgtt gtaaagtgtg cgaagcgggt ttacgacacg catttgatgc acccagttg 40200  
tacgttaaag cattgactaa gaattattta tegttagta acgcaataca atcgtaaaag 40260  
gaaaccgtgc ataaactaac acaagatgaa aaatttttag aggttgccga atacatggac 40320  
gaattaggag aacttatagg cgtaaattat gacttagttc ttaatccatt atttcacgga 40380  
ggggaaccca tcaaagatat ggaaatcatt tttttaaaac tgtttaagaa aacagacttc 40440  
aaagtgtgta aaaaattaa gtttataaga ttacttattt gggcatacct aagcaagaaa 40500  
gatacaggga tagagtttgc ggataatgat agacaagata tatatactct atttcaacaa 40560  
actggtagaa tgcctcatag caatctaaca gaaacgttta gagattatat ctttcccgga 40620  
gataagacta cgtattgggt gtggttaaac gaaagtatag ctaatgatgc ggatacgtt 40680  
cttaatagac cgcgccattac catgtatgat aaaattctta gttatatata ctctgagata 40740  
aaacaaggac gcgttaataa aaacatgctt aagttagttt atatctttga gcctgaaaaa 40800  
gatatacagag aacttctgct agaaatcata tatgatattc ctggagatat cctatctatt 40860  
attgatgcaa aaaacgacga ttggaaaaaa tattttatta gtttttataa agctaatttt 40920  
attaacggta atacatttat tagtgataga acgtttaacg aggacttatt cagagtgtgt 40980  
gttcaaatag atcccgaaata tttcgataat gaacgaatta tgtctttatt ctctacgagt 41040  
gctgcggaaca ttaaacgatt tgatgagtta gatattata acagttatat atctaataa 41100  
atttatggag tgaacgatat cacattagat acaatggatg atatgaagaa gtgtcaaatc 41160  
tttaacgagg atacgtcgta ttatgttaag gaatacaata catacctgtt tttgcacgag 41220  
tcggatccca tggatcataga gaacggaata ctaaagaaac tgtcatctat aaaatccaag 41280  
agtagacggc tgaacttggt tagcaaaaac attttaaaat attattttaga cggacaattg 41340  
gctcgtctag gtcttgtgtt agatgattat aaaggagact tgttagttaa aatgataaac 41400  
catcttaagt ctgtggagga tgtatccgca ttcgttcgat tttctacaga taaaaaccct 41460  
agtattcttc catcgctaatt caaaactatt tttagctagtt ataataattc catcatcgtc 41520  
ttatttcaaa ggtttttgag agataatcta tatcatgtag aagaattctt ggataaaagc 41580  
atocattcaa ccaagacgga taagaaatat atacttcaat tgataagaca cggtagatca 41640  
tagaacagac caaatatatt attaatatatt tgbatataca tagatataat tatcacacat 41700  
ttttgataaa tgggaactgc tgcaacaatt cagactccca ccaaattaat gaataaagaa 41760  
aatgcagaaa tgattttgga aaaaattgtt gatcatatag ttatgtatat tagtgacgaa 41820  
tcaagtgatt cagaaaataa tcttgaatat attgattttc gtaacagata cgaagactat 41880

agatctctca ttataaaaaag tgatcacgag tttgtaaagc tatgtaaaaa teatgcggag 41940  
aaaagttctc cagaaaacgca acaaatgatt atcaaacaca tatacgaaca atatcttatt 42000  
ccagtatctg aagtactatt aaaacctata atgtccatgg gtgacataat tacatataac 42060  
ggatgtaaag acaatgaatg gatgctagaa caactctota ccctaaactt taacaatctc 42120  
cgcacatgga actcatgtag cataggcaat gtaacgcgtc tgtttttatc attttttagt 42180  
tatctgatga aagataaaact aaatatataa gtataatccc attctaatac tttaacctga 42240  
tgtattagca tcttattaga atattaacct aactaaaaga cataacataa aaactcatta 42300  
catagttgat aaaaagcggg aggatataaa tattatggct gccaccgttc cgcgttttga 42360  
cgacgtgtac aaaaatgcac aaagaagaat tctagatcaa gaaacatttt ttagtagagg 42420  
tctaagtaga ccgttaatga aaaacacata tctatttgat aattacgcgt atggatggat 42480  
accagaaact gcaatttggg gtagtagata cgcaaaactt gatgcaagt actattatcc 42540  
catttcggtg ggattactta aaaagttcga gtttctcatg tctctatata aaggctctat 42600  
tccagtatac gaagaaaaag taaatactga attcattgct aatggatctt tctccggtag 42660  
atacgtatca tatcttagaa agttttctgc tcttccaaca aacgagttta ttagtttttt 42720  
gttactgact tccattccaa tctataatat cttgttctgg tttaaaaata ctcagtttga 42780  
tattactaaa cacacattat tcagatacgt ctatacagat aatgccaaac acctggcggt 42840  
ggctaggtat atgtatacaa caggagacta taagcctttg tttagtcgtc tcaaagagaa 42900  
ttatatattt accggtcccg ttccaatatg tatcaaagat atagatcacc ctaatcttag 42960  
tagagcaaga agtccatccg attatgagac attagctaata attagtacta tattgtactt 43020  
taccaagtat gatccggtat taatgttttt attgttttac gtacctgggt attcaattac 43080  
tacaaaaatt actccagccg tagaatatct aatggataaa ctgaatctaa caaagagcga 43140  
cgtacaactg ttgtaaatta ttttatgctt cgtaaaatgt aggttttgaa ccaaacattc 43200  
tttcaaagaa tgagatgcat aaaactttat tatccaatag attgactatt tcggacgtca 43260  
atcggtttaa gttaaacttcg taaaatattc tttgatcact gccgagttta aaacttctat 43320  
cgataattgt ttcataatgt ttaatattta caagtttttt ggtccatggt ccattaggac 43380  
aaatatatgc aaaataatat cgttctccaa gttctatagt ctctggatta tttttattat 43440  
attcagtaac caaataacata ttaggggtat ctgcggattt ataatttgag tgatgcattc 43500  
gactcaacat aaataattct agaggagacg atctactatc aaattcggat cgtaaatctg 43560  
tttctaaaga acggagaata tctatacata cctgattaga attcatccgt ccttcagaca 43620  
acatctcaga cagtctgggt ttgtacatct taatcatatt cttatgaaac ttggaacat 43680  
ctcttctagt ttcactagta cettatttaa ttctctcagg tacagatttt gaattcgacg 43740  
atgctgagta tttcatcggt gtatattttt tcttcgattg cataatcaga ttcttatata 43800  
ccgcctcaaa ctctatttta aaattattaa acaatactct attattaatc agtcgttcta 43860  
actctttcgc tattttctata gacttatcta catcttgact gtctatctct gtaaacacgg 43920  
agtcggtatc tccatacacg ctacgaaaac gaaatctgta atctataggc aacgatgttt 43980  
tcacaatcgg attaatatct ctatcgtcca tataaaatgg attacttaat ggattggcaa 44040  
accgtaacat accgttagat aactctgctc catttagtac cgattctaga tacaagatca 44100  
ttctacgtcc tatggatgtg caactcttag ccgaagcgta tgagtataga gcactatttc 44160  
taaattcccat cagaccatat actgagttgg ctactatctt gtacgtatat tgcattggaat 44220  
catagatggc cttttcagtt gaactggtag cctgttttag catcttttta tatctggctc 44280  
tctctgccaa aaatgttctt aatagctag gaatgggtcc ttctatcgat ctatcgaaaa 44340  
ttgctatttc agagatgagg ttccgtagtc taggttcaca atgaaccgta atatatctag 44400  
gagggtgata tttctgaagc aatagctgat tatttatttc ttcttccaat ctattgggtac 44460  
taacaacgac accgactaat gtttccggag atagatttcc aaagatacac acattaggat 44520  
acagactgtt ataatacaag attaatatct tattactaaa cattttttgt tttggagcaa 44580  
ataccttacc gccttcataa ggaaactttt gttttgtttc tgatctaact aagatagttt 44640  
tagtttccaa caatagcttt aacagtgga ccttgatgac tgtactcgt ctatattcga 44700  
ataccatgga ttgaggaagc acatatgttg acgcaaccgc gtctgttttt gtttctactc 44760  
cataatactc ccacaaatc tgacacaaac aagcatcatg aatacagtat ctagccatat 44820  
ctaaagctat gttttagatta taatccttat acatctgagc taaatcaacg tcatcctttc 44880  
cgaaagataa ttttatgtga tcattaggta aagtaggaca taatagtacg actttaaatc 44940  
cattttccca aatatcttta cgaattactt tacatataat atcctcatca acagtccat 45000  
aattacctgt ggttaaaacc tttgcaaagc cagcggcttt gcctttcgcg tctgtagtat 45060  
cgtcacogat gaacgtcatt tctctaactc ctctatttaa tactttacc atgcaactga 45120  
accggttctt ggatatagaa tccaatttgt acgaatccaa tttttcaaat ttttgaatga 45180

atgaatatag atcgaaaaat atagttccat tattgttatt aacgtgaaac gtagtattgg 45240  
ccatgccgcc tactccctta tgactagact gatttctctc ataaatacag agatgtacag 45300  
cttccttttt gtccggagat ctaaagataa ttttctctcc tgttaataac tctagacgat 45360  
tagtaataata tctcagatca aagttatgtc cgttaaagggt aacgacgtag tctgaacgtta 45420  
gttccaacaa ttgttttagct attcgtaaca aaactatttc agaacataga actagttctc 45480  
gttcgtaatc catttccatt agtgactgta tctcacaaca tctctctatcg acggcttctt 45540  
gtatttctctg ttccgttaac atctcttcat taatgagcgt aaacaataat cgtttaccac 45600  
ttaaatcgat ataacagtaa cttgtatgcg agattgggtt aataaataca gaaggaaact 45660  
tcttatcgaa gtgacactct atatctagaa ataagtaga tcttgggata tctgaatctag 45720  
gtattttttt agcgaaacag ttacgtggat cgtcacaatg ataacatcca ttgttaatct 45780  
ttgtcaataa ttgtctgtcc aacgagtaac atccgtctgg agatatcccg tttagaatat 45840  
aaaaccaact aatattgaga aattcatcca tgggtggcatt ttgtatgctg cgtttctttg 45900  
getcttctat caaccacata tctgacgacgg agcattttct atctttaata tctagattat 45960  
aacttattgt ctctgcaatg tctatagttc tcatctttcc caacggcctc gcattaaatg 46020  
gaggaggaga caatgactga tatatttctg ccgtcactac gtaataaaag taatgaggaa 46080  
atcgataaaa tacgggtctca ccatttccgac atctggattt cagatataaa aatctgtttt 46140  
caccgtgact ttcaaaccaa ttaatgcacc gaacatccat ttatagaatt tagaaatata 46200  
ttttcattta aatgaatccc aaacattggg gaagagccgt atggaccatt atttttatag 46260  
tactttcgca agcgggttta gacggcaaca tagaagcgtg taaacgaaaa ctataacta 46320  
tagttagcac tcttccatgt cctgcatgta gacggcacgc gactatcgct atagaggaca 46380  
ataatgtcat gtctagcgat gatctgaatt atatttatta tttttctatc agattattta 46440  
acaatttggc atctgatccc aaatacgcga tctgatgtgac aaaggttaac cctttataaa 46500  
cttaacccat tataaaactt atgattagtc acaactgaaa taaccgcgtg attatttttt 46560  
ggtataatc tacacggcat ggtttctgtg actatgaatt caaccccggt tacattagtg 46620  
aaatctttta caaacagcaa gggttcgtca aagacataaa actcattgtt tacaatcgaa 46680  
atagaccccc tatcacactt aaaataaaaa atactcttat cctttaccac caaataaaa 46740  
tctgattgggt caatgtgaat gtattcactt aacagttcca caaatttatt tattaactcc 46800  
gaggcacata catcgctcggg attttttatg gcaaaacttta ctcttccagc atccgtttct 46860  
aaaaaaaaat taacgagttc catttatatc atccaatatt attgaaatga cgttgatgga 46920  
cagatgatac aaataagaag gtacggtacc tttgtccacc atctcctcca attcatgctc 46980  
tattttgtca ttaactttaa tgtatgaaaa cagtacgcca catgcttcca tgacagtgtg 47040  
taacactttg gatacaaaat gtttgacatt agtataattg ttcaagactg tcaatctata 47100  
atagatagta gctataatat attctatgat ggtattgaag aagatgacaa ccttggcata 47160  
ttgatcattt aacacagaca tggatcaaac agatagcttg aatgaaagag aatcagtaat 47220  
tggaataaag gtcttctcga tagagtgtcc gtataccaac atgtctgata ttttgatgta 47280  
ttccattaaa ttatttagtt ttttcttttt attctcgtta aacagcattt ctgtcaacgg 47340  
accccaacat cgttgaccga ttaagttttg attgattttt ccgtgtaagg cgtatctagt 47400  
cagatcgtat agcctatcca ataatccatc atctgtgcgt agatcacatc gtacactttt 47460  
taattctcta tagaagagcg acagacatct ggagcaatta cagacagcaa tttctttatt 47520  
ctctacagat gtaagatact tgaagacatt cctatgatga tgcagaattt tggataaac 47580  
ggtattgatg gtatctgtta ccataattcc tttgatggct gatagtgtca gagcacaaga 47640  
tttccaatct ttgacaattt ttagcaccat tatctttgtt ttgatattc tatcacacag 47700  
catggtgcgt ctgacaacac agggattaag acggaagat gaaatgatc tctcaacatc 47760  
ttcaatagat accttgctat tttttctggc attatctata tgtgcgagaa tatcctctag 47820  
agaatcagta tcttttttga tgatagtgga tctcaatgac atgggacgtt taaaccttct 47880  
tattctatca ccagattgca tgggtgatttg tcttctttct tttatcataa tgtaatctct 47940  
aaattcatcg gcaaattgtc tatatctaaa atcataatat gagatgttta cctctacaaa 48000  
tatctgttcg tccaatgtta gagtattttac atcagttttg tattccaaat taaacatggc 48060  
aacggattta attttatatt cctctattaa gtctctctcg ataataacag aatgtagata 48120  
atcatttaat ccactgtaca tgggtggaag atgcttgttg acaaaatctt taattgtctt 48180  
gatgaagggt ggactatctc taacatcttg attaataaaa tttataacat tgtccatagg 48240  
atactttgta actagtttta tacacatctc ttcactcgta agtttagaca gaatctctg 48300  
aacagggtgt atatttatatt catcagatat acgaagaaca atgtccaaat ctatattgtt 48360  
taatataatta tatagatgta gcgtagctcc tacaggaata tctttaacta agtcaatgat 48420  
ttcatcaacc gtttagatcta ttttaaagtt aatcatatag gcattgattt ttaaaaggta 48480

|             |             |             |             |             |             |       |
|-------------|-------------|-------------|-------------|-------------|-------------|-------|
| tgtagccttg  | actacattct  | cattaattaa  | ccattccaag  | tcaactgtgtg | taagaagatt  | 48540 |
| atattctatc  | ataagcttga  | ctacatttgg  | tcccgatacc  | attaaagaat  | tcttatgata  | 48600 |
| taaggaaaca  | gcttttaggt  | actcatctac  | tctacaagaa  | ttttggagag  | ccttaacgat  | 48660 |
| atcagtgacg  | tttattat    | caggaggaaa  | aaaccttaaca | ttgagaatat  | cggaattaat  | 48720 |
| agcttccaga  | tacagtgatt  | ttggcaatag  | tccgtgtaat  | ccataatcca  | gtaacacgag  | 48780 |
| ctgggtgcttg | ctagacacct  | tttcaatggt  | taattttttt  | gaaataagct  | ttgataaagc  | 48840 |
| cttctcgcga  | aattccggat  | acatgaacat  | gtcggcgaca  | tgattaagta  | ttgttttttc  | 48900 |
| attattttct  | caatacccca  | atagatgata  | gaatatcacc  | caatgcgtcc  | atgttgctta  | 48960 |
| tttccaacag  | gtcgttatat  | ccaccaatag  | aagtttttcc  | aaaaaagatt  | ctaggaacag  | 49020 |
| ttctaccacc  | agtaatttgt  | tcaaaatagt  | cacgcaattc  | attttcgggt  | ttaaattctt  | 49080 |
| taatatcgac  | aatttcatac  | gtcctctttt  | tgaactaaa   | cttattttaga | atatccagtg  | 49140 |
| catttctaca  | aaaaggacat  | gtatacttga  | caaaaattgt  | cactttgtta  | ttggccaacc  | 49200 |
| tttggtgtac  | aaattcctcg  | gccattttaa  | tatttaagt   | atataaaact  | atctcgactt  | 49260 |
| atttaactct  | ttagtcgaga  | tatatggacg  | cagatagcta  | tatgatagcc  | aactacagaa  | 49320 |
| ggcaaacgct  | ataaaaaaca  | taattacgac  | gagcatattt  | ataaatattt  | ttattcagca  | 49380 |
| ttacttgata  | tagtaatat   | aggcacagtc  | aaacattcaa  | ccactctcga  | tacattaact  | 49440 |
| ctctcatttt  | ctttaacaaa  | ttctgcaata  | tcttcgtaaa  | aagattcttg  | aaacttttta  | 49500 |
| gaatatctat  | cgactctaga  | tgaatagcg   | ttcgtcaaca  | tactatgttt  | tgtatacata  | 49560 |
| aaggcgccca  | ttttaacagt  | ttctagtac   | aaaatgctag  | cgatcctagg  | atccttttaga | 49620 |
| atcacataga  | ttgacgattc  | gtctctctta  | gtaactctag  | taaaataatc  | atacaatcta  | 49680 |
| gtacgcgaaa  | taattattatc | cttgacttga  | ggagatctaa  | acaatctagt  | tttgagaaca  | 49740 |
| togataagtt  | catcggaat   | gacatacata  | ctatctttaa  | tagaactctt  | ttcatccagt  | 49800 |
| tgaatggatt  | cgctcttaac  | caactgatta  | atgagatctt  | ctattttatc  | attttccaga  | 49860 |
| tgatattgat  | gtccattaaa  | gttaaattgt  | gtagcgcttc  | tttttagtct  | agcagccaat  | 49920 |
| actttaacat  | cactaatatc  | gatatacaaa  | ggagatgatt  | tatctatggt  | attaagaatt  | 49980 |
| cgtttttga   | catccgtcaa  | aaccaattcc  | tttttgctg   | tatcatccag  | ttttccatec  | 50040 |
| tttgtaaaga  | aattattttc  | tactagacta  | tttaataagac | tgataaggat  | tcctccataa  | 50100 |
| ttgcacaatc  | caaacttttt  | cacaaaacta  | gactttacaa  | gatctacagg  | aatgcgtact  | 50160 |
| tcaggttttt  | tagcttggtga | ttttttcttt  | tgcggacatt  | ttcttgtagc  | caactcatct  | 50220 |
| accatttcat  | tgatttttagc | agtgaataaa  | gctttcaatg  | cacgggcact  | gatactattg  | 50280 |
| aaaacgagtt  | gatcttcaaa  | ttccgccatt  | taagctcacc  | aaacaacttt  | taaaatacaaa | 50340 |
| tatttaacat  | gtagtagaat  | aagaactata  | aaaaaaataa  | taattaacca  | ataccaaccc  | 50400 |
| caacaaccgg  | tattattagt  | tgatgtgact  | gtttttctcat | cacttagaac  | agatttaaca  | 50460 |
| attttetataa | agtctgtcaa  | atcatcttcc  | ggagacccca  | taaatacacc  | aaatatagcg  | 50520 |
| gcgtacaact  | tatccattta  | tacattgaat  | attggctttt  | ctttatcgct  | atcttcatca  | 50580 |
| tattcatcat  | caatatcaac  | aagtcccaga  | ttacgagcca  | gatcttcttc  | tacattttca  | 50640 |
| gtcattgata  | cagtttact   | atctccagag  | agtccgataa  | cgttagccac  | cacttctcta  | 50700 |
| tcaatgatta  | gtttcttgag  | cgcgaaagta  | atttttgttt  | ccgttccgga  | tctatagaag  | 50760 |
| acgatagggt  | tgataattgc  | cttgccaat   | tgtctttctc  | ttttactgag  | tgattctagt  | 50820 |
| tcaccttcta  | tagatctgag  | aatggatgat  | tctccagtcg  | aaacatattc  | taccatggat  | 50880 |
| ccgttttaatt | tgttgatgaa  | gatggattca  | tccttaaatg  | ttttctctgt  | aatagtttcc  | 50940 |
| accgaaagac  | tatgcaaaga  | atttggaatg  | cgttccttgt  | gcttaatgtt  | tocatagacg  | 51000 |
| gcttctagaa  | gttgatacaa  | cataggacta  | gccgcggtaa  | cttttatttt  | tagaaagtat  | 51060 |
| ccatcgcttc  | tatcttggtt  | agattttatt  | ttataaagtt  | tagtctctcc  | ttccaacata  | 51120 |
| ataaaagtg   | aagtcatttg  | actagataaa  | ctatcagtaa  | gttttataga  | gatagacgaa  | 51180 |
| caattagcgt  | attgagaagc  | atttagtgta  | acgtattoga  | tacattttgc  | attagattta  | 51240 |
| ctaatoctg   | ttgcatactc  | tataacaccc  | gcacaagtct  | gtagagaatc  | gctagatgca  | 51300 |
| gtagggtctg  | gtgaagtctc  | aactctcttc  | tgattacot   | tactcatgat  | taaacctaaa  | 51360 |
| taattgtact  | ttgtaatata  | atgatataata | ttttcacttt  | atctcatttg  | agaataaaaa  | 51420 |
| tgtttttggt  | taaccactgc  | atgatgtaca  | gatttcggaa  | tcgcaaacca  | ccagtgggtt  | 51480 |
| tattttatcc  | ttgtccaatg  | tgaattgaat  | gggagcggat  | gcgggtttcg  | tacgtagata  | 51540 |
| gtacattccc  | gttttttagac | cgagactcca  | tccgtaaaaa  | tgcatactcg  | ttagtttgga  | 51600 |
| ataactogga  | tctgctatat  | ggatattcat  | agattgactt  | tgatcgatga  | aggctccctt  | 51660 |
| gtctgcagcc  | atttttatga  | tctgtctttg  | tggaaatttc  | caaatagttt  | tataaactcg  | 51720 |
| cttaatatct  | tctggaaggt  | ttgtattctg  | aatggatoca  | ccatctgcca  | taatcctatt  | 51780 |

cttgatctca tcattccata attttctctc ggtaaact ctaaggagat ggggattaac 51840  
tacttgaaat tctccagaca atactctccg agtgtaaata ttactgggat acggttccac 51900  
cgactcatta tttcccaaaa tttgagcagt tgatgcagtc ggcatagggtg ccaccaataa 51960  
actattttcta agaccgtatg ttctgatttt atcttttaga ggttcccaat tccaaagatc 52020  
cgacgggtaca acattccaaa gatcatattg tagaataccg ttactggcgt acgatccctac 52080  
atatgtatcg tatggtcctt ccttctcagc tagttcaca ctccgctcta atgcaccgta 52140  
ataaatgggt tcgaagatct tcttatttag atcttggtgt tccaggctat caaatggata 52200  
atttaagaga ataaacgggt ccgctaattc ttgaacacca ataccgatag gtctatgtct 52260  
cttatttagag atttcagctt ctggaatagg ataataatta atatctataa ttttattgag 52320  
atttctgaca attactttga ccacatcctt cagtttgaga aaatcaaact gccatctat 52380  
tacaacatg ttcaaggcaa cagatgccag attacaaacg gctacctcat tagcatccgc 52440  
atattgtatt atctcagtc aaagattact acacttgata gttcctaaat tttgttgatt 52500  
actctttttg ttacacgcat ccttataaag aatgaatgga gtaccagttt caatctgaga 52560  
ttctataatc gctttccaga cgactcgagc ctttattata gatttgatc tctttctct 52620  
ttcgtatagt gtatacaatc gttcgaactc gtctcccaa acattgtcca atccaggaca 52680  
ttcatccgga cacatcaacg accactctcc gtcatcctc actcgtttca taaagagatc 52740  
aggaatccaa agagctataa atagatctct gggtctatgt tctcgtttc ctgtattctt 52800  
tttaagatcg aggaacgcaa taatatcaga atgcccgggt tccaagtata tggccataac 52860  
tccaggccgt ttgtttcctc cctgatctat gtatctagcg gtgttattat aaactctcaa 52920  
cattggaata ataccgtttg atataccatt ggtaccggag atatagcttc cactggcacg 52980  
aatattacta attgatagac ctattcccc tgccatttta gagattaatg cgcactggtt 53040  
taacgtgtca tagataccct ctatgctatc atcgatcatg ttaagtagaa aacagctaga 53100  
catttggtga cgactagttc ccgcattaaa taaggtagga gaagcgtgcg taaaccattt 53160  
ttcagaaagt agattgtacg tctcaatagc tgagtctata tcccattgat gaattcctac 53220  
tgcgacacgc attaacatgt gctgaggtct ttcaacgacg ttgttggtta ttttcaacaa 53280  
gtaggatttt tccaaagttt taaaaccaa atagttgtat gaaaagtctc gttcgtaaat 53340  
aataaccgag ttgagtttat ccttatattt gtttaactata tccatgggtga tacttgaat 53400  
aatcggagaa tgtttcccat ttttaggatt aacatagttg aataaatcct ccactcctc 53460  
actaaatagt tttttgttt ccttggttag atttgatagc gctattctgg cggctagaat 53520  
ggcataatcc ggatgttggt tagtacaagt ggctgctatt tccgctgcca gagtgtccaa 53580  
ttctacogtt gttactccat tatatattcc ttgaataacc ttcatagcta ttttaatagg 53640  
atctatatga tccgtgttta agccataaca taattttcta ataccgagacg tgattttatc 53700  
aaacatgaca ttttccctgt atccatttcg tttaatgaca aacatttttg ttggtgtaat 53760  
aaaaaatta ttttaacttt cattaatagg gatttgacgt atgtagcgt caaaatgatc 53820  
gttctcgtga tatagataaa gagtccata tatttgaaaa tctgtacggc tctgattaac 53880  
tttaatgatt gcatagtga tatatcatta ggatttaact ccttgactat catggcggcg 53940  
ccagaaatta ccatcaaaag cattaataca gttatgccga tccgagttaa aacgggtata 54000  
gcatccacca tttatatcta aaaattagat caaagaatat gtgacaaagt cctagttgta 54060  
tactgagaat tgacgaaaca atgtttctta catatttttt tcttattagt aactgactta 54120  
atagtaggaa ctggaaagct agacttgatt attctataag tatagataacc cttccagata 54180  
atgttctctt tgataaaaagt tccagaaaat gtagaatttt ttaaaaagtt atcttttgct 54240  
attaccaaga ttgtgttttag acgcttatta ttaatatgag tgatgaaatc cacaccgct 54300  
ctagatatcg cctttatttc cacattagat ggtaaatcca atagtgaac tatcttttta 54360  
ggaatgtatg gactcgcgtt tagaggagtg aacgtcttg gctcggaaa ggatgattcg 54420  
tcaaacgaat aaacaatttc acaaatggat gttaatgtat tagtaggaaa ttttttgacg 54480  
ctagtggaa tgaagattct aatggatgat gttctaccta tttcatccga taacatgtta 54540  
atttccgaca ccaacgggtt taatatttcg atgatatacg gtatgtctct tttcggactt 54600  
atatagctta ttccacaata cgagtcatta tatactccaa aaaacaaaat aactagtata 54660  
aaatctgtat cgaatgggaa aaacgaaatt atcgacatag gtatagaatc cggaacattg 54720  
aacgtattaa tacttaattc tttttctgtg gtaagtaccg ataggttatt gacattgtat 54780  
ggttttaaat attctataac ttgagacttg atagattata gtgatgaatt gaaaattatt 54840  
tttatccaca cgtgtgtttc aggatcatcg tccagcccg attagtattt ttattaatec tttgattatc 54900  
aaataaatat cattaatata tgctctagat attagtattt ttattaatec tttgattatc 54960  
atcttctcgt aggcgaatga ttccatgatc aagagtgaat tgagaadac ctccggagta 55020  
ttaatgggct tagtaaacag tccatcggtg caataataaa agttatccaa gttaaaggat 55080

attatgcatt cgtttaaaga tateacctca tctgacggag acaatttttt ggtaggtttt 55140  
agagactttg aagctacttg titaacaaag ttattcatcg tctgtacta ttctatttaa 55200  
ttttgtagtt aatttatcac atatcacatt aattgacttt ttggtccatt ttccatacag 55260  
tttatattct ttaaatccctg cgttatccgt tccggttata tccagggata gatcttgcaa 55320  
gttaaataga atgctcttaa ataagtcat ttcttatoc gctaaaaatt taaagaatgt 55380  
ataaaccttt ttcagagatt tgaaactctt aggtggtgct ctagtacaca atatcataaa 55440  
caaactaata aacattccac attcagattc caacagctga ttaacttcca cattaatada 55500  
gcctattttc gctccaaatg tacattcgaa aaatctgaat aaaacatcga tgtcacatt 55560  
tgtattatcc aatacagaat gtttgtgatt cgtgttaaaa ccatcggaga aggaatagaa 55620  
ataaaaaatta ttatagtggt ggaattcagt tggaaatttg cctccggagt cataaaagga 55680  
tactaaacat tgttttttat cataaattac acatttccaa tgagacaaat aacaaaatcc 55740  
aaacattaca aatctagagg tagaactttt aattttgtct ttaagtatat acgataagat 55800  
atgtttatct ataaacgcgt caaatttttc atgaatcgt aaggagttta agaattctcat 55860  
gtcaaattgt cctatataat ccacttcgga tccataagca aactgagaga ctaagttctt 55920  
aatacttcga ttgctcatcc aggtcctctc ctccaggctct attttcatct tgacgacct 55980  
tggtttttca ccagtatgta ttcttttacg tgataaatca tggattttca aatccatttg 56040  
tgagaagtct atgccttag atactttttc ccgtagtcga ggtttaaaaa aatacgttaa 56100  
cggataacta gtaggtaact caaagacatc atatatagaa tggtaacgcg tctttaactc 56160  
gtcggttaac tctttctttt gatcgagttc gtcgctacta ttgggtctgc tcagggtgcc 56220  
cgactctact agttccaaca tcataccgat aggaatacaa gacactttgc cagcggttgt 56280  
agatttatca tatttctcca ctacatatcc gttacaattt gttaaaaatt tagatacatc 56340  
tatattgcta cataatccag ctagtgaata tatatgacat aataaattgg taaatcctag 56400  
ttctggtatt ttactaatta ctaaaactgt atatctttcc atttatcatg gaaaagaatt 56460  
taccagatat cttctttttt ccaaaactgcg ttaatgtatt ctcttcaaaa tattcacaag 56520  
atgaattcag taatatgagt aaaacggaac gtgatagttt ctcatggcc gtgtttccag 56580  
ttataaaaca tagatggcat aacgcacacg ttgtaaaaa taaaggaata tacaagtta 56640  
gtacagaagc acgtggaaaa aaagtatctc ctccatcact aggaanaacc gcacacataa 56700  
acctaaccgc gaagcaatat atatacagtg aacacacaaat aagctttgaa tgttatagtt 56760  
ttctaaaaatg tataacaaat acagaaatca attcgttcga tgagtatata ttaagaggac 56820  
tattagaagc tggtaatagt ttacagatat ttccaattc cgtaggtaaa cgaacagata 56880  
ctatagggtg actagggaat aagtatccat ttagcaaaat tccattggcc tcattaactc 56940  
ctaaagcaca acgagagata ttttcagcgt ggattttctca tagacctgta gttttaactg 57000  
gaggaactgg agtgggtaag acgtcacagg taaccaagtt attgctttgg ttttaattatt 57060  
tatttggtgg attctctact ctagataaaa tcaactgactt tcacgaaaga ccagtcattc 57120  
tatctcttcc taggatagct ttagtttagat tgcatagcaa taccatttta aaatcattgg 57180  
gatttaaggt actagatgga tctcctattt ctttacggta cggatctata ccggaagaat 57240  
taataaaca acaaccaaaa aaatatgga ttgtattttc taccataag ttatctctaa 57300  
caaaactatt tagttatggc actcttatta tagacgaagt tcatgagcat gatcaaatag 57360  
gagatattat tatagcagta gcgagaaagc atcatacgaa aatagattct atgtttttaa 57420  
tgactgccac gtttagaggat gacagggaaac ggctaaaagt atttttacct aatcccgcat 57480  
ttatacatat tcttgagat acactgttta aaattagcga ggtatttatt cataataaga 57540  
taaatccatc tccagaatg gcatacatag aagaagaaaa gagaaattta gttactgcta 57600  
tacagatgta tactcctcct gatggatcat ccggtatagt ctttgtggca tccgttgca 57660  
agtgtcacga atataaatca tatttagaaa aaagattacc gtatgatatg tatattattc 57720  
atggtaaggt cttagatata gacgaaatat tagaaaaagt gtattcatca cetaatgtat 57780  
cgataattat ttctactcct tatttggaaat ccagcgttac tatacgcaat gttacacaca 57840  
tttatgatat gggtagagtt ttgtccccg ctctttttgg aggatcgcaa caatttattt 57900  
ctaaatctat gagagatcaa cgaaaaggaa gtagtaggaag agttaatcct ggtacatacg 57960  
tctatttcta tgatctgtct tatatgaagt ctatacagcg aatagattca gaatttctac 58020  
ataattatat attgtacgct aataagttta atctaact cccogaagat ttgtttataa 58080  
tcctacaaa tttggatatt ctatggcgta caaaggaata tatagactcg ttcgatatta 58140  
gtacagaaac atggaataaa ttattatcca attattatat gaagatgata gagtatgcta 58200  
aactttatgt actaagtcct attctcggtg aggagttgga taactttgag aggacgggag 58260  
aattaactag tattgtacga gaagccattt tatctctaaa tttacaaatt aagattttaa 58320  
attttaaaca taaagatgat gatacgtata tacacttttg taaaatatta ttcgggtgtc 58380

|             |             |            |             |             |             |       |
|-------------|-------------|------------|-------------|-------------|-------------|-------|
| ataacggaac  | aaacgctact  | atatattatc | atagacctct  | aacgggatat  | atgaatatga  | 58440 |
| tttcagatac  | tatatattgtt | cctgtagata | ataactaaaa  | atcaaactct  | aatgaccaca  | 58500 |
| tcttttttta  | gagatgaaaa  | attttccaca | tctccttttg  | tagacacgac  | taaacatttt  | 58560 |
| gcagaaaaaa  | gtttattagt  | gtttagataa | tcgtatactt  | catcagtgtg  | gatagtaaat  | 58620 |
| gtgaacagat  | aaaaggtatt  | cttgctcaat | agattggtaa  | attccataga  | atatattaat  | 58680 |
| cctttcttct  | tgagatccca  | catcatttca | accagagacg  | ttttatccaa  | tgattttacct | 58740 |
| cgtactatac  | cacatacaaa  | actagatttt | gcagtgcagt  | cgtacctggg  | attcctacca  | 58800 |
| aacaaaattt  | tacttttagt  | tcttttagaa | aattctaagg  | tagaatctct  | atttgccaat  | 58860 |
| atgtcatcta  | tggaattacc  | actagcaaaa | aatgatagaa  | atataatattg | atacatcgca  | 58920 |
| gctgggtttg  | atctactata  | ctttaaaaac | gaatcagatt  | ccataattgc  | ctgtatatca  | 58980 |
| tcagctgaaa  | aactatgttt  | tacacgtatt | ccttcggcat  | ttctttttaa  | tgatatactt  | 59040 |
| tgtttagaca  | atgataaagt  | tatcatgtcc | atgagagacg  | cgtctccgta  | tcgtataaat  | 59100 |
| atttcattag  | atgtagacg   | cttcattagg | ggtatacttc  | tataagggtt  | cttaatcagt  | 59160 |
| ccatcattgg  | ttgctcaag   | aactactatc | ggatgttgtt  | gggtatctct  | agtgttacac  | 59220 |
| atggccttac  | taaagtttgg  | gtaaataact | atgatatctc  | tattaattat  | agatgcata   | 59280 |
| atttcattcg  | tcaaggatat  | tagtatcgac | ttgctatcgt  | cattaatacg  | tgtaatgtaa  | 59340 |
| tcataataat  | catgcgatag  | ccaaggaaaa | tttaaataga  | tgttcatcat  | ataatcgctg  | 59400 |
| ctataattca  | tattaatcac  | ttgacattga | ctaatttgta  | atatagcctc  | gccacgaaga  | 59460 |
| aagctctcgt  | attcagtttc  | atcgataaag | gataccgtta  | aatataactg  | gttgccgata  | 59520 |
| gtctcatagt  | ctattaagtg  | gtaagtttcg | tacaaataca  | gaatccctaa  | aatattatct  | 59580 |
| aatgttggat  | taatctttac  | cataactgta | taaaatggag  | acggagtcac  | aactatttta  | 59640 |
| ccgtttgtac  | ttactggaat  | agatgaagga | ataatctccg  | gacatgctgg  | taaagacca   | 59700 |
| aatgtctgtt  | tgaagaaatc  | caatgttcca | ggtccctaac  | tcttaacaaa  | aattacgata  | 59760 |
| ttcgatcccg  | atatectttg  | cattctattt | accagcatat  | cacgaactat  | attaagatta  | 59820 |
| tctatcatgt  | ctattctccc  | accgtttatc | aaatcgctc   | cgctaagaaa  | cgtagtata   | 59880 |
| tccatacaat  | ggaatacttc  | atttctaata | tagtattcgt  | tttctaattc  | tttaatttga  | 59940 |
| aatcgtatac  | tagaaaggga  | aaaattatct | ttgagttttc  | cgtagaaaa   | gaaccacgaa  | 60000 |
| actaatgttc  | tgattgcgtc  | cgattccgtt | gctgaattaa  | tggaattaca  | ccaaaaactc  | 60060 |
| atataacttc  | tagatgtaga  | agcattegct | aaaaaattag  | tagaatcaaa  | ggatataagt  | 60120 |
| agatgttcca  | acaagtgagc  | aattcccaag | atttcatcta  | tatcattctc  | gaatccgaaa  | 60180 |
| ttagaaatto  | ccaagtagat  | atcctttttc | atccgatcgt  | tgatgaaaat  | acgaacttta  | 60240 |
| ttcggtaaaga | caatcattta  | ctaaggagta | aaataggaag  | taatgttcgt  | atgtcgttat  | 60300 |
| catcgtataa  | attaaagggtg | tgttttttac | cattaaagtga | cattataatt  | ttaccaatat  | 60360 |
| tggaattata  | atatagggtg  | atttgcgcac | tcgcgacggg  | tgatgcacgc  | gtaaatatag  | 60420 |
| ctgtatctaa  | tgttctagtc  | ggtatttcat | catttcgctg  | totaataata  | gcgttttctc  | 60480 |
| tatctgtttc  | cattacagct  | gctgaagtt  | tattggtcgg  | ataatatgta  | aaataataag  | 60540 |
| aaatacatat  | gaataacaaa  | aataaaataa | gatataataa  | agatgccatt  | tagagatcta  | 60600 |
| attttgttta  | acttgtccaa  | attcctactt | acagaagatg  | aggaatcggt  | ggagatagtg  | 60660 |
| tcttccttat  | gtagaggatt  | tgaaatatct | tataatgact  | tgataactta  | ctttccagat  | 60720 |
| aggaaatacc  | ataaatatat  | ttataaagta | tttgaacatg  | tagattttatc | ggaggaatta  | 60780 |
| agtatggaat  | tccatgatac  | aactctgaga | gatttagtct  | atcttagatt  | gtacaagtat  | 60840 |
| tccaagtgtg  | tacggccgtg  | ttataaatta | ggagataatc  | taaaaggcat  | agttgttata  | 60900 |
| aaggacagga  | atatttatat  | tagggaagca | aatgatgact  | tgatagaata  | tctcctcaag  | 60960 |
| gaatacactc  | ctcagattta  | tacatattct | aatgagcgcg  | tccccataac  | tggttcaaaa  | 61020 |
| tttaattcttt | gtggattttc  | tcaagttaca | tttatggcgt  | atacaacgct  | gcatataaca  | 61080 |
| acaaataaaa  | aggtagatgt  | tctcgtttcc | aaaaaatgta  | tagatgaact  | agtcgatcca  | 61140 |
| ataaattatc  | aaatacttca  | aaatttattt | gataaaggaa  | gcggaacaat  | aaacaaaata  | 61200 |
| ctcaggaaga  | tattttatct  | ggtaacaggt | ggccaaactc  | cataggttagc | tttttctatt  | 61260 |
| toggatttta  | gaatttccaa  | attcaccagc | gatttatcgg  | ttttggtgaa  | atccaaggat  | 61320 |
| ttattaatgt  | ccacaaatgc  | catttggttt | gtctgtggat  | tgtatttgaa  | aatggaaacg  | 61380 |
| atgtagttag  | atagatgccc  | tgcgaagttt | cctattaggg  | ttccgcgctt  | cacgtcacc   | 61440 |
| agcatacttg  | aatcaccatc  | ctttaaaaaa | aatgataaga  | tatcaacatg  | gagtatatca  | 61500 |
| tactcggatt  | ttaattcttc  | tactgcatca | ctgacatttt  | cacaaatact  | acaatacggg  | 61560 |
| ttaccgaaaa  | taatcagtac  | gttcttcatt | tatgggtatc  | aaaaacttaa  | aatcgttact  | 61620 |
| gctggaaaa   | aaatcactga  | cgatattaga | tgataattta  | tacaaagtat  | acaatggaat  | 61680 |

atttgtggat acaatgagta tttatatagc cgtcgccaat tgtgtcagaa acttagaaga 61740  
gttaactacg gtattcataa aatacgtaaa cggatgggta aaaaaggag ggcagtgaac 61800  
cctttttatc gatagaggaa gtataaaaat taaacaagac gttagagaca agagacgtaa 61860  
atatttctaaa ttaaccaagg acagaaaaat gttagaatta gaaaagtgt catccgaaat 61920  
acaaaatgtt accggattta tggaagaaga aataaaggca gaaatgcaat taaaaatcga 61980  
taaaactcaca tttcaaatat atttatctga ttctgataac ataaaaatat cattgaatga 62040  
gatactaaca catttcaaca ataatgagaa tgttacatta ttttattgtg atgaacgaga 62100  
cgcagaattc gttatgtgtc tgcaggctaa aacacatttc tctaccacag gagaatggcc 62160  
gttgataata agtaccgatc aggatactat gctattttgca tctgctgata atcatcctaa 62220  
gatgataaaa aacttaactc aactgtttta atttgttccc tcggcagagg ataactattt 62280  
agcaaaaatta accggcgttag tgaatggatg tgatttcttt cctggactct atggggcactc 62340  
tataacacccc accaacttaa acaaaaataca attgtttagt gatttttaca tcgataatat 62400  
agtcactagt ttggcaatta aaaattatta tagaaagact aactctaccg tagacgtgcg 62460  
taatattgtt acgtttataa acgattacgc taatttagac gatgtctact cgtatatttc 62520  
tccttgtcaa tgcactgttc aagaatttat attttccgca ttagatgaaa aatggaatga 62580  
atttaaataca tcttatttag aaagcgtgoc gttaccctgc caattaatgt acgcgttaga 62640  
accacgcaag gagattgatg tttcagaagt taaaacttta tcatcttata tagatttcga 62700  
aaataactaaa tcagatatcg atgttataaa atctatatcc tcgatcttcg gatattcctaa 62760  
cgaaaactgt aacacgatag tattcggcat ctataaggat aatttactac tgagtataaa 62820  
taattcattt tactttaacg atagtctgtt aataaccaat actaaaagt ataataaat 62880  
aaatataggt tactagatta aaaatggtgt tccaactcgt gtgctctaca tgcggtaaaag 62940  
atattttctca cgaacgatat aaattgatta tacgaaaaaa atcattaaag gatgtactcg 63000  
tcagtgtaaa gaacgaatgt tgtagggtta aattatctac acaaatagaa cctcaacgta 63060  
acttaacagt gcaacctcta ttggatataa actaatatgg atccggttaa ttttatcaag 63120  
acatatgcgc ctagagggtc tattatTTTT attaatata ccatgtcatt aacaagtcatt 63180  
ttgaatccat cgatagaaaa acatgtgggt atttattatg gtacgttatt atcggaaacac 63240  
ttggtagttg aatctaccta tagaaaagga gttcgaatag tcccattgga tagttttttt 63300  
gaaggatata ctagtgcaaa agtatacatg tttagaataa ttcaagtat gaaaatagca 63360  
gctgatacgt cattaacttt attgggtatt cgttatggat ttggtcataa tagaatgtat 63420  
tgTTTTaaat tggtagctga ctgttataaa aatgccggta ttgatacatc gtctaaacga 63480  
atattgggca aagatatTTT tctgagccaa aacttcacag acgataatag atggataaag 63540  
atatatgatt ctaataattt aacatttttg caaattgatt accttaaagg gtgagttaat 63600  
atgcataact actcctccgt tgttttttcc ctggttcttt ttcttaacgt tgtttgccat 63660  
cactctcata atgtaaagat attctaaaat ggtaaaacttt tgcatatcgg acgcagaaat 63720  
tggatataaa gttgtaattg tattatttcc cgtcaatgga ctagtcacag ctccatcagt 63780  
tttatatcct ttagagtatt tctcactcgt gtctaacatt cttagacatt ccatgatctg 63840  
tttatcgttg atattggcg gaaagataga ttttttattt ttattatat tactattggc 63900  
aattgtagat ataactctg gtaaatattt ttctaccttt tcaatctctt ctattttcaa 63960  
gccggctata tattctgcta tattgttgc agtatcaata ccttttctgg ctaagaagtc 64020  
atattgtggt ttcactatat cagttttaac tggtagttcc attagccttt cacttctgc 64080  
agaataatca gaaattggtt ctttaccaga aaatccagct actataatag gctcaccgat 64140  
gatcattggc aaaatcctat attgtaccag attaatgaga gcatatttca tttccaataa 64200  
ttctgctagt tcttgagaca ttgatttatt tgatgaatct agttggtct ctagataactc 64260  
taccatttct gccgcataca ataactgtt agataaaaatc agggttatca aagtgttttag 64320  
cgtggctaga atagtgggt tgcattgtatt aaagaatgcg gtagtatgag taaaccgttt 64380  
taacgaatta tatagtctcc agaaatctgt gccgttacat acatgagccg aatgacatcg 64440  
aagattgtcc aatattttta atagctgctc tttgtccatt atttctatat ttgactcgca 64500  
acaattgtag ataccattaa tcaccgatc ctttttcgat gccggacaat agcacaattg 64560  
tttagctttg gactctatgt attcagaatt aatagatata tctctcaata cagattgcac 64620  
tatacatttt gaaactatgt caaaaattgt agaacgacgc tgttctgcag ccatttaact 64680  
ttaaataatt tacaaaaatt taaaatgagc atccgtataa aaatcgataa actgcgcca 64740  
attgtggcat atttttcaga gttcagtgaa gaagtatcta taaatgtaga ctgcagggat 64800  
gagttaatgt atatttttgc cgccttgggc ggtctgttaa acatttgggc cattataact 64860  
ctcagtgc atgtgtttta ccgaggagcc gaaaatattg tgtttaatct tctgtgtcc 64920  
aaggtaaaat cgtgtttgtg tagttttcac aatgatgcca tcatagatat agaacctgat 64980

|             |            |            |             |             |             |       |
|-------------|------------|------------|-------------|-------------|-------------|-------|
| ctggaaaata  | atctagtaaa | actttctagt | tatcatgtag  | taagtgtcga  | ttgtaacaag  | 65040 |
| gaactgatgc  | ctattaggac | agatactact | atttgtctaa  | gtatagatca  | aaagaaatct  | 65100 |
| tacgtgttta  | attttcacaa | gtatgaagaa | aaatgttgtg  | gtagaaccgt  | cattcattaa  | 65160 |
| gtgacattat  | aattttacca | atattggaat | tataatatag  | gtgtatttgc  | gcacttgcca  | 65220 |
| cggttgatgc  | atcggtaaat | atagctgtat | ctaattgttct | agtcgggtatt | tcattcatttc | 65280 |
| gctgtctaat  | aatagcgttt | tctctatctg | tttccattac  | agctgcctga  | agtttattgg  | 65340 |
| tcggataata  | tgtaaaataa | taagaaatac | atacgaataa  | caaaaataaa  | ataagatata  | 65400 |
| ataaagatgc  | catttagaga | tctaattttg | tttaacttgt  | ccaaattcct  | acttacagaa  | 65460 |
| gatgaggaat  | cgttggagat | agtgtcttcc | ttatgtagag  | gatttgaaat  | atcttataat  | 65520 |
| gacttgataa  | cttactttcc | agataggaaa | taccataaat  | atatttataa  | agtatttgaa  | 65580 |
| catgtagatt  | tatcggagga | attaagtatg | gaattccatg  | atacaactct  | gagagattta  | 65640 |
| gtctatctta  | gattgtacaa | gtattccaag | tgtatacggc  | cgtgttataa  | attaggagat  | 65700 |
| aatctaaaag  | gcatagtgtg | tataaaggac | aggaatat    | atattaggga  | agcaaagat   | 65760 |
| gacttgatag  | aatatctcct | caaggaatac | actcctcaga  | tttatacata  | ttctaattgag | 65820 |
| cgcgtcccca  | taactgggtc | aaaattaatt | ctttgtggat  | tttctcaagt  | tacatttatg  | 65880 |
| gcgtatacaa  | cgtcgcatat | aacaacaaat | aaaaaggtag  | atgttctcgt  | ttccaaaaaa  | 65940 |
| tgtatagatg  | aactagtcga | tccaataaat | tatcaaatac  | ttcaaaat    | atttgataaa  | 66000 |
| ggaagcggaa  | caataaacaa | aatactcagg | aagatatatt  | attcggtaac  | aggtggccaa  | 66060 |
| actccatagg  | tagctttttc | tatttcggat | tttagaattt  | ccaaattcac  | cagcgattta  | 66120 |
| tcggttttgg  | tgaatccaa  | ggatttatta | atgtccacaa  | atgccatttg  | ttttgtctgt  | 66180 |
| ggattgtatt  | tgaaaatgga | aacgatgtag | tttagatagat | gcgctgcgaa  | gtttcctatt  | 66240 |
| agggttccgc  | gcttcacgtc | accagcata  | cttgaatcac  | catcctttaa  | aaaaaatgat  | 66300 |
| aagatatcaa  | catggagtat | atcatactcg | gattttaatt  | cttctactgc  | atcactgaca  | 66360 |
| ttttcacaaa  | tactacaata | cggtttaccg | aaaataatca  | gtacgttctt  | catttatggg  | 66420 |
| tatcaaaaac  | ttaaaatcgt | tactgctgga | aaataaatca  | ctgacgatat  | tagatgataa  | 66480 |
| tttatacaaa  | gtatacaatg | gaatatttgt | ggatacaatg  | agtatttata  | tagcgcgtcg  | 66540 |
| caattgtgtc  | agaaacttag | aagagttaac | tacgggtattc | ataaaaatag  | taaacgggatg | 66600 |
| ggtaaaaaag  | ggagggcatg | taaccctttt | tatcgataga  | ggaagtataa  | aaattaaaca  | 66660 |
| agacgttaga  | gacaagagac | gtaaatattc | taaattaacc  | aaggacagaa  | aaatgctaga  | 66720 |
| attagaaaag  | tgtacatccg | aaatacaaaa | tgttaccgga  | tttatggaa   | aagaaataaa  | 66780 |
| ggcagaaatg  | caattaaaaa | tcgataaact | cacatttcaa  | atatatttat  | ctgattctga  | 66840 |
| taacataaaa  | atatcattga | atgagatact | aacacatttc  | aacaataatg  | agaatgttac  | 66900 |
| attattttat  | tgtgatgaac | gagacgcaga | attcgttatg  | tgtctcgagg  | ctaaaacaca  | 66960 |
| tttctctacc  | acaggagaat | ggcgttgat  | aataagtacc  | gatcaggata  | ctatgctatt  | 67020 |
| tgcattctgt  | gataatcatc | ctaagatgat | aaaaaactta  | actcaactgt  | ttaaatatgt  | 67080 |
| tcattctgca  | gaggataact | atttagcaaa | attaacggcg  | ttagtgaatg  | gatgtgattt  | 67140 |
| ctttctggga  | ctctatgggg | catctataac | accaccacac  | ttaaacaaaa  | tacaattggt  | 67200 |
| tagtgatttt  | acaatcgata | atatagtcac | tagtttgcca  | attaaaaatt  | attatagaaa  | 67260 |
| gactaactct  | accgtagacg | tgcgtaatat | tggtacgttt  | ataaacgatt  | acgctaattt  | 67320 |
| agacgatgtc  | tactcgtatg | ttcctccttg | tcaatgcact  | gttcaagaat  | ttatatattt  | 67380 |
| cgcattagat  | gaaaaatgga | atgaatttaa | atcatcttat  | ttagagaccg  | tgcggttacc  | 67440 |
| ctgtcaatta  | atgtacgcgt | tagaaccacg | taaggagatt  | gatgtttcag  | aagttaaaac  | 67500 |
| tttatcatct  | tatatagatt | tcgaaaatac | taaatacagat | atcgatgtta  | taaaatctat  | 67560 |
| atcctcgatc  | ttcggatatt | ctaacgaaaa | ctgtaacacg  | atagtattcg  | gcattctataa | 67620 |
| ggataattta  | ctactgagta | taaataattc | attttacttt  | aacgatagtc  | tgtaataaac  | 67680 |
| caataactaaa | agtgataata | taataaatat | aggttactag  | attaaaaatg  | gtgttccaac  | 67740 |
| tcgtgtgctc  | tacatgcggt | aaagatat   | ctcaccgaacg | atataaattg  | attatacgaa  | 67800 |
| aaaaatcatt  | aaaggatgta | ctcgtcagtg | taaagaacga  | atgttgtagg  | ttaaaattat  | 67860 |
| ctacacaaat  | agaacctcaa | cgtaacttaa | cagtgcaccc  | tctattggat  | ataaactaat  | 67920 |
| atggatccgg  | ttaattttat | caagacatat | gcgcctagag  | gttctattat  | ttttattaat  | 67980 |
| tataccatgt  | cattaacaag | tcatttgaat | ccatcgatag  | aaaaacatgt  | gggtatttat  | 68040 |
| tatggtaact  | tattatcgga | acacttggtg | gttgaatcta  | cctatagaaa  | aggagtccga  | 68100 |
| atagtcctat  | tggaatgttt | ttttgaagga | tatcttagtg  | caaaagtata  | catgttagag  | 68160 |
| aatattcaag  | ttatgaaaat | agcagctgat | acgtcattaa  | ctttattggg  | tattccgtat  | 68220 |
| ggatttgggc  | ataatagaat | gtattgtttt | aaattggtag  | ctgactgtta  | taaaaatgcc  | 68280 |

ggatttgata catcgtctaa acgaatattg ggcaaagata tttttctgag ccaaaacttc 68340  
acagacgata atagatggat aaagatatat gattctaata atttaacatt ttggcaaat 68400  
gattacctta aagggtgagt taatatgcat aactactcct ccgttggttt tccctcgtt 68460  
ctttttctta acgttggttg ccatcactct cataatgtaa agatattcta aaatggtaaa 68520  
cttttgcata tcggacgcag aaattggtat aaatgttgta attgtattat ttccatatta 68580  
ttatgaagac tcttggtaat actgatggcg ttttccaggg aatattctat gactgaatgt 68640  
tctcaagaac tacaaaagtt ttctttcaaa atagctatct cgtctctcaa caaactacga 68700  
ggattcaaaa agagagtcaa tgtttttgaa actagaatcg taatggataa tgacgataac 68760  
attttaggaa tgttgttttc ggatagagtt caatccttta agatcaacat ctttatggcg 68820  
tttttagatt aatactttca atgagataaa tatgggtggc ggagttaagt ttgagctccc 68880  
taaacgggat ccgcaccogg gagtaccac tgatgagatg ttattaaacg tggataaaat 68940  
gcatgacgtg atagctcccg ctaagctttt agaatatgtg cataaggac cactagcaaa 69000  
agataaagag gataaagtaa agaaaagata tccagagttt agattagtca acacaggacc 69060  
cgggtggtctt tcggcattgt taagacaatc gtataatgga accgcacca attgctgtcg 69120  
cacttttaat cgtactcatt attggaagaa ggatggaaag atatcagata agtatgaaga 69180  
gggtgcagta ttagaatcgt gttggccaga cgttcacgac actggaaaat gcgatgttga 69240  
ttttatcgac tgggtgcagg gggatagctt cgatagaaac atatgccatc agtggatcgg 69300  
ttcagccttt aataggagta atagaactgt agaggggtcaa caatcgttaa taaatctgta 69360  
taataagatg caaacattat gtagtaaaga tgtagtgta ccaatatgcg aatcattttt 69420  
gcattattta cgcgcacaca atacagaaga tagcaaagag atgatcgatt atattctaag 69480  
acaacagtct gcggacttta aacagaaata tatgagatgt agttatcca ctagagataa 69540  
gttagaagag tcattaaaat atgcggaacc tcgagaatgt tgggatccag agtggtcgaa 69600  
tgccaatgtt aatttcttac taacacgtaa ttataataat ttaggacttt gcaatattgt 69660  
acgatgtaat accagcgtga acaacttaca gatggataaa acttctcat taagattgtc 69720  
atgtggatta agcaatagtg atagattttc tactgttccc gtcaatagag caaaagtagt 69780  
tcaacataat attaaacact cgttcgacct aaaattgcat ttgatcagtt tattatctct 69840  
cttggttaata tggatactaa ttgtagctat ttaaatgggt gccgcggcaa gcatacagac 69900  
gacgggtgaat acactcagcg aacgtatctc gtctaaatta gaacaagaag cgaacgctag 69960  
tgctcaaaaca aaatgtgata tagaaatcgg aaatttttat atccgacaaa accatggatg 70020  
taacctoact gttaaaaata tgtgctctgc ggacgcggat gctcagttgg atgctgtgtt 70080  
atcagccgct acagaaacat atagtggatt aacaccggaa caaaaagcat acgtgccagc 70140  
tatgtttact gctgcgttaa acattcagac actgttgta gagattttga 70200  
aaattatgtg aaacagactt gtaattctag cgcggtcgtc gataacaaat taaagataca 70260  
aaacgtaatc atagatgaat gttacggagc cccaggatct ccaacaaatt tggaaattat 70320  
taatacagga tctagcaaag gaaattgtgc cattaaagcg ttgatgcaat tgacgactaa 70380  
ggccactact caaatagcac ctagacaagt tgctggtaca ggagtccagt tttatatgat 70440  
tgttatcggg gttataatat tggcagcgtt gtttatgtac tatgccagc gtatgttggt 70500  
cacatccacc aatgataaaa tcaaacttat tttagccaat aaggaaaacg tccattggac 70560  
tacttacatg gacacattct ttagaacttc tccgatgggt attgctacca cggatatgca 70620  
aaactgaaaa tatattgata atattttaat agattaacat ggaagttatc gctgategtc 70680  
tagacgatat agtgaacaa aatatagcgg atgaaaatt tgtagatttt gttatacacg 70740  
gtctagagca tcaatgtcct gctatacttc gaccattaat taggttggtt attgatatac 70800  
tattatttgt tatagtaatt tatattttta cggtaactgt agtaagtaga aattatcaaa 70860  
tggtgttggt ggtgctagtc atcacattaa ctatttttta ttactttata ctataatagt 70920  
actagactga cttctaaca acatctcacc tgccataaat aaatgcttga tattaagtc 70980  
ttctattttc aacactattc catctgtgga aaataatact ctgacattat cgctaattga 71040  
cacatcggtg agtgatatgc ctataaagta ataactttct ttgggcacat ataccagtgt 71100  
accaggttct aacaacctat ttactggtgc tctgttagca tactttttct ttaccttgag 71160  
aatatccatc gtttgcctgg tcaatagcga tatgtgattt tttatcaacc actcaaaaaa 71220  
gtaattggag ttttcataac ctctacgggc tattgtctca tggcctgtga tgaaatttaa 71280  
gtaacacgac tgtggtagat ttgtttctata gagccgattg ccgcaaatag atagaactac 71340  
caatatgtct gtacaaatgt taaacattaa ttgattaaca gaaaaaaca tgttcgttct 71400  
gggaatagaa accagatcaa aacaaaattc gttagaatat atgccacgtt tatacatgga 71460  
atataaaata actacagttt gaaaaataac agtatcattt aaacatttaa cttgcggggt 71520  
taatctcaca actttactgt ttttgaactg ttcaaaatat agcatcgatc cgtgagaaat 71580

|             |             |              |             |             |             |       |
|-------------|-------------|--------------|-------------|-------------|-------------|-------|
| acgttttagcc | gcctttaata  | gaggaaatcc   | cacgccttt   | ctggatctca  | ccaacgacga  | 71640 |
| tagttctgac  | cagcaactca  | tttcttcac    | atccacctgt  | tttaacatat  | aataggcagg  | 71700 |
| agatagatat  | ccgtcattgc  | aatattcctt   | ctcgtaggca  | cacaatctaa  | tattgataaa  | 71760 |
| atctccattc  | tcttctctgc  | atcttattatc  | ttgtctcggt  | ggctgattag  | gctgtgggtct | 71820 |
| tgggttaggc  | cttggtctat  | cgttgttgaa   | tctatttttg  | tcattaaatc  | tttcatttct  | 71880 |
| tcctgggata  | tttctatcac  | ctcgtttggg   | tggatttttg  | tctatattat  | cgtttgaac   | 71940 |
| atcggtacgg  | gtattcattt  | atcacaaaa    | aaacttctct  | aaatgagtct  | actgctagaa  | 72000 |
| aacctcatcg  | aagaagatac  | catatttttt   | gcaggaagta  | tatctgagta  | tgatgattta  | 72060 |
| caaagtgtta  | ttgccggcgc  | aaaatccaaa   | tttccaagat  | ctatgctttc  | tatttttaat  | 72120 |
| atagtacctt  | gaacgatgtc  | aaaatatgag   | ttggagtga   | ttcataacga  | aaatatcaca  | 72180 |
| ggagcaatgt  | ttaccacaat  | gtataatata   | agaaaacaatt | tgggtctagg  | agatgataaa  | 72240 |
| ctaactattg  | aagccattga  | aaactatttc   | ttggatccta  | acaatgaagt  | tatgctctct  | 72300 |
| attattaata  | atacggatat  | gactgcgcgc   | attcctaaaa  | aaagtggtag  | gagaaagaat  | 72360 |
| aagaacatgg  | ttatcttccg  | tcaaggatca   | tcacctatct  | tgtgcatttt  | cgaaactcgt  | 72420 |
| aaaaagatta  | atattttataa | agaaaatatg   | gaatccgcgt  | cgactgagta  | tacacctatc  | 72480 |
| ggagacaaca  | aggtcttgat  | atctaaatat   | gcgggaatta  | atgtcctgaa  | tgtgtattct  | 72540 |
| ccttccacat  | ccataagatt  | gaatgccatt   | tacggattca  | ccaataaaaa  | taaaactagag | 72600 |
| aaacttagta  | ctaataagga  | actagaatcg   | tatagttcta  | gccctcttca  | agaaccctatt | 72660 |
| aggttaaatg  | atcttctggg  | actattggaa   | tgtgttaaaa  | agaatattcc  | tctaacagat  | 72720 |
| attccgacaa  | aggattgatt  | actataaatg   | gagaatgttc  | ctaattgtata | ctttaatcct  | 72780 |
| gtgtttatag  | agcccacggt  | taaacattct   | ttattaagtg  | tttataaaca  | cagattaata  | 72840 |
| gttttatttg  | aagtattcgt  | tgtattcatt   | ctaataatg   | tatttttttag | atctgaatta  | 72900 |
| aatatgttct  | tcattgcctaa | acgaaaaata   | cccgatccta  | ttgatagatt  | acgcgtgct   | 72960 |
| aatctagcgt  | gtgaagacga  | taaatattaatg | atctatggat  | taccatggat  | gacaactcaa  | 73020 |
| acatctgcgt  | tatcaataaa  | tagtaaacgc   | atagtgtata  | aagattgtgc  | aaagcttttg  | 73080 |
| cgatcaataa  | atggatcaca  | accagtatct   | cttaacgatg  | ttcttcgcag  | atgatgatcc  | 73140 |
| atcttttaag  | tattttggcta | gtcaagatga   | tgaatcttca  | ttatctgata  | tattgcaaat  | 73200 |
| cactcaatat  | ctagactttc  | tgttattatt   | attgatccaa  | tcaaaaaata  | aattagaagc  | 73260 |
| tgtgggcat   | tgttatgaat  | ctctttcaga   | ggaatacaga  | caattgacaa  | aattcacaga  | 73320 |
| ctttcaagat  | tttaaaaaac  | tgtttaacaa   | ggcccttatt  | gttacagatg  | gaagggtcaa  | 73380 |
| acttaataaa  | ggatatttgt  | tgcactttgt   | gattagtgtg  | atgctgattca | aaaaagaatc  | 73440 |
| ctctctagct  | accaccgcaa  | tagatcctgt   | tagatacata  | gatcctcgtc  | tgatatacgc  | 73500 |
| atcttctaac  | gtgatggata  | tattaaagtc   | gaataaagtg  | aacaataatt  | aattctttat  | 73560 |
| tgtcatcatg  | aacggcggac  | atattcagtt   | gataatcggc  | cccatgtttt  | caggtaaaag  | 73620 |
| tacagaatta  | attagacgag  | ttagacgtta   | tcaaatagct  | caatataaat  | gcgtgactat  | 73680 |
| aaaatattct  | aacgataata  | gatacggaa    | gggactatgg  | acgcattgata | agaataattt  | 73740 |
| tgaagcattg  | gaagcaacta  | aactatgtga   | tgtcttgga   | tcaattacag  | atttctccgt  | 73800 |
| gataggtatg  | gatgaaggac  | agttctttcc   | agacattgtt  | taattctgtg  | agcgtatggc  | 73860 |
| aaacgaagga  | aaaatagtta  | tagtagccgc   | actcgatggg  | acatttcaac  | gtaaacctgt  | 73920 |
| taataatatt  | ttgaatctta  | ttccattatc   | tgaatgggtg  | gtaaaactaa  | ctgctgtgtg  | 73980 |
| tatgaaatgc  | tttaaggagg  | cttctttttc   | taaacgattg  | ggtagaggaa  | ccgagataga  | 74040 |
| gataatagga  | ggtaatgata  | tgtatcaatc   | gggtgttaga  | aagtgttacg  | tcggctcata  | 74100 |
| atattatatt  | ttttatctaa  | aaaactaaaa   | ataaacattg  | attaaatttt  | aatataatac  | 74160 |
| ttaaaaatgg  | atgttgtgtc  | gttagataaa   | ccgtttatgt  | atcttgagga  | aattgataat  | 74220 |
| gagttagatt  | acgaaccaga  | aagtgc aaat  | gaggtcgcaa  | aaaaactgcc  | gtatcaagga  | 74280 |
| cagttaaaac  | cttactagg   | agaattattt   | tttcttagta  | agttacagcg  | acacggtata  | 74340 |
| ttagatgggtg | ccaccgtagt  | gtatatagga   | tcggctcctg  | gtacacatat  | acgttatttg  | 74400 |
| agagatcatt  | tctataattt  | aggagtgatc   | atcaaatgga  | tgctaattga  | cggccgccat  | 74460 |
| catgatccta  | ttttaaatgg  | attgcgtgat   | gtaactctag  | tgactcgggt  | cgttgatgag  | 74520 |
| gaatatctac  | gatccatcaa  | aaaacaactg   | catccttcta  | agattatttt  | aatttctgat  | 74580 |
| gtaagatcca  | aacgaggagg  | aaatgaacct   | agtacggcgg  | atttactaag  | taattacgct  | 74640 |
| ctacaaaatg  | tcattgattg  | tatttttaaac  | cccggtggcat | ctagtcttaa  | atggagatgc  | 74700 |
| ccgtttccag  | atcaatggat  | caaggacttt   | tatatccac   | acggtataaa  | aatgttacaa  | 74760 |
| ccttttgctc  | cttcatattc  | agctgaaatg   | agattattaa  | gtatttatac  | cgggtgagaac | 74820 |
| atgagactga  | ctcgagttac  | caaattagac   | gctgtaaatt  | atgaaaaaaa  | gatgtactac  | 74880 |

cttaataaga tcgtccgtaa caaagtagtt gtttaactttg attatocctaa tcaggaatat 74940  
gactattttc acatgtactt tatgctgagg accgtgtact gcaataaaac atttcctact 75000  
actaaagcaa aggtactatt tctacaacaa tctatatctt gtttctttaa tattccaaca 75060  
acatcaactg aaaaagttag tcatgaacca atacaacgta aaatatctag caaaaattct 75120  
atgtctaaaa acagaaatag caagagatcc gtacgcggta ataaatagaa acgtactact 75180  
gagatatact accgatatag agtataatga ttttagttact ttaataaccg ttagacataa 75240  
aattgattct atgaaaactg tgtttcaggt atttaacgaa tcatccataa attatactcc 75300  
ggttgatgat gattatggag aaccaatcat tataacatcg tatcttcaaa aaggtcataa 75360  
caagtttctt gtaaattttc tatacataga tgtggtaata tctgacttat ttcctagctt 75420  
tgttagacta gatactacag aaactaatat agttaatagt gtactacaaa caggcgatgg 75480  
taaaaagact cttcgtcttc ccaaaatgtt agagacggaa atagttgtca agattctcta 75540  
tcgcccta ataccattaa aaattgttag atttttccgc aataacatgg taactggagt 75600  
agagatagcc gatagatctg ttatttcagt cgtgattaa tcaattagta gagatgagat 75660  
aagaacatta taataatcaa taatatatct tatatcttat atcttatatc ttatatcttg 75720  
tttagaaaa tgctaattat aaaatagcta acgctagtaa tccaatcgga agccatttga 75780  
tatctataat aggttatcta atttcctgat ttaaatacgg gacagctata ttctcggtag 75840  
ctactcgttt ggaatcacaa acattattta catctaattt actatctgta atggaaacgt 75900  
ttcccaatga aatggtaaaa tccgatacat tgcattttgt tatattttt tttaaagagg 75960  
ctggtaacaa cgcacgctt cgtttacatg gctcgtacca acaataatag ggtaactctg 76020  
tatctattcc tatccgtact atgcttttat caggataaat acatttacat cgtatatcgt 76080  
ctttgttagc atcacagaat gcataaattt gttcgtccgt catgataaaa atttaagtg 76140  
taaataatac tattattttt atagttgtaa taaaaggga aatttgattg tatactttcg 76200  
gttcttttaa agaaactgac ttgataaaaa tggctgtaat ctctaagggt acgtatagtc 76260  
tatatgatca aaaagagatt aatgctacag atattatcat tagtcatgtt aaaaatgacg 76320  
acgatatcgg tapcgttaaa gatggtagac taggtgctat ggatggggca ttatgtaaga 76380  
cttgtgggaa aacggaattg gaatgtttcg gtcactgggg taaagtaagt atttataaaa 76440  
ctcatatagt taagcctgaa tttatttcag aaattattcg tttactgaat catatatgta 76500  
ttcactcggg attattgcgt tcacgagaac cgtattocga cgatattaac ctaaaagagt 76560  
tatcgggaca cgtcttagg agattaaagg ataaaaatatt atccaagaaa aagtcatgtt 76620  
ggaacagtga atgtatgcaa ccgtatcaaa aaattacttt ttcaaagaaa aaggtttgtt 76680  
tcgtcaacaa gttggatgat attaacgttc ctaattctct catctatcaa aagttaattt 76740  
ctattcatga aaagttttgg ccattattag aaattcatca atatccagct aacttatttt 76800  
atacagacta ctttcccatc cctcgttga tttattagacc ggctattagt ttttgtagat 76860  
atagtatacc aagagagacc aatgaattaa aaattattt aggtatgac gttaagaatt 76920  
gtaacttgaa tgctgatgaa caggttatcc agaaggcggg aatagaatac gatgatatta 76980  
aaattatttc taataacact accagtatca atttatcata tattacatcc ggcaaaaata 77040  
atatgattag aagttatata gtcgcccgc gaaaagatca gaccgctaga tctgtaattg 77100  
gtoccagtac atctatcacc gttaatgagg taggaatgcc cgcataatatt agaaatacac 77160  
ttacagaaaa gatatttggt aatgccttta cagtggataa agttaacaa ctattagcgt 77220  
caaaccaagt taaattttac ttttaataaac gattaaacca attacaaga atacgccaa 77280  
gaaagtttat taaaaataaa atacatttat tgccgtggtga ttgggtagaa gtagctgttc 77340  
aagaatatac aagtattatt tttggaagac agccgtctct acatagatac aacgtcatcg 77400  
cttcatctat cagagctacc gaaggagata ctatcaaaat atctcccga attgccaa 77460  
ctcaaaatgc tgatttcgac ggggatgagg aatggatgat attagaacaa aatcctaaag 77520  
ctgtaattga acaaagtatt cttatgtatc cgacgacgtt actcaaacac gatattcatg 77580  
gagccccctt ttatggatct attcaagatg aaatcgtagc agcgtattca ttgttttagga 77640  
tacaagatct ttgtttagat gaagtattga acatcttggg gaaatatgga agagagttcg 77700  
atcctaaagg taaatgtaaa ttcagcggta aagatatcta tacttacttg ataggtgaaa 77760  
agatttaatta tccgggtctc ttaaaggatg gtgaaattat tgcaaacgac gtagatgta 77820  
actttgttgt ggctatgagg catctgtcat tggctggact cttatccgat cataagtcga 77880  
acgtggaagg tatcaacttt attatcaagt catcttatgt ttttaagaga tatctatcta 77940  
tttacggttt tggggtgaca ttcaaagatc tgagaccaaa ttcgacgttc actaataaat 78000  
tggaggccat caacgtagaa aaaatagaac ttatcaaaga agcatacgcc aaatatctca 78060  
acgatgtaag agacgggaaa atagttccat tatctaaagc tttagaggcg gactatgtgg 78120  
aatccatgtt atccaacttg acaaatctta atatccgaga gatagaagaa catatgagac 78180

aaacgctgat agatgatcca gataataacc tcctgaaaat ggccaaagcg gggtataaag 78240  
taaatccacac agaactaatg tatattctag gtacgtatgg acaacaaagg attgatgggtg 78300  
aaccagcaga gactcgagta ttgggtagag ttttacctta ctatcttcca gactctaagg 78360  
atccagaagg aagaggttat attcttaatt cttaacaaa aggattaacg gggtctcaat 78420  
attacttttc gatgctggtt gcaagatctc aatctactga tatcgtctgt gaaacatcac 78480  
gtaccggaac actggctaga aaaatcatta aaaagatgga ggataggtg gtcgacggat 78540  
acggacaagt agttataggt aatacgtc tcaagtagcg cgccaattat accaaaattc 78600  
taggctcagt atgtaaacct gtagatctta tctatccaga tgagtccatg acttgggtatt 78660  
tggaatttag tgctctgtgg aataaaataa aacagggatt cgtttactct cagaaacaga 78720  
aacttgcaaa gaagacattg ggcgcgttta atttcttagt attcgtcaaa cccaccactg 78780  
aggataatgc tattaagggtt aaggatctgt acgatatgat tcataacgtc attgatgatg 78840  
tgagagagaa atacttcttt acggtatcta atatatagatt tatggagtat atattcttga 78900  
cgcatcttaa tccttctaga attagaatta caaaagaaac ggctatcact atctttgaaa 78960  
agttctatga aaaactcaat tatactctag gtgggtggaac tcctattgga attatttctg 79020  
cacaggtatt gtctgagaag ttacacaaac aagccctgtc cagttttcac actactgaaa 79080  
aaagtgggtc cgtcaacaa aaacttgggt tcaacgagtt taataacttg actaatttga 79140  
gtaagaataa gaccgaaatt atcactctgg tatccgatga tatctctaaa cttcaatctg 79200  
ttaagattaa tttcgaattt gtatgtttgg gagaattaaa tccaaacatc actcttcgaa 79260  
aagaaacaga taggtatgta gtagatataa tagtcaatag attatacatc aagagagcag 79320  
aaataaccga attagtctgc gaatatatga ttgaacgatt catctccttt agcgtcattg 79380  
taaagggaatg gggtagggag acattcattg aggacgagga taatattaga tttactgtct 79440  
acctaaattt cgttgaaccg gaagaattga atcttagtaa gtttatgatg gttcttccgg 79500  
gggcagccaa caagggaaag attagtaaat tcaagattcc tatctctgac tatacgggtg 79560  
atgacgactt caatcaaaca aaaaagctca ataagatgac tgtagaactc atgaatctaa 79620  
aagaattggg tttcttctgat ttggaaaacg tcaacgtgta tcctggagta tggaatacat 79680  
acgatatctt cggtagctgag gccgctcgtg aatacttgtg cgaagccatg ttaaaccctt 79740  
atggagaagg gttecgattat ctgtatcagc cttgtgatct tctcgtagt ttactatgtg 79800  
ctagtacga accagaatca gtgaataaat tcaagttcgg cgcagctagt actcttaaga 79860  
gagctacgtt cggagacaat aaagcattgt taaacgcggc tcttcataaa aagtcagaac 79920  
ctattaacga taatagtagc tgccactttt ttagcaaggc ccctaataa ggaactggat 79980  
attacaaata ctttatcgac ttgggtcttc tcatgagaat ggaaaggaaa ctatctgata 80040  
agatatcttc tcaaaagatc aaggaaatgg aagaaacaga agacttttaa ttcttatcaa 80100  
taacatattt ttctatgatc tgtcttttaa acgatggatt ttccacaaat gcgcctctca 80160  
agtcctcat agaatgatac acgtataaaa aatatagcat aggcaatgac tccttatttt 80220  
tagacattat atatgccaaa atcatagccc cgcttctatt tactcccgca gcacaatgaa 80280  
ccaacacggg ctggtttcgt tgatcacatt tagataaaaa ggcggttacg tcgtcaaaat 80340  
atttactaat atcggtagtt gtatcatcta ccaacgggtat atgaataata ttaattatag 80400  
agttaggtaa tgtatattta tccatcgta aattttaaac atatttgaac ttaacttcag 80460  
atgatgggtc atccatagca tttttataat ttcccaata cacattattg gttacccttg 80520  
tcattatagt gggagatttg gctctgtgca tatctccagt tgaacgtagt agtaagtatt 80580  
tatacaaact tttcttatcc atttataacg tacaaatgga taaaactact ttatcggtaa 80640  
acgcgtgtaa tttagaatac gttagagaaa aggctatagt aggcgtacaa gcagccaaaa 80700  
catcaacact tatattcttt gttattatat tggcaattag tgcgctatta ctctggtttc 80760  
agacgtctga taatccagtc tttaatgaat taacgagata tatgcaatt aaaaatcagg 80820  
ttaacgattg gaaatcatta acggatagca aaacaaaatt agaaagtgat agaggtagac 80880  
ttctagccgc tggtaaggat gatatatctg acttcaaatg tgtggatttc ggccctatt 80940  
ttatagctat gcgattggat aagaaaacat atctgccgca agctattagg cgaggtactg 81000  
gagacgcgtg gatgggttaa aaggcggcaa aggtcgatcc atctgctcaa caattttgtc 81060  
agtatttgat aaacacaaag tctaataatg ttattacttg tggtaatgag atgttaaagt 81120  
aattaggtta tagcgggttat tttatgtcac cgcattgggtg ttccgatttt agtaatatgg 81180  
aatagtgtta gataaatgcg gtaacgaatg ttctgtgaag gaaccataac agcttagatt 81240  
taacgttaaa gatgagcata aacataataa acaaaattac aatcaaacct ataacattaa 81300  
tatcaaacaa tccaaaaaat gaaatcagtg gagtagtaaa cgcgtacata actcctggat 81360  
aacgttttagc agctgccgtt cctattctag accaaaaatt cggtttcatg ttttcgaaac 81420  
ggatttctgc aacaagtcga ggatcgtgtt ctacatattt ggccggcatta tccagtatct 81480

gcctattgat cttcatttcg ttttcaattc tggctatttc aaaataaaat cccgatgata 81540  
gacctccaga ctttataaatt tcatctacga tgttcagcgc cgtagtaaat ctaataatat 81600  
aggctgataa gctaacatca taccctcctg tatatgtgaa tatggcatga tttttgtcca 81660  
ttacaagctc gggttttaact ttattgcctg taataatttc tctcatctgt aggatatcta 81720  
tttttttgc atgcattgcc ttcaagacgg gacgaagaaa cgtaatatcc tcaataacgt 81780  
tatcgttttc tacaataaact acatattcta cctttttatt ttctaactcg gtaaaaaaat 81840  
tagaatccca tagggctaaa tgtctagcga tttttctttt cgtttcctct gtacacatag 81900  
tgttacaaaa cctgaaaag aagtgagtat acttgcctac atttctaatt tttcctccag 81960  
tccactgtat aaacgcataa tccttgtaat gatctggatc atccttgact accacaacat 82020  
ttcttttttc tggcataaact tcgttgctct ttacatcatc gaacttctga tcattaatat 82080  
gctcatgaac attaggaaat gtttctgatg gaggtctatc aataactggc acaacaataa 82140  
caggagtttt caccgcgcgc atttagttat tgaaattaat catatacaac tctttaatac 82200  
gagttatatt ttctgtctatc cattgtttca cattgacata tttcgacaaa aagatataaa 82260  
atgcgtattc caatgcttct ctgtttaatg aattactaaa atatacaaac acgtcactgt 82320  
ctggcaataa atgatattctt agaattttgt aacaatgtaa ggaaccataa cagtttagat 82380  
ttaacgttaa agatgagcat aaacataata aacaaaatta caatcaaacc tataacatta 82440  
atatcaaaac atccaaaaaa tgaaatcagt ggagtagtaa acgcgtacat aactcctgga 82500  
taacgttttag cagctgcgt tcctattcta gacaaaaaat ttggtttcat gttttcgaaa 82560  
cggatttctg caacaagtcg gggatcgtgt tctacatatt tggcggcatt atccagatc 82620  
tgctattga tcttcatttc gttttcgatt ctggctattt caaaataaaa tcccgatgat 82680  
agacctccag actttataat ttcactacg atgttcagcg ccgtagtaac tctaataata 82740  
taggctgata agctaacatc ataccctct gtatatgtga atatggcatg attttgtcc 82800  
attacaagct cggttttaac tttattgcct gtaataattt ctctcatctg taggatct 82860  
atttttttgt catgcattgc cttcaagacg ggacgaagaa acgtaatatc ctcaataacg 82920  
ttactgttt ctacaataac tacatattct acctttttat tttctaacte ggtaaaaaaa 82980  
ttagaatccc atagggctaa atgtctagcg atatttctt tctgttctctc tgtacacata 83040  
gtgttacaaa accctgaaaa gaagttagta tacttgtcat catttctaatt gtttctctca 83100  
gtccactgta taaacgcata atccttgtaa tgcctggat catccttgac taccacaaca 83160  
tttcttttt ctggcataaac ttctgtgtcc ttacatcat cgaacttctg atcattaata 83220  
tgctcatgaa cattaggaaa tgtttctgat ggaggtctat caataactgg cacaacaata 83280  
acaggagtth tcaccgcgcg catttagtta ttgaaattaa tcatatacaa ctctttaata 83340  
cgagttatat tttctgtctat ccattgtttc acattttacat atttcgacaa aagatataaa 83400  
aatgcgtatt ccaatgcttc tctgtttaat gaattactaa aatatacaaa cagctcactg 83460  
tctggcaata aatgatattc tagaatattg taacaattta ttttgtattg cacatgttctg 83520  
tgatctatga gttctttctc gaatggcata ggatctccga atctgaaaa gtataaatag 83580  
gagttagaat aataatattt gagagtattg gtaatatata aactcttttag cggatataatt 83640  
agtttttttc tctcaatttc tatttttaga tgtgatggaa aaatgactaa tttttagtga 83700  
ttagtatcat gaactctaatt caaaatctta atatcttctg caccagtttag ctctttgaag 83760  
tttttaagag atgcatcagt tggttctaca gatggagttag gtgcaacaat tttttgttct 83820  
acacatgtat gtactggagc cattgtttta actataatgg tgcttgatc gaaaaacttt 83880  
aatgcagata gcggaagctc ttgcgcgga ctttctacgt cgtaatggg ttctaacgcc 83940  
gatctctgaa tggatactag ttttctaagt tctaattgta ttctctgaaa atgtaaatcc 84000  
aattcctccg gcattataga tgtgtatata tgggtaata aaactatagt atccaacgat 84060  
cccttctcgc aaattctagt ctttaacaaa aaatcgtata taaccacgga gatggcgtat 84120  
ttaagagtgg attctctac cgttttgttc ttggatgtca tataggaaac tataaagtcc 84180  
gcactactgt taagaatgat tactaacgca actatatagt ttaaatttag cattttggaa 84240  
acataaaaata actctgtaga cgatacttga ctttcgaata agtttgcaga caaacgaaga 84300  
aagaacagac ctctcttaatt ttcagaagaa aacttttttt cgtattcctg acgtctagag 84360  
tttatatcaa taagaaagtt aagaattagt cggttaatgt tgtatttcat taccgaagt 84420  
tgagattttc taatattatc aaaagacatg ataatttaa agataaagcg ctgactatga 84480  
acgaaatagc tatatggttc gctcaagaat atagtcttgt taaacgtgga aacgataact 84540  
gtatttttaa tcacgtcagc ggcactctaaa tttaatatag gtatatttat tccacacact 84600  
ctacaatatg ccacaccatc ttcataataa ataaattcgt tagcaaaatt attaatttta 84660  
gtgaaatagt tagcgtcaac tttcatagct tcttcaatc taatttgatg ctacacggg 84720  
gcgaattcca ctctaacatc ccttttccat gcctcaggtt catcgatctc tataatatct 84780

agttttttgc gtttcacaaa cacaggctcg tctctcgcga tgagatctgt atagtaacta 84840  
tgtaaatgat aactagatag aaagatgtag ctatatagat gacgatcctt taagagaggt 84900  
ataataactt taccccaatc agatagactg ttgttatggg cttcggaaaa agaattttta 84960  
taaatttttc cagtattttc caaatatacg tacttaacat ctaaaaaatc cttaatgata 85020  
ataggaatgg ataatccgto tattttataa agaaatacat atcgcacatt atactttttt 85080  
ttggaaatgg gaataccgat gtgtctacat aaatatgcaa agtctaaata ttttttagag 85140  
aatcttagtt ggtccaaatt cttttccaag tacggtaata gatttttcat attgaacggt 85200  
atcttcttaa tctctgggtc tagttccgca ttaaatgatg aaactaagtc actattttta 85260  
taactaacga ttacatcacc tctaaccatca tcatttacca gaatactgat cttcttttgt 85320  
cgtaaataca tgtctaattgt gttaaaaaaa agatcataca agttatacgt catttcatct 85380  
gtggatttct tgtcattgaa ggataaaactc gtactaatct cttctttaac agcctgttca 85440  
aatttatatc ctatatacga aaaaatagca accagtgttt gatcatccgc gtcaatattc 85500  
tggtctatcg tagtgtataa caatcgtata tcttcttctg tgatagtcga tacgttataa 85560  
agggtgataa cgaaaatatt tttatttcgt gaaataaagt catcgtagga ttttggaact 85620  
atattcgcgt ctagttagata tgcttttatt ttgtgaatga tctcaattag aatagtcctt 85680  
ttagagtcca tttaaagtta caaacaacta ggaaattggg ttatgatgta taattttttt 85740  
agtttttata gattctttat tctatactta aaaaatgaaa ataaatacaa aggttcttga 85800  
gggttggtgt aaattgaaag cgagaaataa tcataaatta tttcattatc gogatatccg 85860  
ttaagtttgt atcgtaatgg cgtggtcaat tacgaataaa gcggatacta gtagcttcac 85920  
aaagatggct gaaatcagag ctcatctaaa aaatagcgt gaaaaataag ataaaaacga 85980  
ggatattttc ccggaagatg taataattcc atctactaag cccaaaacca aacgagccac 86040  
tactcctcgt aaaccagcgg ctactaaaag atcaaccaaa aaggaggaag tggagaaga 86100  
agtgttata gaggaatata atcaaaacaac tgaaaaaat tctccatctc ctggagtcag 86160  
cgacattgta gaaagcgtgg ccgctgtaga gctcgatgat agcgacgggg atgatgaacc 86220  
tatggtacaa gttgaagctg gtaaagtaaa tcatagtgtc agaagcgatc tctctgacct 86280  
aaagtggtgt accgacaata tcgttaaaga tcttaagaaa attattacta gaatctctgc 86340  
agtatcgacg gttctagagg atgttcaagc agctggtatc tctagacaat ttacttctat 86400  
gactaaagct attacaacac tatctgatct agtcaccgag ggaaaatcta aagttgttcg 86460  
taaaaaagtt aaaacttgta agaagtaaat gcgtgcactt ttttataaag atggtaaaact 86520  
ctttaccgat aataattttt taaatcctgt atcagacgat aatccagcgt atgaggtttt 86580  
gcaacatggt aaaattccta ctcatttaac agatgtagta gtatatgaac aaacgtggga 86640  
ggagggcgtta actagattaa tttttgtggg aagcgattca aaaggacgta gacaatactt 86700  
ttacggaaaa atgcatgtac agaatcgcaa cgctaaaaga gatcgtattt ttggttagagt 86760  
atataacggt atgaaacgaa ttaattgttt tataaaccaaa aatataaaga aatcgtccac 86820  
agattccaat tatcagttgg cggtttttat gttaatggaa actatgtttt ttattagatt 86880  
tggtaaaatg aaatatctta aggagaatga aacagtaggg ttattaacac taaaaataa 86940  
acacatagaa ataagtcocg atgaaatagt tatcaagttt gtaggaaagg acaaagtttc 87000  
acatgaattt gttgttcata agtctaatag actatataaa ccgctattga aactgacgga 87060  
tgattctagt cccgaagaat ttctgttcaa caaactaagt gaacgaaagg tatacgaatg 87120  
tatcaaacag tttggtatta gaatcaagga tctccgaacg tatggagtca attatacgtt 87180  
tttatataat ttttggaaca atgtaaagtc catatctcct cttccgtcac caaaaaagtt 87240  
aatagcgtta actatcaaac aaactgctga agtggttaggt catactocat caatttcaaa 87300  
aagagcttat atggcaacga ctattttaga aatggtaaaag gataaaaatt ttttagatgt 87360  
agtatctaaa actacgttcg atgaattcct atctatagtc gtagatcacg ttaaatcatc 87420  
tacggatgga tgatatagat ctttacacaa ataattacaa gaccgataaa tggaaatgga 87480  
taagcgtatg aaatctctcg caatgacagc tttcttcgga gagctaagca cattagatat 87540  
tatggcattg ataattgtcta tttttaaacg ccattccaaac aataaccattt tttcagtgga 87600  
taaggtatgg cagtttatga ttgatttcga atacgataat tataaggctt ctcaatattt 87660  
ggatctgacc ctactccga tatctggaga tgaatgcaag actcacgcat cgagtatagc 87720  
cgaacaattg gcgtgtgtgg atattattaa agaggatatt agcgaatata tcaaaactac 87780  
tccccgtctt aaacgattta taaaaaataa ccgcaataga tcagatactc gcatcagtcg 87840  
agatacagaa aagcttaaaa tagctctagc taaaggcata gattacgaat atataaaaga 87900  
cgcttggtta taagtaaatg aaaaaaaact agtcgtttat aataaaacac gatatggatg 87960  
ccaacgtagt atcatcttct actattgcga cgtatataga cgcttttagcg aagaatgctt 88020  
cagaattaga acagaggtct accgcatacg aaataaataa tgaattggaa ctagtattta 88080

ttaagcgcgc attgattact ttgacaaatg tagtgaatat ctctacgatt caggaatcgt 88140  
ttattcgatt taccggttact aataaggaag gtgtttaaagt tagaactaag attccattat 88200  
ctaagggtaca tgggtctagat gtaaaaaatg tacagttagt agatgctata gataacatag 88260  
tttggtgaaaa gaaatcatta gtgacggaaa atcgtcttca caaagaatgc ttgttgagac 88320  
tatcgacaga ggaacgtcat atatttttgg attacaagaa atatggatcc tctatccgac 88380  
tagaattagt caatcttatt caagcaaaaa caaaaaactt tacgatagac ttttaagctaa 88440  
aatatttttct aggatccggt gcccaatcta aaagttcttt gtgacacggt attaatcatc 88500  
caaagtcaag gcctaataca tctctggaaa tagaattcac acctagagac aatgaaacag 88560  
ttccatatga tgaactaata aaggaattga cgactctctc gcgtcatata tttatggctt 88620  
ctccagagaa tgaatttctt tctccgccta ttaacgcgcc tataaaaaacc tttatgttgc 88680  
ctaaacaaga tatagtaggt ttggatctgg aaaactctata tggcgtaact aagactgacg 88740  
gcattcctat aactatcaga gttacatcaa acgggttgta ttgttatttt acacatcttg 88800  
gttatattat tagatatcct gttaagagaa taatagattc cgaagtagta gtctttgggtg 88860  
aggcagttaa ggataagaac tggaccgtat atctcattaa gctaatagag cccgtgaatg 88920  
ctatcagtaga tagactagaa gaaagtaagt atgttgaatc taaactagtg gatattttgtg 88980  
atcgtagagt attcaagtca aagaaatcag aaggctccgtt tactacaact agtgaagtgc 89040  
tcgatatgtt atctacatat ttaccaaaagc aaccagaagg tgttattctg ttctattcaa 89100  
agggacctaa atctaacatt gatttttaaaa ttaaaaagga aaatactata gaccaaaactg 89160  
caaagttagt atttaggtac atgtccagtg aaccaattat ctttggagaa tgcgtctatct 89220  
ttgttagagta taagaaaattt agcaacgata aaggctttcc taaagaatat gggtctggta 89280  
agattgtgtt atataacggc gttaattatc taaataatat ctattgtttg gaatatatta 89340  
atacacataa tgaagtgggt attaatgctc tgggtgtacc tattaagttt atagcagaat 89400  
tcttagttaa tggagaaata cttaaaccta gaattgataa aacctgaaa tatattaact 89460  
cagaagatta ttatggaaat caacataata tcatagtcga acatttaaga gatcaaagca 89520  
tcaaaatagg agatatcttt aacgaggata aactatcgga tgtgggacat caatacgcca 89580  
ataatgataa atttagatta aatccagaag ttagttattt tacgaataaa cgaactagag 89640  
gaccgttggg aattttatca aactacgtca agactctctt tatttctatg tattgttcca 89700  
aaacattttt agacgattcc aacaaacgaa aggtattggc gattgatttt ggaaacgggtg 89760  
ctgacctgga aaaatacttt tatggagaga ttgctgtatt ggtagcgacg gatccggatg 89820  
ctgatgctat agctagagga aatgaaagat acaacaaatt aaactctgga attaaaacca 89880  
agtactacaa atttgactac attcaggaaa ctattcgatc cgatacattt gtctctagtg 89940  
tcagagaagt attctatttt ggaaagttta atatcatcga ctggcagttt gctatccatt 90000  
attcttttca tccgagacat tatgctaccg tcatgaataa cttatccgaa ctaactgctt 90060  
ctggaggcaa ggtattatc actaccatgg acggagacaa attatcaaaa ttaacagata 90120  
aaaagacttt tataattcat aagaatttac ctgtagcgga aaactatatg tctgtagaaa 90180  
aaatagctga tgatagaata gtggtatata atccatcaac aatgtctact ccaatgactg 90240  
aatacattat caaaaagaac gatatagtca gagtgtttta cgaatacggga tttgttcttg 90300  
tagataacgt tgatttcgct acaattatag aacgaagtaa aaagtttatt aatggcgcat 90360  
ctacaatgga agatagaccg tctacaaaaa actttttcga actaaataga ggagccatta 90420  
aatgtgaagg tttagatgtc gaagacttac ttagttacta tgttgtttat gtcttttcta 90480  
agcggtaaat aataatatgg tatgggttct gatatccccg ttctaaatgc attaaataat 90540  
tccaatagag cgatttttgt tectatagga ccttccaaact gtggatactc tgtattgtta 90600  
atagatatat taatactttt gtccgggtaac agaggttcta cgtcttctaa aaataaaagt 90660  
tttataacat ctggcctgtt cataaataaa aacttggcga ttctatatat actcttatta 90720  
tcaaacttag ccattgtctt atagatgtga gctactgtag gtgtaccatt tgattttctt 90780  
tctaatacta tatatttctc tcgaagaagt tcttgacat catctgggaa taaaatacta 90840  
ctgttgagta aatcagttat tttttttata tcgatattga tggacatttt tatagtttaag 90900  
gataataagt atcccaaagt cgataacgac gataacgaag tattttatct tttaggaaat 90960  
cacaatgact ttatcagatt aaaattaaaca aaattaaagg agcatgtatt tttttctgaa 91020  
tatattgtga ctccagatac atatggatct tttatgctcg aattaaatgg gtctagtttt 91080  
cagcacgggtg gtgatatat agaggtggag gaatttatag atgetggaag acaagttaga 91140  
tgggtgtcta catccaatca tatatctaaa gatatacccg aagatatgca caetgataaa 91200  
tttgtcattt atgatata cacttttgac gctttcaaga ataaacgatt ggtattcgta 91260  
caggtacctc cgctgtagg agatgatagt catttgacta atccgttatt gtctccgtat 91320  
tatcgtaatt cagtagccag acaaatgggtc aataatatga tttttaatca agattcattt 91380

|             |            |             |            |             |            |       |
|-------------|------------|-------------|------------|-------------|------------|-------|
| ttaaaatatt  | tattagaaca | tctgattaga  | agccactata | gagtttctaa  | acataataca | 91440 |
| atagtttagat | acaaggatac | cgaagaatta  | aatctaacga | gaatatgtta  | taatagagat | 91500 |
| aagtttaagg  | cgtttgatt  | cgcttggtt   | aacggcggtt | cggaaaatga  | aaaggtacta | 91560 |
| gatacgtata  | aaaaggatc  | taatttgata  | taatgaattc | agtgactgta  | tcacacgcgc | 91620 |
| catatactat  | tacttatcac | gatgattggg  | aaccagtaat | gagtcaattg  | gtagagtttt | 91680 |
| ataacgaagt  | agccagttgg | ctgctacgag  | acgagacgtc | gcctattcct  | gataagttct | 91740 |
| ttatacagtt  | gaaacaaccg | cttagaaata  | aacgagtatg | tgtgtgcggt  | atagatccgt | 91800 |
| atccgaaaga  | tggaaactgg | gtaccgttcg  | aatcaccaaa | ttttacaaaa  | aaatcaatta | 91860 |
| aggagatagc  | ttcatctata | tctagattaa  | ccggagtaat | tgattataaa  | ggttataacc | 91920 |
| ttaatataat  | agaagggtt  | ataccctgga  | attattactt | aagttgtaaa  | ttaggagaaa | 91980 |
| caaaaagtca  | cgcgatctac | tgggataaga  | tttccaagtt | actgctgcag  | catataacta | 92040 |
| aacacgttag  | tgttctttat | tgtttgggta  | aaacagattt | ctcgaatata  | cgggcaaatg | 92100 |
| tagaatcccc  | ggtaactacc | atagtcggat  | atcatccagc | ggctagagac  | cgccaattcg | 92160 |
| agaaagatag  | atcatttgaa | attatcaacg  | ttttactgga | attagacaac  | aaggcaccta | 92220 |
| taaattgggc  | tcaagggtt  | atttattaat  | gccttagtga | aattttaact  | tgtgttctaa | 92280 |
| atggatgagg  | ctattagagg | taatgatgtt  | atctttgttc | ttaaagactat | aggtgtcccg | 92340 |
| tcagcgtgca  | gaacaaatga | agatccaaga  | tttgtagaag | catttaaatg  | cgacgagtta | 92400 |
| gaaagatata  | ttgagaataa | tccagaatgt  | acactattcg | aaagtcttag  | ggatgaggaa | 92460 |
| gcatactcta  | tagtcagaat | tttcatggat  | gtagatttag | acgctgtct   | agacgaaata | 92520 |
| gattatttaa  | cggctattca | agattttatt  | atcgagggtg | caaactgtgt  | agctagattc | 92580 |
| gcgtttacag  | aatgcgggtg | cattcatgaa  | aatgtaataa | aatccatgag  | atctaatttt | 92640 |
| tcatttgacta | agtctacaaa | tagagataaa  | acaagtttct | atattatctt  | tttagacacg | 92700 |
| tataccacta  | tggatacatt | gatagctatg  | aaacgaacac | tattagaatt  | aagttagtca | 92760 |
| tctgaaaatc  | cactaacaag | atcgatagac  | actgccgtat | ataggagaaa  | aacaactctt | 92820 |
| cgggttgtag  | gtactaggaa | aaatccaaat  | tgcgacacta | ttcatgtaat  | gcaaccaccg | 92880 |
| catgataata  | tagaagatta | cctattcact  | tacgtggata | tgaacaacaa  | tagttattac | 92940 |
| ttttctctac  | aacaacgatt | ggaggattta  | gttcttgata | agttatggga  | accagggttt | 93000 |
| atttcattcg  | aagaegctat | aaaaagagtt  | tcaaaaatat | tcattaattc  | tataataaac | 93060 |
| tttaatgatc  | tcgatgaaaa | taattttaca  | acggtaccac | tggtcataga  | ttacgtaaca | 93120 |
| ccttgtgcat  | tatgtaaaaa | acgatcgcat  | aaacatccgc | atcaactatc  | gttggaataa | 93180 |
| ggtgctatta  | gaattttaca | aactggtaat  | ccacatagtt | gtaaagttaa  | aattgttccg | 93240 |
| ttggatggta  | ataaactggt | taattttgca  | caaagaattt | tagacactaa  | ctctgtttta | 93300 |
| ttaaccgaac  | gaggagacca | tatagtttgg  | attaataatt | catggaaatt  | taacagcgaa | 93360 |
| gaacccttga  | taacaaaact | aattttgtca  | ataagacatc | aactacetaa  | ggaatattca | 93420 |
| agcgaattac  | tctgtccgag | gaaacgaaag  | actgtagaag | ctaacatacg  | agacatgtta | 93480 |
| gtagattcag  | tggagaccga | tacctatccg  | gataaacttc | cgtttaaaaa  | tggtgtattg | 93540 |
| gacctggtag  | acggaatggt | ttactctgga  | gatgatgcta | aaaaatatac  | gtgtactgta | 93600 |
| tcaaccggat  | ttaaaattga | cgatacaaa   | ttcgtcgaag | acagtcacga  | aatggaagag | 93660 |
| ttaatgaata  | tcattaacga | tatccaacca  | ttaacggatg | aaaataagaa  | aaatagagag | 93720 |
| ctatatgaaa  | aaacattatc | tagttgttta  | tgcggtgcta | ccaaaggatg  | tttaacattc | 93780 |
| ttttttggag  | aaactgcaac | tggaaagtgc  | acaaccaaac | gtttgttaaa  | gtctgctatc | 93840 |
| ggtgacctgt  | ttgttgagac | gggtcaaaca  | attttaacag | atgtattgga  | taaaggacct | 93900 |
| aatccattta  | tcgctaacat | gcatttgaaa  | agatctgtat | tctgtagcga  | actacctgat | 93960 |
| tttgcatgta  | gtgggtcaaa | gaaaatcaga  | tctgataata | ttaaaaagtt  | gacagaacct | 94020 |
| tgtgtcattg  | gaagaccgtg | tttctccaat  | aaaattaata | atagaaacca  | tgcgacaatc | 94080 |
| attatcgata  | tcaattacaa | acctgttttt  | gataggatag | ataacgcatt  | aatgagaaga | 94140 |
| attgccgtcg  | ctcgattcag | aacacacttt  | tctcaacctt | ctggtagaga  | ggctgctgaa | 94200 |
| aataatgacg  | cgtacgataa | agtcaaaacta | ttagacgagg | ggttagatgg  | taaaatacaa | 94260 |
| aataatagat  | atagattcgc | atttctatac  | ttgttggtga | aatgggtacag | aaaatatcat | 94320 |
| gttcttatta  | tgaactata  | tcctacaccc  | gaagagattc | ctgactttgc  | attctatctc | 94380 |
| aaaataggta  | ctctgttggt | atctagctct  | gtaaagcata | ttocattaat  | gacggacctc | 94440 |
| tccaaaaagg  | gatatatatt | gtacgataat  | gtggtcactc | ttccgttgac  | tactttccaa | 94500 |
| cagaaaatat  | ccaagtattt | taattctaga  | ctatttggac | acgatataga  | gagcttcac  | 94560 |
| aatagacata  | agaaatttgc | caatgttagt  | gatgaatac  | tgcaatatat  | attcatagag | 94620 |
| gatatttcat  | ctccgtaaat | atatgctcat  | atatttatag | aagatatcac  | atatctaaat | 94680 |

gaataccgga atcatagatt tatttgataa tcatgttgat agtatacca ctatattacc 94740  
tcatacagta gctactctag attatctagt tagaactatc atagatgaga acagaagcgt 94800  
gttattgttc catattatgg gatcaggtaa aacaataatc gctttgttgt tgcgcttgg 94860  
agcttccaga tttaaaaagg tttacattct agtgccgaac atcaacatct taaaaatttt 94920  
caattataat atgggtgtag ctatgaactt gtttaatgac gaattcatag ctgagaatat 94980  
ctttattcat tccacaacaa gttttttatc tcttaattat aacgataacg tcattaatta 95040  
taacggatta tctcgctaca ataactctat ttttatcggt gatgaggcac ataatatctt 95100  
tggaataat actggagAAC ttatgaccgt gataaaaaat aaaaacaaga ttcctttttt 95160  
actattgtct ggatctccca ttactaacac acctaatact ctgggtcata ttatagattt 95220  
aatgtccgaa gagacgatag attttgggtga aattattagt cgtggttaaga aagtaattca 95280  
gacacttctt aacgaacgcg gtgtgaatgt acttaaggat ttgcttaaaag gaagaatatc 95340  
atattacgaa atgcttgata aagatctacc aacgataaga tatcacggac gtaagtttct 95400  
agatactaga gtagtattat gtcacatgtc taaacttcaa gagagagatt atatgattac 95460  
tagacgacag ctatgttatc atgaaatgtt tgataaaaaat atgtataacg tgtcaatggc 95520  
agtattggga caacttaatc tgatgaataa tttagatact ttatttcagg aacaggataa 95580  
ggaattgtac ccaaactctga aaataaataa tggcgtgtta tacggagaag aattggtaac 95640  
gttaaacatt agttccaaat ttaaataact tattaatcgg atacagacac tcaacggaaa 95700  
acattttata tacttttcta attctacata tggcggattg gtaattaaat atatcatgct 95760  
cagtaatgga tattctgaat ataattggtc tcagggaact aatccacata tgataaacgg 95820  
caaaccaaaa acatttgcta tcgttactag taaaatgaaa tcgtctttag aggatctatt 95880  
agatgtgtat aattctcctg aaacgatga tggtagtcaa ttgatgtttt tgttttcatc 95940  
aaacattatg tccgaatcct atactctaaa agaggtaagg catatttgggt ttatgactat 96000  
cccagatact ttttctcaat acaaccaaat tcttgagcga tctattagaa aattctctta 96060  
cgccgatatt tctgaaccag ttaatgtata tcttttagcc gccgtatatt ccgatttcaa 96120  
tgacgaagtg acgtcattaa acgattacac acaggatgaa ttaattaatg tttttaccatt 96180  
tgacatcaaa aagctgttgt atctaaaatt taagacgaaa gaaacgaata gaataactc 96240  
tattcttcaa gagatgtctg aaacgtattc tcttccacca catccatcaa ttgtaaaagt 96300  
ttttatggga gaattggtca gacaattttt ttataataat tctcgtatta agtataacga 96360  
tccaagtta cttaaaatgg ttacatcagt tataaaaaat aaagaagacg ctaggaatta 96420  
catagatgat attgtaaacg gtcacttctt tgtatcgaat aaagtatttg ataaatctct 96480  
tttatacaaa tacgaaaacg atattattac agtaccgttt agactttcct acgaaccatt 96540  
tgtttgggga gttaactttc gtaaagaata taacgtggta tcttctccat aaaactgatg 96600  
agatatataa agaaataaat gtcgagcttt gttaccaatg gatacctttc cgttacattg 96660  
gaacctcatg agctgacgtt agacataaaa actaatatta ggaatgccgt atataagacg 96720  
tatctocata gagaaattag tggtaaaatg gccagaataa tagaaattcg tgaagacgtg 96780  
gaattacctc tcggcgaaat agttaataat cctgtagtta taaacgttcc gtgtgttaata 96840  
acctacgcgt attatcacgt tggggatata gtcagaggaa cattaacatc cgaagatgaa 96900  
tcaaatgtaa ctattcaatg tggagattta atctgtaaac taagtagaga ttogggtact 96960  
gtatcattta gcgattcaaa gtactgcttt tttcgaaatg gtaatgcgta tgacaatggc 97020  
agcgaagtca ctgccgttct aatggaggct caacaaggta tcgaatctag tttgtttttt 97080  
ctcgcgaata tcgtcgactc ataaaaaaga gaatagcggg aagtataaac acgaatacta 97140  
tggaataat tgcgaatggt ttattctctt cgatatattt ttgataatat gaaaaacatg 97200  
tctctctcaa atcggacaac catctcataa aatagtcttc gcgcgctgga gaggtagtgt 97260  
ccgctcgtat aatctctcca gaataatata cttgcgtgtc gtctgtcaat ttatacggat 97320  
ttctatagtt ctctgttata taatgcgggt tgccctcatg attagacgac gacaatagt 97380  
ttctaaattt agatagttga tcagaatgaa tgtttatttg cgttggaata attatccata 97440  
cagegtctgc agagtgggtg atagtgttct ctagatatgt aaaataatcc aacttactag 97500  
gcagcaaatt gtctagataa aatactgaat caaacgggtc agacgtattg gcggatctaa 97560  
tggaatccaa ttgattaact atcttttgaa aatatacatt tttatgatec aatacttgta 97620  
agaatataga aataatgata agtccatcat cgtgtttttt tgccctctca taagaactat 97680  
attttttctt attccaatga acaagattaa tctctccaga gtatttgtac acatctatca 97740  
agtgaattgga tccataatcg tcttctttc cccaatatat atgtagtgtat gataacacat 97800  
attcatggg gagaaacct ccacttatat atctctcttt aaaattaatc cttactagt 97860  
ttccagtgtt ctggatagtg gttggttctg actcattata atgtatgtct aacggcttca 97920  
atcgcgcgtt agaaattgct ttttagttt ctatattaat aggagatagt tgttgccgca 97980

tagtaaaaaat gaaatgataa ctgttttaaaa atagctctta gtatgggaat tacaatggat 98040  
gaggaagtga tatttgaaac tcttagagaa ttaatatcta ttaaacgaat aaaagatatt 98100  
ccaagatcaa aagacacgca tgtgtttgct gcgtgtataa caagtgcagg atatccgtta 98160  
ataggagcta gaagaacttc attcgcgttc caggcgatat tatctcaaca aaattcagat 98220  
tctatcttta gagtatccac taaactatta cggtttatgt actacaatga actaagagaa 98280  
atcttttagac gggttgagaaa aggttctatc aacaatatcg atcctcactt tgaagagtta 98340  
atattattgg gtggtaaact agataaaaag gaatctatta aagattgttt aagaagagaa 98400  
ttaaagagg aaagtgatga acgtataaca gtaaaagaat ttggaaatgt aattctaaaa 98460  
cttacaacac gggataaatt atttaataaa gtatatataa gttattgcat ggctgtttt 98520  
attaatcaat cgttgaggga tttatcgcat actagtattt acaatgtaga aattagaaag 98580  
attaaatcat taaatgattg tattaacgac gataaatacg aatatctgtc ttatattttat 98640  
aatatgctag ttaatagtaa atgaactttt acagatctag tataattagt cagattatta 98700  
agtataatag acgactagct aagtcctatta tttgcgagga tgactctcaa attattacac 98760  
tcacggcatt cgttaaccaa tgcctatggt gtcataaacg agtatccgtg tccgtatttt 98820  
tattaactac tgataacaaa atattagtat gtaacagacg agatagtttt ctctattctg 98880  
aaataattag aactagaaac atgttttagaa agaacgatt atttctgaat tattccaatt 98940  
atttgaaaca acaggaaaga agtatactat cgtcattttt ttctctagat ccagctacta 99000  
ctgataatga tagaatagac gctattttatc cgggtggcat acccaaaagg ggtgagaatg 99060  
ttccagagt tttatccagg gaaattaaag aagaagttaa tatagacaat tcttttgtat 99120  
tcatagacac tcggtttttt attcatggca tcatagaaga taccattatt aataaatttt 99180  
ttgaggtaat cttctttgtc ggaagaatat ctctaacgag tgatcaaatc attgatacat 99240  
ttaaagtaa tcatgaaatc aaggatctaa tatttttaga tccgaattca ggtaatggac 99300  
tccaatacga aattgcaaaa tatgctctag atactgcaaa acttaaatgt tacggccata 99360  
gaggatgtta ttatgaatca ttaaaaaaat taactgagga tgattgatta aaaaatataa 99420  
attaatttac catcgtgtat ttttataacg ggattgtccg gcataatcat tagatagtta 99480  
ccgtctacat cgtatactcg accatctacg cctttaaatc ctctattttat tgacattaat 99540  
ctattagaat tggaaatacca aatattagta ccctcaatta gtttattggg aatatttttt 99600  
ttagacgata gatcgatggc tcttgaaacc aaggtttttc aaccggactc attgtcgatc 99660  
ggtgagaagt ctttttcatt agcatgaatc cattctaatt atgtatgttt aaactactca 99720  
aacaattgga caaattcttt tgatttgctt tgaatgattt caaataggtc ttcgtctaca 99780  
gtaggcatac cattagataa tctagccatt ataaagtgcg cgtttacata tctacgttct 99840  
ggaggagttaa gaacgtgact attgagacga atggctcttc ctactatctg acgaagagac 99900  
gcctcgttcc atgtcatatc taaaatgaag atatcattaa ttgagaaaaa actaatccc 99960  
tcgctccac tagaagagaa tacgcatgtt ttaatgcatt ctccgttagt gtttgattct 100020  
tggttaaact cagccaccgc cttgattcta gtatcttttg ttctagatga gaactctata 100080  
ttagagatac caaagacttt gaaatagatg aataagattt ctattcctga ctgattaaca 100140  
aatggttcaa agactagaca tttaccatgg gatgctaata ttcccaaca tacatctata 100200  
aatttgacgc ttttctcttt taattcagta aatagagaga tatcagccgc actagcatcc 100260  
cctttcaata gttctccctt tttaaaggta tctaagcgg atttagaaaa ctctctatct 100320  
cttaatgaat ttttaaaatc atttatatag gtgtctatct cttgcgcgta ttgcgccgga 100380  
tcacgatttt gtcttttcagg aaagctatcg aacgtaaacg tagtagccat acgtctcaga 100440  
attctaaatg atgatatacc tgtttttatt tcagcgagtt tagccttttg ataaatttct 100500  
tcttgctttt tcgacatatt aacgtatcgc attaatactg ttttcttagc gaatgatgca 100560  
gacctttcta cgtcatcaaa aatagaaaaac tcgttattaa ctatatacga acatagtcct 100620  
cctagtgttg agactaattc tttttcatcg actagacgtt tattctcaaa tagtgattgg 100680  
tgttgtaagg atcctgggtc tagtaagtta accaacatgg tgaattcttg cacactattg 100740  
acgatagggt tagccgataa acaaatcatc ttatggtttt ttaacgcaat ggtcttagat 100800  
aaaaaattat atactgaacg agtaggacgg atcttaccat cttctttgat taatgattta 100860  
gaaatgaagt tatgacattc atcaatgatg acgcataatc tactcttgga attaatagtt 100920  
ttgatattag taaaaaattt atttctaaaa ttttgatcat cgtaattaat aaaaatacaa 100980  
tcttcgtta tctctggagc gtatctgagt atagtgttca tccaaggatc ttctatcaaa 101040  
gcctttttca ccaataagat aatagcccaa ttcgtataaa tatccttaag atgtttgaga 101100  
atatatacag tagtcattgt tttaccgaca cccgtttcat ggaacaataa aagagaatgc 101160  
atactgtcta atcctaagaa aactcttgct acaaatgtt gataatcctt gaggcgtact 101220  
acgtccgacc ccatcatttc aacgggcata ttagtagttc tgcgtaaggc ataactcgata 101280

taggccgcgt gtgatttact catttatgag tgataagtaa taactatggt ttaaaaaatca 101340  
cagcagtagt ttaactagtc ttctctgatg ttgttttctg atactttttg aatcagaagt 101400  
cactactaga taaagcaacg agtgaacgta atagagagct tcgtatactc tattcgaaaa 101460  
ctctaagaac ttattaatga attccgtatc cactggattg tttaaaatac taaattgaac 101520  
actgttcaca tccttccaag aagaagactt agtgacggac ttaacatgag acataaataa 101580  
atccaaattt tttttacaaa catcactagc caccataatg gcgctatctt tcaaccagct 101640  
atcgcttacg catttttagca gtctaacatt tttaaagaga ctacaatata ttctcatagt 101700  
atcgattaca cctctaccga ataaagttgg aagttaata atacaatatt ttctgtttac 101760  
aaaatcaaat aatggctgaa acacgtcgaa ggtaacatc ttataatcgc taatgtatag 101820  
attgttttca gtgagatgat tattagattt aatagcatct cgttcacgtt tgaacagttt 101880  
attgcgtgcg ctgaggtcgg caactacggc gtccgcttta gtactcctcc cataactatt 101940  
tacgctatta atcttttaaaa ttccatagac ttatctaga tcgctttctg gtaacatgat 102000  
atcatgtgta aaaagtttta acatgtcggg cggcattcta tttagatcat taactctaga 102060  
aatctgaaga aagtaattag ctccgtatct cagactaggt aatgggcttt tacctagaga 102120  
cagattaagt tctggcaatg tttcataaaa tggaagaagg acatgcgttc cctcccggt 102180  
attttttaca atttcatcca tttacaactc tatagtttgt tttcattatt attagttatt 102240  
atctcccata atcttggtaa tacttaccct ttgatcgtaa gataccttat acaggtcatt 102300  
acatacaact accaattggt tttgtacata atagattgga tgggtgacat ccatgggtgga 102360  
ataaactact cgaacagata gtttatcttt ccccttagat acattagccg taatagttgt 102420  
cggcctaag aatatctttg gtgtaaagtt aaaagtttag gttctgttgc cattattgct 102480  
ttttgtcagt agttcattat aaattctcga gatgggtccg ttctctgaat atagaacatc 102540  
atttccaaat ctaacttcta gtctagaaat aatatcggtc ttattcttaa aatctattcc 102600  
cttgatgaag ggatcggtta tgaacaaatc cttggccttt gattcggctg atctattatc 102660  
tccgctatag acgttacgtt gactagtcca aagacttaca ggaatagatg tatcgatgat 102720  
gttgatacta tgtgatatgt gagcaaagat tgttctctta gtggcatcac tatatgttcc 102780  
agtaatggcg gaaaactttt tagaaatggt atatatataa gaattttttc gtgttccaaa 102840  
cattagcaga atagtcttgaa gataaacact catattatca ggaacattat caatttttac 102900  
atacacatca gcatcttgaa tagaaacgat accatcttct ggaacctcta cgtatcggc 102960  
agactccgga taaccagtcg gtgggccatc gtaacaata actagatcat ccaacaatct 103020  
actcacatat gcatctatat aatctttttc atcttgtag taccctggat acgaaataaa 103080  
tttattatcc gtatttccat aataagggtt agtataaaca gagagagatg ttgccgcatg 103140  
aacttcagtt acagtcgcgcg ttggttggtt tatttgacct attactctcc taggtttctc 103200  
tataaacgat ggtttaattt gtacattctt aaccatatat ccaataaagc tcaattcagg 103260  
aacataaaca aattctttgt tgaacgtttc aaagtcgaac gaagagtcac gaataacgat 103320  
atcggatact ggttggaagg ttaccgtttc ggttaatttt gaatcggata gtttaagact 103380  
gctgaatgta tcttccacat caaacggagt tttaatataa acgtatactg tagatgggtc 103440  
tttaatagtg tcattaggag ttaggccaat agaaatatca ttaagttcac tagaatatcc 103500  
agagtgtttc aaagcaattg tattattgat acaattatta tataattctt cgccctcaat 103560  
ttcccaaata acaccgttac acgaagagat agatacgtga ttaatacatt tatatocaa 103620  
atatggtacg taactgaatc ttcccatacc ttttaacttct ggaagttcca aactcagaac 103680  
caaatgatta agcgcagtaa tatactgatc ctaattttcg aagctagcga tagcctgatt 103740  
gtctggacca tcgtttgtca taactccgga tagagaaata tattgcggca tatataaagt 103800  
tggaatttga ctatcgactg cgaagacatt agaccgttta atagagtcac cccacccgat 103860  
caaagaatta atgatagtat tattcatttt ctatttaaaa tggaaaaagc ttacaataaa 103920  
ctccgtagag aaatatctat aatttgtgag ttttcttaa agtaacagct tccgtaaacg 103980  
ccgtctttat ctcttagtag gtttattgta ttatgacct tttcttata ttcatagaat 104040  
actaaaggca acaaagaaat ttttggttct tctctaagag ctacgtgaga cttaaccata 104100  
gaagccaacg aatccctaca tatttttagaa cagaaatacc ctacttcacc acccttgat 104160  
gtctcaatac taataggtct aaaaacaaa tcttgattac aaaaccaaca cttatcaatt 104220  
acactatttg tcttaataga cacatctgcc atagatttat aatactttgg tagtatacaa 104280  
ggagtgctt ctctcttagc gggcttaagg actgctttag gtgctgaaat aaccacatct 104340  
ggaaggctta ctccgttagc catttaatta cggaaactatt tttttatact tctaatagag 104400  
aagtagaaaa cctctcatct acaaaaaagc actcgtgtcc ataactctct accatagtta 104460  
cacgtttttt agatctcata tgtgctaaaa agttttccca tactaattgg ttactattat 104520  
tttctgtata atttttaaca gtttgaggtt ttagattttt agttacagaa gtgatatoga 104580

atattttatc caaaaagaat gaataattaa ttgtcttaga aggagtgttt tcttggcaaa 104640  
agaataccaa gtgcttaaata atttctacta cttcattaat cttttctgta ctcagattca 104700  
gtttctcatc ttttacttga ttgattatct caaagactaa cttataatcc tttttattba 104760  
ttctctcggt agccttaaga aaactagata caaaatttgc atctacatca tccgtggata 104820  
tttgattttt ttccatgata tccaagagtt ccgagataat ttctccagaa cattgatgag 104880  
acaataatct ccgcaataca tttotcaaat gaataagttt attagacacg tggaggtttg 104940  
actttttttg tacctttgta catttttgaa ataccgactc gcaaaaaata caatattcat 105000  
atccttggtc agatactata ccgttatgtc tacaaccgct acataatcgt agattcatgt 105060  
taacactcta cgtatctcgt cgtocaatat tttatataaa aacattttat ttctagacgt 105120  
tgccagaaaa tccgtgtaata tttttagttt tttgggctgt gaataaagta tcgocctaata 105180  
attgttaccg tcttccgcca atatatagtag taaattatcc gcacatgcag aagaacaccg 105240  
cttaggcgga ttcagtacaa tgttatattt ttctgaccaa ctcattttaa tatcataatc 105300  
taaaatagtt ctgtaatatg tctagcgtca atataattgat cataatcctg tgcataaatt 105360  
aagatacaac aatgtctcga aatcatcgac atggcttctt ccatagttag aagatcgctc 105420  
tcaaagttag caacgtgatt catcaacatt tgcgtttttg aggcagcaaa tactgaaaccg 105480  
tcgccattca accattcata aaaaccatcg tctgaatcca ttgataattt cttgtactgg 105540  
tttttgagag ctgcgcataa tctagcattt cttagctccc gattgaaaac agaaaagga 105600  
tcgtacatcc aggggtccatt ttctgtaaat agaactcgtat aatgtccctt caagaagata 105660  
tcagacgac cacaatcaaa gaattggtct ccgagtttgt aacaaactgc ggacttttaac 105720  
ctatacatga taccgttttag cataatttct ggtgatacgt caatcggagt atcatctatt 105780  
agagatctaa agccggtgta acattctcca ccaaacatat tcttattctg acgtcgttct 105840  
acataaaaaca tcattgctcc attaacgata acaggggaat gaacagcact acccatcaca 105900  
ttagttccca atggatcaat gtgtgtaact ccagaacatc ttccatagcc tatgttagga 105960  
ggagcgaaca ccactcttcc actattgcca tcgaatgcca tagaataaat atccttgga 106020  
ttgatagaaa tcggactgtc ggatgttgtg atcatcttca taggattaac aactatgtat 106080  
gggtccgcct gaagtctcat atcgttaact atgcctgtta taggtctagc cacagaaacc 106140  
aacgtaggtc taaatccaac tatagacaaa atagaagcca atatctgttc ctcatctgtc 106200  
ataacttgag agcatccagt atgaataatc ttcattagat ggggatctac cgcacatca 106260  
tcgttacaat aaaaaattcc cattctaatg ttcataattg cttttctaat catggtatgc 106320  
atgtttgtc tctgaatctc tgtggaaatt agatctgata cacctgtaat cactatcggg 106380  
ttatcctccg taagacgatt aaccaacaac atataattat aagactttac ttttctaaat 106440  
tcataaagtt gctggattag gctataggtg tctccatgta catacgcgtt ctcgagcgca 106500  
ggaagtttaa taccgaatag tgccatcaga ataggatgaa tatagtaatt agtttctggt 106560  
tttctataaa taaaagacaa atcttgtgaa ctagacatat cggtaaaatg catggattgg 106620  
aatcgtgtag tcgacagaag aatatgatga ttagatggag agtatatttt atctaactct 106680  
ttgagttggt caccgattct aggactagct cgagaatgaa taagtactaa aggatgagta 106740  
catttcacag aaacactagc attgttcaat gtgctcttta catgggtaag gagttgaaat 106800  
agctcgtttc tatttgtttc gacaatattt agtttattca taatgttaag catatcctga 106860  
atagtaaagt tagatgtgtc atacttgta gtagttagat atttagcaat tgcattccca 106920  
tcatttctca atctcgtact ccaatcatgt gtagatgcta cttcgtcgat ggaaccata 106980  
caatcctttt tgataggctg ttgagattga tcatttctcg cacgtttagg tttggtacgt 107040  
tgatttctag cccctgcgga tataaagtca tgcgtacaaa tttgggacaa tgaattgcat 107100  
acactacaag acaagattt atcagaagtg tgaatatgat cttcatctac caaagaaaga 107160  
gtttgattag tataactaga ttttagtctt gcgttagatg ttaaaaaaac atcgctattg 107220  
accacggctt ccattattta tttcgtagt ttttactcga aagcgtgatt ttaatatcca 107280  
atcttattac ttttggaatc gttcaaaacc tttgactagt tgtagaattt gatctattgc 107340  
octacgcgta tactcccttg catcatatac gttcgtcaccc agatcgtttg tttcgccctg 107400  
aagttggtgc atattctctt caacattcga catgagatcc ttaagggcca tatcgtctag 107460  
attttgttga gatgctgtc ctggatttgg attttgttgt gctgtgtgac atactgtacc 107520  
accagtaggt gtaggagtag atacagtggc cacaatagga ggttgagaaa gtgtaaccgt 107580  
tgagtagta caagaaatac ttccatccga ttgttgtgta catgtagtgt ttggttaacc 107640  
ctgagaaggt tgggttagat gggcgctcgt cgttttttga tctttattaa atttagagat 107700  
aatatcctga acagcattgc tcggcgtcaa cgctggaagg agtgaactcg ccggcgcac 107760  
agtatcttca gacagccaat caaaaagatt agacatatca gatgatgtat tagtttgttg 107820  
tcgaggtttt ggtgtaggag cagtactact aggtagaaga ataggagccg atgtagctgt 107880

tggaaccggc tgtggagtta tatgaatagt tgggtgtagc ggttggatag gctgtctgct 107940  
ggcgcccatc atattatctc tagctagtgt ttctcgcaac tgtctttgat aatacgactc 108000  
ttgagacttt agtectatct caatcgcttc atcctttttc gtatccggat ccttttcttc 108060  
agaataatag attgacgact ttggtgtaga ggattctgcc agcctctgtg agaacttggt 108120  
aaagaagtcc atttaaggct ttaaaattga attgcgatta taagattaaa tggcagacac 108180  
agacgatatt atcgactatg aatccgatga tctcaccgaa tacgaggatg atgaagaaga 108240  
ggaagaagat ggagagtcac tagaaactag tgatatagat cccaaatctt cttataagat 108300  
tgtagaatca gcatccactc atatagaaga tgcgcattcc aatcttaaac atatagggaa 108360  
tcataatctt gctcttaaac gacgctatac tagacgtata agtctatttg aaatagcggg 108420  
tataatagca gaaagctata acttgcttca acgaggaaga ttacctctag tttcagaatt 108480  
ttctgacgaa acgatgaagc aaaatatgct acatgttaatt atacaagaga tagaggaggg 108540  
ttcttgtcct atagtcatcg aaaagaacgg agaattgttg tcggtaaaccg attttgacaa 108600  
agatgggtcta aaattccatc tagactatat tatcaaaatt tggaaacttc aaaaacgata 108660  
ttagaattta tacgaatata gttctctaaa tgtcacaaac aagtctcgca tgttcagcaa 108720  
tttattgtcg tactttatat cgtgttcatt aacgatattc tgcaaaatag taatgattct 108780  
atcttctctc gatagatatt cttcagagat tattgtctta tattctttct tgttatcaga 108840  
tatgaatttg ataagacttt gaacattatt gataccgctc tgtttaattt tttctacaga 108900  
tatttttagtt ttggcagatt ctatcgtatc tgtcaataga catccaacat cgacattcga 108960  
cgtcaattgt ctataaatca acgtataaat tttagaaata acattagcga attgttgtgc 109020  
gttgatgtcg ttattctgaa acagtatgat tttaggtagc attttcttaa caaagagaac 109080  
gtattttattg ttactcagtt gaacagatga tatatccaga ttactaacgc atctgattcc 109140  
gtataccaaa ctttcagaag aaatgggata caattgtttg tattcattca atgtctcttt 109200  
ttcagaaatt agtttagagt cgaatactgc aataattttc aagagatagt tttcatcaga 109260  
taagatttta tttagtgtag atatgataaa actattgttt tgttggagaa cttgatcgc 109320  
cgcgttctct gtagtcgacg ctctcaaatg ggaaacaatc tccattattt ttttggaaac 109380  
ggatactata tcttcgggat cttgacgcag tctagtatac atagagttaa gagagattag 109440  
agtttgtaca ttaagcaaca tgtctctaaa tgtggctaca aacttttctt ttttcacatc 109500  
atctagttta ttatataccg atttcacaac ggcaaccagat ttaaggaacc agaatgaaaa 109560  
actctgataa ctacaatatt tcatcatagt tacgatttta tcatcttcta tagttgggtg 109620  
aatagcgc atctttttct ccaagactgg aaccaacgtc ataaaaatgt ttaaatcaaa 109680  
atccatatca acatctgatg cgctaagacc agtctcgct tcaagattat ctttactaat 109740  
ggtgacgaac tcatcgtata aaactctaag tttgtccatt atttatttac agatttagtt 109800  
gttttaattta tttgtgctct tccagagtgt ggatagtatt tttctaactg cggattata 109860  
ttattaggat ctacgttcat atgtatcata atattaatca tccacgtttt gataaatcta 109920  
tcttttagctt ctgaaataac gtatttaaac aaaggagaaa aatatttagc tacggcatca 109980  
gacgcaataa cattttttgt aaatgtaacg tatttagacg acagatcttc gttaaaaagt 110040  
tttccatcta tgtagaatcc atcggttggt aacaccattc cgcgctcaga ttgaatagga 110100  
gtttgaatag tttgtttgg aaatagatcc ttcaataact tatagttggg tgggaaaaaa 110160  
togattttat cactagactc tttctttttt actatcatta cctcatgaac tatttcttga 110220  
atgagtatat gtattttctt tcttatatcg gacgcgttca ttggaaaata taccatgtcg 110280  
ttaactataa gaatattttt atcctcgttt acaaaactgaa taatatcaga tgtagttcgt 110340  
aaacgaacta tatcatcacc agcacaacat ctaactatat gatatccact agtttccttt 110400  
agcggtttat tatcttggtc catattagca gtcattccat catttaagaa ggcgtcaaaag 110460  
ataataggga gaaatgacat tttggattct gttacgaact taccaaaatt aaggatatac 110520  
ggacttacta tctttttctc aacgtcaatt tgatgaacac acgatgaaa tgtgcttcta 110580  
tgagattgat catgtagaaa acaacaaggg atacaatatt tccgcataac atgaaatata 110640  
ttaagaaatc ccaccttatt atatttccc aaaggatoca tgcacgtaaa cattatgccg 110700  
ttatcattaa taaagacttc tttctcatcg gatctgtaaa agttgttact gatttttttc 110760  
attccaggat ctagataaatt aataatgatg ggttttctat tcttattctt tgtattttgg 110820  
catatcctag accagtaaac agtttccact ttggtaaaaat cagcagactt ttgaacgcta 110880  
ttaaacatgg cattaatggc aataactaaa aatgtaaaaa atttttctat gtttaggaata 110940  
tggtttttca cttaataga tatatggttt ttggccaaaa tgatagatat ttttttatcc 111000  
gaggatagta aaatattatt agtcgcgctc tctataaaaa tgaagctagt ctogatatcc 111060  
aattttatcc tagaattgat aggagtcgac aaatgtacct tatacgttat atctcccttg 111120  
atgcggtcca tttgtgtatc tatatcggac acaagatctg taaatagttt tacgttatta 111180

atcatcacgg tategccgtc gctagataac gctaagtgtac catccaagtc ccaaattggag 111240  
agattttaact gttcatcggt tagaataaaa tgattaccgg tcatattaat aaagtgttca 111300  
tcgtatctag ataacaacga cttataatta atgtccaagt cttgaactcg ctgaatgatc 111360  
ttttttaacc cagttagttt tagattggta cgaaatatat tgttaaactt tgattctaca 111420  
gtaatgtcca aatctagttg tggaaatact tccatcaaca ttgtttcaaa cttgataata 111480  
ttattatcta catcttcgta cgatccaaat tccggaatag atgtatcgca cgctctggcc 111540  
acccagataa ccaaaaagtc acacgctcca ggatatacat tgtataaaaa gctatcgttt 111600  
tttagtaggg ttttttctg cgtgtatacg aagggtataa aaatagtatt atcaacgtaa 111660  
ctatattcca aattattctt atgagaatag ataataatat cgtccttaat atctaacaaa 111720  
tttctaaat atccctttaa ttgagtcatt cgaagcgta atagaatag tctcttaact 111780  
atttccggct gttgtatatt taaatgact cgtaaaaaat aatatatggg cgacttctca 111840  
tctatgtaat catatggagt gagatatagg gtcgttcta cctcctgccc cttaccacc 111900  
tgtaatacca attgaggact tactatataat cgcatattta tatcgtgggg taaagtgaag 111960  
atctactacc gatgatgtaa gtcttacaat gttcgaacca gtaccagatc ttaatttgga 112020  
ggcctccgta gaactagggg aggtaaatat agatcaaca acacctatga taaaggagaa 112080  
tagcggtttt atatcccgca gtagacgtct attcggccat agatctaagg atgatgagag 112140  
aaaactagca ctacgattct ttttacaag actttatatt ttagatcata gagagattca 112200  
ttatttggtc agatggttg acgctgtaaa agacgtcact attacaaaaa aaaataacat 112260  
tatcgtggcg ccttatatag cacttttaac tatcgcatca aaaggatgca aacttacaga 112320  
aacaatgatt gaagcattct tccagaact atataatgaa catagtaaga aatttaatt 112380  
caactctcaa gtatccatca tccaagaaaa actcggatac cagtttgga actatcacgt 112440  
ttatgatttt gaacggtatt actctacagt agctctggct attcgagatg aacattctc 112500  
tggtattttt aatatccgct aagagagtta tctggtaagt tcattatctg aaataacata 112560  
tagattttat ctaattaatc taaaatctga tcttggtcaa tggagtgtca gtacggggcg 112620  
tgtaattaat caaatggtaa atactgtatt attggctgtg gaatcaaac ttccaataaa 112680  
catagaaaat gattcacaat ttacatgttc aattattttac aaaggttaa aaagaccag 112740  
attacttaaa gatagaaatg aattattttac taccgataagga tacgctacta aaatatttta cttaggactg 112800  
ttcattcaag ataagcaaac gcgataagga ttatcattgt tactattatt actattacta 112860  
gagttagaat ttatagacga ctcatctcgt ttatcattgt tactattatt actattacta 112920  
tcattattag tgttggcatt attagtattc ttcttgctat ctgtgtcaga aatatacagc 112980  
aatgctatac ctaataactaa atacattatc atgctcgcaa tggctctaac aacaacgaac 113040  
caaaatgaat ttggtcgtag cttttgttca caaaaataca taaagaaatg tctacataaa 113100  
tctatggcg cattggctac ttgaaatagc gccagtcctc ctacagattt taatatagct 113160  
gtataacatg acattttatc atcatcaaaa gagacagagt caccatctgt catatttaga 113220  
ttttttttca tgtgttcaaa gtatcctcta ctcatttcat tataatagtt tatcattctt 113280  
agaattttag gacggatcaa tgagtaagac ttgactagat cgtcagtagt aatttgtgca 113340  
tcgtctatc tgcacccgtc tgcgcgaata atgtatagca tcgctttgag attctccata 113400  
gctatcaagt ctttatacaa tgacatggaa atactctgtga atactttata cttctccaac 113460  
atcgatgcct taacatcatc gcctacttta gcattgaaaa tacgttctat tgtgtagatg 113520  
gatgtagcaa gattttttaa caacaatgcc atcttacacg atgattgcct caagtctcca 113580  
atcgtttgtt tagaacgatt agctacagag tccaatgctt ggctgactag catattatta 113640  
tctttagaaa ttgtattctt caatgaggcg tttatcatat ctgtgatttc gttagtata 113700  
ttacagtctg actgggttgg aatgttatcc aacatacac ctatggatac ggtacacgta 113760  
ccagcatttg taataatcct atctaagatg ttgtatggca ttgcccagaa aatatcttct 113820  
cctgtaatat ctccactctc gataaatcta ctcagattat tcttaaatgc cttattctct 113880  
ggagaaaaga tatcagtgtc catcatttca ttaatatgat acgcagaaaa gataccacga 113940  
gtatcaattc tatccaagat acttatcggt tccgagtcac agataatggg ttctctctct 114000  
tcgggagatc ctgcatagaa atatctagga caatagtttc tatactgtct gtaactctga 114060  
taatctctaa agtcactaac tgataccatg aaattgagaa gatcaaacgc tgaagtaatt 114120  
aatttttctg cctcgttttt actacaacta gttttcatca atgtagtgc gatgtattgt 114180  
ttagtattct ttggtctaact actgatgata gagatattat tgcctccat aatggatctt 114240  
ctagtagtca ccttaaagcc cattgatgcg aatagcagat agataaagtc ttggtatgac 114300  
tcctttctaa tatagtacgg actacctttg tcaccaact ttatacccac ataagccata 114360  
acaacctctt taatagccgt ttcattgagg ttatcagcca tgagcctgag tagttggaag 114420  
aatctcatga atcccgctc agaaagtcct atatgcatga tagattttatc tttcctggga 114480

aactctcgta tagtcataga tgaaatactc tttaaagttt ctgaaataag attagtaaca 114540  
gtcttacctc cgactactct aggttaacaaa caaactctaa taggtgtttt ctctgcggag 114600  
ataatatcag aaaggataga gcaataagta gtattattgt gattataaag accgaatata 114660  
taacaggtag aattttataaa catcatgtcc tgaagggttt tagacttgta ttcctcgtaa 114720  
tccataccgt cccaaaacat ggatttggtta actttgatag ccgtagatct ttgttccttc 114780  
gccaacagg taaagaaatt aataaagaat ttgttgtttc tatttatgtc cacaattgc 114840  
acgtttggaa gcgccacggt tacattcact gcagcatttt gaggatcgcg agtatgaagt 114900  
acgatgttat tgtttactgg tatactctgga aagaaatcta ccagtctagg aataagagat 114960  
tgatatcgca tagaaatagt aaagtttata atctcatcat cgaagagcat tttgttacca 115020  
ttgtaataaa tatccactct gteatatgta taaatgaagt actgttcaaa catgatgaga 115080  
tgtttatatg ttggcatagt agtgagatcg acgtttggta atggcaatgt attaagatta 115140  
actccataat gtctagcagc atctgcgatg ttataagcgt cgtcaaagcg gggtcgatct 115200  
tctattgtta tatattgtct aacacctata agattatcaa aatcttgtct gcttaatata 115260  
cgtttaacaa tttttgcctt gaattctttt attgggtgat taataacatc cttatagagg 115320  
atgttaacaa aataagtgtt atcaaagtta agatctggat atttcttttc tgctagaaca 115380  
tccattgagt cggagccatc tgggttaata taaccaccga taaatctagc tctgtattct 115440  
gtatccgtca atctaattt aagaagggtg tgagtgaag gtggaagatc gtaaaagctg 115500  
tgagtattaa tgataggatt agtttccgaa ctaatgttaa ttggggattt aataatatct 115560  
atatttccag cgttaagtgt aacattaaac agttttaatt cacgtgacgt ggtatcaatt 115620  
aaataattaa tgcccaattt ggatatagca gcctgaagct catcttgttt agttacggat 115680  
cctaattgagt tattaagcaa tatactgaac ggatgaacga aggttgtttt agtttggtca 115740  
catactttgt aacttagaca tagatgcgga agaacggtag aaactatacg aaataaatat 115800  
tcagagtcct ctaattgatc aagagtaact attgacttaa taggcacatc ttatttagta 115860  
ttaaatgacg accgtaccag tgacggatat acaaaacgat ttaattacag agttttcaga 115920  
agataattat ccatctaaca aaaattatga aataactctt cgtcaaagt ctattctaac 115980  
tcacgttaac aacgtggtag atagagaaca taatgccgcc gtagtgatcat ctccagagga 116040  
aatatcctca caacttaatg aagatctatt tccagatgat gattcacccg ccaactattat 116100  
cgaacgagta caacctcata ctactattat tgacgatact ccacctcta cgtttcgtag 116160  
agagttatta ataecggaac aacgtcaaca acgagaaaa agatttaata ttacagatc 116220  
gaaaaatgct gaagcaataa tggaaatctag atctatgata acttctatgc caacacaaac 116280  
accatccttg ggagtagttt atgataaaga taaaagaatt cagatgttag aggatgaagt 116340  
ggttaatctt agaaatcaac gatctaatac aaaatcatct gataatttag ataattttac 116400  
caaaatacta tttggtaaga ctccgtacaa atcaacagaa gttataaagc gtatagccat 116460  
cgttaattat gcaaaatttg acgggtcccc cttatcagtc gaggacttg atgtttgttc 116520  
ggaggatgaa atagatagaa tctataaaac gattaacaa tatcacgaaa gtagaaaacg 116580  
aaaaattatc gtcactaacg tgattattat tgtcataaac attatcgagc aggcattgct 116640  
aaaactcgga tttgaagaaa tcaaaaggact gagtaccgat atcacttcag aaattatcga 116700  
tgtggagatc ggagatgact gcgatgctgt agcatctaaa ctaggaaatc gtaacagtc 116760  
ggttcttaat attgtattgt ttataactca gatattcggt aaacgaatta aaattattta 116820  
atttaataca ttcocatatc cagacaacaa tctgtctggat taatctgttc ctgtcgtctc 116880  
ataccggacg acatattaat ctttttatta gtgggcacat ttttagatgg tttctttttc 116940  
ccagcattaa ctgagtcgat acctagaaga tctgtgattga tctctccgac cattccacga 117000  
acttctaatt ggccgtctct gacggtaacca taaactattt taccagcatt agtaacagct 117060  
tggaacaatc gaccatccat cgcattgtac gatgtagtag taactgttgt tctacgtcta 117120  
ggagcaccag aagtattttt ggagcccttg gatgttgatg tagaagaaga cgaggatttt 117180  
gattttgggt tacatgtaat acattttgaa ctctttgatt ttgtatcaca tgcgcccga 117240  
gtcacatctg tttgagaatt aagattattg ttgcctcctt tgacggctgc atctccaccg 117300  
atttgcgcta gtagattttt aagctgtggt gtaactcttat taactgtttc gatataatca 117360  
tcgtaactgc ttctaacggc taaatttttt ttatccgcca tttagaagct aaaaatattt 117420  
ttatttatgc agaagattta actagattat acaatgaact aatatgatcc ttttccagat 117480  
tatttacaaa cttggtattt tttggttctg gaggaggcga atttaaatc ggacttggat 117540  
tcggattttg taagtctctg atottattat acatcgagta taggatggcg acagtaactg 117600  
ctacacaaat accgatcaaa agaagaatac caatcattta ttgacaataa cttcactatt 117660  
gatcaagtat gcaatatatc atctttcac taaataagta gtaataatga ttcaacaatg 117720  
tcgagatata tggacgataa taatttagtt catggaaata tcgctatgat tgggtgtaat 117780

gactccgcta actctgtggg gtgcgcagtg ctttcccac atagaataaa tttagcattcc 117840  
gactgtgata ataataccaa gtataaacgc cataatactc aatactttcc atgtacgagt 117900  
gggactggta gacttactaa agtcaataaa ggcgaagata cacgaaagaa tcaaaagaat 117960  
gattccagcg attagcacgc cggaaaaata atttccaatc ataagcatca tgteccattta 118020  
actaataaaa attttaaatc gccgaatgaa caaagtggaa tataaaccat ataaaaacaa 118080  
tagtttgtag tgcaaaaata atattctatct ttgttttcga agatatggta aaattaaata 118140  
gtagtacaca gcatgttata actaacagca gcaacggctc gtaattactt atcattttact 118200  
agacgaaaag gtggtgggat attttcttgc tcaataataa cgaatatatc acccatccat 118260  
tttatgogag gtttatatac tctaattctt aatagatcta tagacgacgg gtttaccac 118320  
aatatagatt ttatcgattc atctaattta aaccttccct taaacgtgaa tgatctatta 118380  
tctggcataa cgatgaccct acctgatgaa tccgacaatg tactgggcca tggtagaataa 118440  
attatcaacg aattatcgct tacgaacatt tatatcattt gttttaattt taggacgcga 118500  
ataaatggat ataaaataga aaataacaga tattacaacc agtggtatgg ccgcgcccaa 118560  
ccaggtaggc agttttatct tatcttttac tacaggttct cctggatgta cgtcaccaac 118620  
ggcggacgta gttctagtac aattagacgt aagttccgct tgggaatttt ttaacgctaa 118680  
agagttaacg ttaatcgctc acccaacgta ttacatcta gttcgttgaa catcttgatt 118740  
ataatataac cattttctat ctctagattc gtcagtgcac tcatgtaacc aacataccct 118800  
aggtcctaaa tatttatctc cgggaattaga ttttgataaa ttccgcgcac aacaatttct 118860  
atttctttta tgategttac aaaagacgta taatgccgta tccccaaaag taaaataatc 118920  
aggacgaata attctaataa actcagaaca atatctcgca tccatatgtt tggagcaaat 118980  
atcggataaa gtagacatag ccggtttccg ttttgacgt aaccttcta aacaattggg 119040  
gtttccagga tctgtttctac aaaatccagt catgaaatcg tcacaatgtt ctgtcttgta 119100  
attattatta aatatttttg gacagtgttt ggtatttgct ttagaacaac attttgccac 119160  
gctatcacta tgcgccagga gataatcctt ttttataaaa tgacatcgtt gcccgatgc 119220  
tatataatca gtacggtgtt ttaaactcct aatatattca ggagttacct cgttctgata 119280  
atagattaat gatccaggac gaaatttgaa agaactacat ggttctccat gaattatac 119340  
atattgttta gcaaatcag gaactataaa actactacaa tgatctatcg acataccatc 119400  
tatcaaacia aacttgggtt taattttctc cggagatgtt tcataatagt acgtataact 119460  
ttcttctgca aacttaacag ctctattata ttcaggataa ttaaaacctt attccatata 119520  
tttgtctcgt atatctgcta ttctgtgtgc tatttttgatt ctattaagag taacagctgc 119580  
ccccattctt aataatcgct agtattttaa ctgttaaagt ttggtatata aacatctacc 119640  
ttatttcccg cagtataagg tttgttgacg gtatactgtt caggaaatgg ttacatttata 119700  
cttcttctat agtctgtct ttcgatgttc atcacatag caaagaacag aataaacaaa 119760  
ataatgtaag aaataatatt aaatatctgt gaattcgtaa atacattgat tggcataata 119820  
attacagcag ctacaataca cacaatagac attccecacag tgttgccatt acctccacga 119880  
tacatttgag ttactaagca ataggtaata actaagctag taagaggcaa tagaaaagat 119940  
gagataaata tcatcaatat agagattaga ggagggtat atagagccaa gacgaacaaa 120000  
atcaaaccga gtaacggtct aacatcatta tttttgaaga ttcccaaata atcattcatt 120060  
cctccataat cgttttgcat catacctcca tctttaggca taaacgattg ctgctgttcc 120120  
tctgtaataa aatctttatc aagcactcca gcacccgcag agaagtcgtc aagcatattg 120180  
taatatctta aataactcat ttatatatta aaaaatgtca ctattaaga tggagtataa 120240  
tctttatgcc gaactaaaa aaatgacttg tggccaacc ctaagtctt ttaacgaaga 120300  
cggggatttc gtagaagttg aaccgggac atcctttaag tttctgatac ctaagggatt 120360  
ttacgctct ccttcgtaa agacgagct agtattcgag acattaacaa cgaccgataa 120420  
taaaatcact agtatcaatc caacaaatgc gccaaagtta tatcctctc aacgcaaagt 120480  
cgtatctgaa gtagtttcta atatgaggaa aatgatcgaa tcaaacgctc ctctatacat 120540  
tactcttcac ttggcggtg gatttggtaa gactattacc acgtgttatc ttatggctac 120600  
acacggtaga aaaaccgtca tttgcgtacc caataaaatg ttaatacatc aatggaagac 120660  
acaggtagag gcagtcggat tggaaacata gatattccata gatggagtaa gtagtctatt 120720  
aaaggaaact aagactcaaa gtccggatgt attaatagta gtcagtagac atctgacaaa 120780  
cgatgccttt tgtaaatata tcaataagca ttatgatttg ttcactttgg atgaatcaca 120840  
tacgtataat ctgatgaaca atacagcagt tacaagattt ttagcgtatt atcctccgat 120900  
gatgtgttat tttttaactg ctacacctag accatctaac cgaatttatt gtaacagtat 120960  
tattaatatt gccaaagtat ccaatctaaa aaaaactatc tatgcagtag atagtttttt 121020  
tgagccatat tccacagata atattagaca tatggtaaaa cgactagatg gaccatctaa 121080

|             |             |             |             |             |             |        |
|-------------|-------------|-------------|-------------|-------------|-------------|--------|
| taaatatcat  | atatataaccg | agaagttatt  | atctgtagac  | gagcctagaa  | atcaacttat  | 121140 |
| tcttgatacc  | ctggtagaag  | aattcaagtc  | aggaactatt  | aatcgcat    | tagttattac  | 121200 |
| taaactacgt  | gaacatatgg  | tattattcta  | caaacgatta  | ttagattttt  | tcggaccaga  | 121260 |
| ggttgtattt  | ataggagacg  | cccaaaatag  | acgtactcca  | gatatggcca  | aatcaatcaa  | 121320 |
| ggaactaaat  | agattttatat | tcgtatccac  | cttatttttat | tcgggtactg  | gttttagatat | 121380 |
| tcttagtttg  | gattcgttgt  | tcatttgctc  | ggcagtaatc  | aacaatatgc  | aaatagagca  | 121440 |
| attactaggg  | agggtatgtc  | gagaaacaga  | actattagat  | aggacggtat  | atgtatttcc  | 121500 |
| taacacatcc  | atcaaagaaa  | taaagtacat  | gataggaaat  | ttcatgcaac  | gaattattag  | 121560 |
| tctgtctgta  | gataaactag  | gatttaaaca  | aaaaagtatt  | cggaaacatc  | aagaatccga  | 121620 |
| tcccacttct  | gcatgtacaa  | catcatccag  | agaagaacgt  | gtattaaata  | gaatatttaa  | 121680 |
| ctcgcaaaat  | cgtaaagaag  | tttaagcgac  | gatccgcatg  | ctgcgccaggc | cagtgtatta  | 121740 |
| cccctcatag  | tattaatata  | atccaatgat  | acttttgtga  | tgctcgaaat  | cttaaccaat  | 121800 |
| ttagactgac  | aggcagaaca  | cgctatgcaa  | tcatcatcgt  | catcgataac  | tgtagtcttg  | 121860 |
| ggcttctttt  | tgcgactcct  | cattccggaa  | cgcacattgg  | tgctatccat  | ttaggtagta  | 121920 |
| aaaaataagt  | cagaatatgc  | cctataacac  | gatcgtgcaa  | aacctggtat  | atcgtctcta  | 121980 |
| tctttatcac  | aatatagtg   | atcgacattt  | ttattattat  | tgacctcgtt  | tatcttgga   | 122040 |
| catggaatgg  | gaacattttt  | gttatcaacg  | gccatctttg  | ccttaattcc  | agatgttgta  | 122100 |
| aaattataac  | taaacagtc   | atcatcgaca  | caaatgaaat  | tcttgtttag  | acgtttgtag  | 122160 |
| tttacgtatg  | cggtcgttc   | gcgtctcatt  | ttttcagata  | ttgcaggtag  | tataatatta  | 122220 |
| aaaaataagaa | tgaaataaca  | taggattaaa  | aataaagtta  | tcatgacttc  | tagcgtgat   | 122280 |
| tttaactaact | taaaagaatt  | acttagtctg  | tacaaaagtt  | tgagattttc  | agattctcgc  | 122340 |
| gctatagaaa  | agtataattc  | tttggtagaa  | tggggaacat  | ctacttactg  | gaaaataggc  | 122400 |
| gtgcaaaagg  | tagctaattg  | cgagacgtca  | atatctgatt  | attatgatga  | ggtaaaaaat  | 122460 |
| aaaccgttta  | atattgatcc  | gggctattac  | atcttcttac  | cggatatatt  | tgggagcgtc  | 122520 |
| tttattttatt | cgaagggtaa  | aaatatggta  | gaacttggat  | ctggaaactc  | ttttcaaaata | 122580 |
| ccagatgata  | tgcggaagtgc | gtgtaacaaa  | gtattagaca  | gcgataacgg  | aatagacttt  | 122640 |
| ctgagatttg  | ttttgttaaa  | caatagatgg  | ataatggaag  | atgctatata  | aaaaatcag   | 122700 |
| tctccagtta  | atataatttaa | actagctagt  | gagtagcgat  | taaacatacc  | caaataattta | 122760 |
| gaaattgaaa  | tagaggaaga  | cacattattt  | gacgacgagt  | tatactctat  | tatagaacgc  | 122820 |
| tctttcgatg  | ataaaatttc  | aaaaatatcc  | atatcgtata  | ttaggtggg   | agaacttaga  | 122880 |
| cggcaagttg  | tagacttttt  | caaattctca  | ttcatgtata  | ttgagtcct   | caaggtagat  | 122940 |
| cgtataggag  | ataatatttt  | tattcctagc  | gttataacaa  | aatcaggaaa  | aaagatatta  | 123000 |
| gtaaaagatg  | tagaccattt  | aatacgaatc  | aagggttagag | aacatacatt  | tgtaaaagta  | 123060 |
| aaaaagaaaa  | acacattttc  | catttttatac | gactatgatg  | gaaacggaac  | agaaactaga  | 123120 |
| ggagaagtta  | taaaacgaat  | tatagacact  | ataggacgag  | actattatgt  | taacggaaag  | 123180 |
| tattctctca  | agggtggtag  | tgcaaggctta | aagcaattga  | ctaataaatt  | agatattaat  | 123240 |
| gagtgcgcaa  | ctgtcgatga  | gttaggtgat  | gagattaata  | aatccggaac  | tgtaaaacga  | 123300 |
| aaaataaaaa  | accaatcagc  | atgtgattta  | agcagagaat  | gtttgggata  | tccagaagcg  | 123360 |
| gattttataa  | cgtttagtta  | taacatgcgg  | ttcaaaatag  | aaaattgtaa  | ggttgtaaat  | 123420 |
| ttcaatattg  | aaaatactaa  | ttgttttaaat | aacccgagta  | ttgaaactat  | atatggaaac  | 123480 |
| tttaaccagt  | tcgtctcaat  | ctttaatgtc  | gtcaccgatg  | tcaaaaaaag  | attattcgag  | 123540 |
| tgaaataata  | tgcgcccttg  | atataggtgc  | aaaaaatcct  | gccagaactg  | ttttagaagt  | 123600 |
| caaggataac  | tcggttaggg  | tattggatat  | atcaaaatta  | gactggaggt  | ctgattggga  | 123660 |
| aaggcgcata  | gctaaagatt  | tgccacaata  | tgaatacact  | acagttcttc  | tagaacgtca  | 123720 |
| gcctagaagg  | tcgcccgtatg | ttaaaatttat | ctatttttatt | aaaggctttt  | tatatcctac  | 123780 |
| atcggtctgc  | aaagttattt  | gcgtctcgcc  | tgctatgtct  | ggtaattcat  | atagagatcg  | 123840 |
| aaaaaagaga  | tcggtcgaag  | cattttcttga | ttggatggac  | acattcggat  | tgcgagactc  | 123900 |
| cgttcgggat  | agacgcaaat  | tagacgatgt  | agcggatagt  | ttcaatttgg  | ctatgagata  | 123960 |
| cgtatttagat | aaatggaata  | ctaattatac  | accttataat  | aggtgtaaat  | ctagaaatta  | 124020 |
| cataaaaaaa  | atgtaataac  | gttagtaacg  | ccattatgga  | taatctattt  | acctttctac  | 124080 |
| atgaaataga  | agatagatat  | gccagaacta  | tttttaactt  | tcacttaata  | agttgcgatg  | 124140 |
| aaataggaga  | tatatatggg  | cttatgaaag  | acgcattttc  | ctcagaggat  | atgtttgata  | 124200 |
| atatatgtta  | taataaagat  | atacatcctg  | ccattaagaa  | actagtgtat  | tgcgacatcc  | 124260 |
| aacttactaa  | acacattatt  | aatcagaata  | cgtatccggg  | atttaacgat  | tcttcacaag  | 124320 |
| tgaaatgttg  | tcattatttc  | gacataaact  | cagataatag  | caatatttag  | tctcgtacag  | 124380 |

|            |             |             |             |             |             |        |
|------------|-------------|-------------|-------------|-------------|-------------|--------|
| tagagatatt | tgagagggaa  | aagtcattctc | ttgtatcata  | tattaaaact  | accaataaga  | 124440 |
| agagaaaggt | caattacggc  | gaaataaaga  | aaactgttca  | tggaggcact  | aatgcaaatt  | 124500 |
| acttttcggg | taaaaagtct  | gacgagtatc  | tgagtactac  | agttagatcc  | aacattaatc  | 124560 |
| aaccttggat | caaaaccatc  | tctaagagga  | tgagagttga  | tatcattaat  | cactctatag  | 124620 |
| taacgcgtgg | aaaaagctct  | atattacaaa  | ctatagaaat  | tatttttact  | aatagaacat  | 124680 |
| gtgtgaaaat | attcaaggat  | tctactatgc  | acattattct  | atccaaggac  | aaggatgaaa  | 124740 |
| aggggtgtat | acacatgatt  | gacaaattat  | tctatgtcta  | ttataattta  | tttctgttgt  | 124800 |
| tcgaagatat | catccaaaac  | gagtacttta  | aagaagtagc  | taatgttgta  | aaccacgtac  | 124860 |
| tcacggctac | ggcattagat  | gagaaattat  | tcctaattaa  | gaaaatggct  | gaacacgtatg | 124920 |
| tttatggagt | tagcaatttc  | aaaatagggg  | tgtttaacct  | gacattttatt | aagtcgttgg  | 124980 |
| atcataccgt | tttccctct   | ctgttagatg  | aggatagcaa  | aataaagttt  | tttaagggga  | 125040 |
| aaaagctcaa | tattgtagca  | ttacgatctc  | tggaggattg  | tataaattac  | gtgactaaat  | 125100 |
| ccgagaatat | gatagaaatg  | atgaaggaaa  | gatcgactat  | tttaaatagc  | atagatatag  | 125160 |
| aaacggaatc | ggtagatcgt  | ctaaaagaat  | tgcttctaaa  | atgaaaaaaa  | acactaattc  | 125220 |
| agaaatggat | caacgactag  | ggtataagtt  | tttgggtgct  | gacctaag    | ccggagtttt  | 125280 |
| ttatagaccg | ttacatttcc  | aatatgtatc  | gtattctaat  | tttatattgc  | atcgattgca  | 125340 |
| tgaaatcttg | accgtcaagc  | ggccactctt  | atcgtttaag  | aataatacag  | aacgaattat  | 125400 |
| gatagaaatt | agcaatgtta  | aagtgactcc  | tccagattac  | tcacctataa  | tcgcgagtat  | 125460 |
| taaaggtaag | agttatgacg  | cattagccac  | gttcactgta  | aatatcttta  | aagaggtaat  | 125520 |
| gaccaaagag | ggtatatcca  | tcactaaaat  | aagtagttat  | gagggaaaag  | attctcattt  | 125580 |
| gataaaaatt | ccgtacttaa  | taggatacgg  | gaataaaaat  | ccacttgata  | cagccaagta  | 125640 |
| tcttgttctc | aatgtcatag  | gtggagtctt  | tatcaataaa  | caatctgtcg  | aaaaagtagg  | 125700 |
| aattaatcta | gtagaaaaga  | ttacaacatg  | gccaaaattt  | agggttgtta  | agccaaactc  | 125760 |
| attcactttc | tcgttttctc  | ccgtatcccc  | tcctaattgta | ttaccgacaa  | gatatcgcca  | 125820 |
| ttacaagata | tcctcggata  | tatcacaaat  | ggaagcgttg  | aatatatcat  | cgacaaagac  | 125880 |
| atttataacg | gtcaaatattg | ttttgetgtc  | tcaatattta  | tctagagtga  | gtctagaatt  | 125940 |
| cattagacgt | agtttatcat  | acgatatgcc  | tccagaagtt  | gtctatctag  | taaacgcgat  | 126000 |
| aatagatagt | gctaaacgaa  | ttactgaatc  | tattactgac  | tttaattattg | atacatacat  | 126060 |
| taatgacctg | gtggaagctg  | aacacattaa  | acaaaaatct  | cagttaacga  | ttaacgagtt  | 126120 |
| caaatatgaa | atgctgcata  | actttttacc  | tcatatgaac  | tatacacccg  | atcaactaaa  | 126180 |
| gggattttat | atgatattct  | tactaagaaa  | gtttctctac  | tgtatcttcc  | acacttctag  | 126240 |
| atatccagat | agagattcga  | tggtttgtca  | tcgcactcta  | acgtacggca  | aatattttga  | 126300 |
| gacgttggca | catgatgaat  | tagagaatta  | cataggcaac  | atccgaaacg  | atatcatgaa  | 126360 |
| caatcacaa  | aacagaggca  | cttacgcggt  | aaacattcat  | gtactaacia  | ctcccggaact | 126420 |
| taatcacgct | ttttctagct  | tattgagtgg  | aaagttcaaa  | aagtcagacg  | gtagttatcg  | 126480 |
| aacacatcct | cactattcat  | ggatgcagaa  | tatttctatt  | cctaggagtg  | ttggatttta  | 126540 |
| tccggatcaa | gtaaagattt  | caaagatggt  | ttctgtcaga  | aaataccatc  | caagtcaata  | 126600 |
| tctttacttt | tgttcatcag  | acgttccgga  | aagaggtcct  | caggtaggtt  | tagtatctca  | 126660 |
| attgtctgtc | ttgagttcca  | ttacaaatat  | actaacgtct  | gagtatttgg  | atttggaaaa  | 126720 |
| gaaaatttgt | gagtatatca  | gatcatatta  | taaagatgat  | ataagttact  | ttgaaacagg  | 126780 |
| atttccaatc | actatagaaa  | atgctctagt  | cgcatctctt  | aatccaaata  | tgatatgtga  | 126840 |
| ttttgtaact | gacttttagac | gtagaaaacg  | gatgggattc  | ttcggttaact | tggaggtagg  | 126900 |
| tattacttta | gttagggatc  | acatgaatga  | aattcgcatt  | aatattggag  | cgggagagatt | 126960 |
| agtcagacca | ttcttggttg  | tggataacgg  | agagctcatg  | atggatgtgt  | gtccggagtt  | 127020 |
| agaaagcaga | ttagacgaca  | tgacattctc  | tgacattcag  | aaagagtttc  | cgcatgtcat  | 127080 |
| cgaaatggta | gatatagaac  | aatttacttt  | tagtaacgta  | tgtgaatcgg  | ttcaaaaatt  | 127140 |
| tagaatgatg | tcaaaggatg  | aaagaaagca  | atacgattta  | tgtgactttc  | ctgccgaatt  | 127200 |
| tagagatgga | tatgtagcat  | cttcactagt  | gggaatcaat  | cacaattctg  | gaccagagac  | 127260 |
| tattcttggg | tgtgctcaag  | ctaaacaagc  | tatctcttgt  | ctgagttcgg  | atatacgaaa  | 127320 |
| taaaatagac | aatggaattc  | atttgatgta  | tccagagagg  | ccaatcgtga  | ttagtaaggc  | 127380 |
| tttagaaact | tcaaagattg  | cggctaattg  | cttcggccaa  | catgttacta  | tagcattaat  | 127440 |
| gtcgtacaaa | ggtatcaatc  | aagaggatgg  | aattatcatc  | aaaaaacaat  | ttattcagag  | 127500 |
| aggcgtctc  | cagcattgta  | cagccaagaa  | acatcaagta  | gaaattccat  | tggaaaactt  | 127560 |
| taataacaaa | gaaagagata  | ggtctaacgc  | ctattcaaaa  | ttagaaagta  | atggattagt  | 127620 |
| tagactgaat | gctttcttgg  | aatccggaga  | cgctatggca  | cgaaatatct  | catcaagaac  | 127680 |

tcttgaagat gattttgcta gagataatca gattagcttc gatgtttccg agaaatatac 127740  
cgatatgtac aaatctcgcg ttgaacgagt acaagtagaa cttactgaca aagttaaggt 127800  
acgagtatta accatgaaag aaagaagacc cattctagga gacaaattta ccactagaac 127860  
gagtcaaaag ggaacagtcg cgtatgtcgc ggatgaaacg gaacttccat acgacgaaaa 127920  
tggtatcaca ccagatgtca ttattaattc tacatccatc ttctctagaa aaactatatc 127980  
tatgttgata gaagttatct taacagccgc atattctgct aagccgtaca acaataaggg 128040  
agaaaaccga cctgtctgtt ttcttagtag taacgaaaca tccatcgata catatatgca 128100  
attcgctaaa caatgttatg agcattcaaa tccgaaattg tccgatgaag aattatcgga 128160  
taaaatcttt tgtgaaaaga ttctctatga tccgaaacg gataagcctt atgcatccaa 128220  
agtatttttt ggaccaatct attacttgcg tctgaggcat ttaactcagg acaaggcaac 128280  
cgttagatgt agaggtaaaa agacgaagct cattagacag gcgaatgagg gacgaaaacg 128340  
tggaggagggt atcaagttcg gagaaatgga gagagactgt ttaatagcgc atggtgcagc 128400  
caatactatt acagaagttt tgaagattc ggaagaagat tatcaagatg tgtatgtttg 128460  
tgaaaattgt ggagacatag cagcacaat caagggtatt aatacatgtc ttagatgttc 128520  
aaaacttaat ctctctctc tcttaacaaa aattgatacc acgcacgtat ctaaagtatt 128580  
tcttactcaa atgaacgcca gaggcgtaaa agtcaaatta gatttcgaac gaaggcctcc 128640  
ttcgttttat aaaccattag ataaagtga tctcaagcgc tcttttctgg tghtaatctc 128700  
tagtttggtg gtagatacat atcaatatca tcaaatctga gatccgaatt ataaaatggg 128760  
cgtggattgt taactataga atcggaagtc tgatattcga aaatctgtgg agtttcagggt 128820  
tttgggtggg gtgtaactgc tacttgggat actgaagtc gatattcaga aagctgtgga 128880  
tgttctgggt cggcatccac cgatgggtgc acatcactaa tccggttcgg aacgtctgtg 128940  
gatggagggt ctactctac agaactgtga gcctcagttg tcaacggaga tacattttta 129000  
atgcgagaaa atgtataatt tggtaatggg ttcttatgtg gatctgaaga agaggtaaga 129060  
tatctactag aaagataccg atcacgttct agttctcttt tgtagaactt aactttttct 129120  
ttctcggcat ctagtgtgata ttccaacctc ttcacgttac tacgttcaga ttccaattca 129180  
cgttcgcgtg gggtacctcc gcagttttta cgagcgattt caggttcagc cttcatgcgt 129240  
ctctccctct ctctatcgag ttatcagag cagtccttct gaaggcgatc gaactccata 129300  
aatttctcca acgctttgat tgtttccata gatttccgaa gttcagcttt taggaactgtg 129360  
attctttttt ctactctac acagctggat gtacaaccgt ttccattacc gccatctcta 129420  
agtttctttt ctagatcggc aacatttcat ccccatgcct ttacattcc tccagctctac 129480  
tgtcgtcgaa atatcgttcc agtctcttt cgacatcaat aacttttagc cgttgtctct 129540  
caagctctct tttgtagtta tctgattccc tggcagcttt aagatcttca tgcaattgag 129600  
tcagctctta acttctctc ttgctcttc gtcatagtag gcgcaatcac tgtgagatcc 129660  
attgttacca cgtctacact cggcgagctc gcgtttaaga gattcaattt cccgtttgta 129720  
ttggtccatg ttccattgc taccaccatt agatttacag gctgctagtt gtcgttcgag 129780  
atcagaaata cgggtttttt tggaaattgt ttctcgtatg tacttggcat cgaaacactt 129840  
attaagttct ttttccaatt ctacgatttt atttcttctc cgagtcaatt cctcctgtga 129900  
gtaactatct gttttgtcag attcacgtc tctacgtaga ctttcttgca agttactaat 129960  
ttgttcccta gcacgtccga gtttagtttt atatgctgaa tagagttctg attcatcctt 130020  
tgagcagatc tctagcgatc gtttaagatt cctaattcta gtcttttagc tatttacctc 130080  
ctcagaagat gttccggttac cgttgcgttt acactcggtt agctgtctat caagatccat 130140  
gattctatct ctaagacgtt gcattctctt ttccatatca gcattgcttt cattattacg 130200  
tctgcagtca ctcaactgtc ttccaatata tgagattcta tctctaagac gtcgcattct 130260  
tctctgtttc ggcatttggg tcattattac gtcacagtc gttcaactgt ctttcaagat 130320  
ctgatattct agattggagt ctgctaattc ctgtagcatt ttcacggcat tcaactcagt 130380  
gtctttcaag atctgaaatt ttagattgga gtctgctaatt ctctgtaaga tttcctctc 130440  
cgctctcgat gcagtcggtc aacttattct ctagtctctt aatacgcgaa cgcagtgcat 130500  
caacttcttg cgtgtcttcc tgggtgctg tacattcact gagtctagat tccgagatctc 130560  
taacgcgtcg tggttcttcc tcaagttctc tgcgtactac agaaagcgtg tcttatctt 130620  
gttgatattt agcaatttct gattctagag tactgatttt gcttacgtag ttactaatat 130680  
ttgtcttggc cttatcaaga tctccttgt atttgcgca ttccttgata tccctacgaa 130740  
gtctggacag ttccattcg acattacgac gtttctcgat ttcagctcgg agatcgtcat 130800  
cgcgttgttt tagccacata cgactgagtt caagttctcg ttgacaagat ccatctactt 130860  
ttccattcct aatagtatcc agttcctttt ctagtcttga acgcatttct cgttccctat 130920  
caagcgattc tctcaattct cggatagttt tcttatcaat ttctaataaa tctgaaccat 130980

catctgtccc attttgaata tccctgtggt ctttgatctc ttttgtaagt cggctcgattc 131040  
tttcgggtttt ataaacagaa tccctttcca aagtcctaatt cttactgagt ttatcactaa 131100  
gttctgcatt caattcgggt agttttctct tggcttcttc caactctggt taaactctc 131160  
cactatttcc gcatttctc tcgcatttat ctaaccattc aattagttta ttaataacta 131220  
gttggtaatc agcgattcct atagcgttc ttgtaattgt gggaacataa ttaggatctt 131280  
ctaattggatt gtatggcttg atagcatcat ctttatcatt attaggggga tggacaacct 131340  
taattgggtg gtcctcatct cctccagtag cgtgtgggtt ttcaatacca gtgttagtaa 131400  
taggcttagg caaatgcttg tcgtacgcgg gcacttcttc atccatcaag tatttataat 131460  
cgggttctac ttcagaatat tcttttctaa gagacgcgac ttccgggagt agtagaagaa 131520  
ctctgtttct gtatctatca acgctggaat caatactcaa gttaaggata gcgaatacct 131580  
catcgtcatc atccgtatct tctgaaacac catcatatga catttcatga agtctaact 131640  
attgataaat agaattcagat ttagtattaa acagatcctt aaccttttta gtaaaccgat 131700  
atgtatattt tagatctcca gatttcataa tatgatcaca tgccttaaat gtcagtgtt 131760  
ccatgatata atctggaaca ctaatgggtg acgaaaaaga tacagcacca tatgctaact 131820  
tgataaataa atctgaacca ctaagtagat aatgattaat gttaagaaag aggaaatatt 131880  
cagtgtatag gtatgtcttg gcgtcatatc ttgtactaaa cagcctaaac agtttggtta 131940  
tgtgatcaat ttccaataga ttaattagag cagcgggaat accaacaac atattaccac 132000  
atccgtattt tctatgaata tcacatatca tgttaaaaaa tcttgataga agagcgaata 132060  
tctcgtctga cttaatgagt cgtagtctag cagcaacata agtcataact gtaaatagaa 132120  
catactttcc tgtagtgttg attctagact ccacatcaac accattatta aaaatagttt 132180  
tatatacatc tttaattctg tctccgttaa tcgtcgaaac ttctagtata cggaaacact 132240  
ttgatttctt atctgtagtt aatgacttag tgatatcagc aagaatatta cgaattacat 132300  
ttcttggttt tcttgagaga cctgattcag aactcaactc atcgttccat agtttttcta 132360  
cctcagtggc gaaatctttg gagtgtctgg tacatttttc aataagggtc gtgacctcca 132420  
tttattataa aaaatttatt caaaacttaa ctacaatcgg gtaattataa aatcgtagat 132480  
ctcccatgtg gcggaatact accatctatc gcatgtggat ggacagtagg taatggccat 132540  
gggaacagta atgtttgcat atttatcttt cttgccagta ttactgcata ttgtcccaat 132600  
aaatgaagat gatgtcttaa cctatcaact gccgtgtat cacaacaata gtgtccgatg 132660  
tcttttttga caggatcagg ttcttctaca ggaagaagtt tcggcctctt atgatattca 132720  
tgtctgggaa acggtggtct aggggtgaggc tccgggtatc gagtgggttt tggattataa 132780  
tcatcatcgt ctatgacatc atcttctgact tcgatattta ttttgctatc ttgatgatgt 132840  
cctgtatcag ttgcattttc agcactcgac tgaatattag cgcattcatt gtctattatt 132900  
accatatttc taaacccaaa atgtatgtgt tgaacatcag tactatcgtt gatgagtctt 132960  
atagcatgaa ttcgcttatc gttatcgggt ttactctctg tcaccttagc aattcctttt 133020  
ttattaaact ctacataatc atatccattt ctattgtttg ttctaataa aacgagtata 133080  
gcatcattgc taaatttttc aatagtatca aaaacagaat atcctaacc atataatata 133140  
tattcaggaa cactcaaaact aaatgtccag gattctctca aatacgtaaa ctttaatagt 133200  
gcgaaatcat tcaaaaatct accacttata gatagatagt acataaatgc gtatagtagt 133260  
ctacctatct ctttattatg aaaaccggca ttacgatcat atatgtcgtg atatacctgt 133320  
gatccgttta cgttaaacca taaatacatg ggtgatccta taaacatgaa tttatttcta 133380  
attctcagag ctatagttaa ttgaccgtgt aatatttgct tacatgcata cttgatcgc 133440  
ttattaataa gattttttatc attgtctggt atttcagaat cgtatatata aggagtacca 133500  
tcgtgattct taccagatat tatacaaaat actatatata aaatatattg acccagttta 133560  
gtaatcatat aaatgtttta cgttttaaat tttgtattca atgatccatt atcatacgtt 133620  
atcatggtct tgaattatct attctttaa atataatatt gtgttagcca ttgcattgga 133680  
gctcctaagt gagattttct attctcatcc atttttaggt aggttttcat aaagtcoccta 133740  
ataacttctg gaataatgtt tctatgtttt ctactgatgc atgtatttgc ttcgattttt 133800  
ttatcccatg tttcatctat catagattta aacgcagtaa tgctcgcaac attaacatct 133860  
tgaaccgttg gtacaattcc gttccataaa tttataatgt tcgatattga ggcgtccagt 133920  
tttttgaata tacttttaaa taacaaaaga gttaagttac tcatatggac ggcgtccagt 133980  
ctgaacatca atcttttttag ccagagatat catagccgct cttagagttt cagcgtgatt 134040  
ttccaacct aatagaactt catcgttgcg tttacaacac ttttctattt gttcaaaact 134100  
tgttgttaca ttagtaactt ttttttcaa attagtttag cgttggttga gaggttctc 134160  
attgtcgtct tcatcggctt taacaattgc ttcgcgttta gcctctggct ttttagcagc 134220

ctttgtagaa aaaaattcag ttgctggaat tgcaagatcg tcatctccgg ggaaaagagt 134340  
tccgtccatt taaagtacag attttagaaa ctgacactct gcgttattta tatttggtac 134400  
aacacatgga ttataaatat tgatgttaat aacatcagaa aatgtaaagt ctatacattg 134460  
ttgcatcggtg ttaaattttc taatggatct agtattattg ggtccaaact ctgectgaaa 134520  
tccaaatatg gaagcggata caaaaccggt tccctggataa accacacatc tccacttttg 134580  
ctttacatca gaaattgtgt cgttgacatc ttgaactctc ctatctaagt ccggtgttcc 134640  
acctatagat tttgaatatt cgaatgctgc atgagtagca ttaaattcct taatattgcc 134700  
ataattttca tatattgagt aaccctggat aaaaagtaaa cacaccgcag ccgtcgtac 134760  
cacaataaaa aaaattgata gagagttcat ttataatcta ttagaagctg acaaaatttt 134820  
tttacacgca tcagacaatg ctttaataaa tagttcaaca tctacttttg tcatatcgaa 134880  
ccgatggat gattctaacc tagaattaca tccgaaaaag ttgactatgt tcatagtcat 134940  
taagtcatta acaacaaca ttccagactc tggattataa gacgatactg tttcgtcaca 135000  
ataactacc ttaatcatgt gattatgaat attggctatt agagcacctt ctaagaaatc 135060  
tataatatct ttgaacacg atttaaaaac aaaccacgaa tataacttcta cgaagaaagt 135120  
tagtttacc ataggagaaa taactataaa tggagatcta aatacaaaat ccggtatctat 135180  
gatagtttta acattattat attctctatt aaatacctcc acatctaaaa atgttaattt 135240  
tgaaactatg tcttcgttta ttaccgtacc tgaactaaac gctataagct ctattgtttg 135300  
agaactcttt aaacgatatt cttgaaatac atgtaacaaa gtttccttta actcggctcg 135360  
tttatctacc atagttacag aatttgtatc cttatctata atataataat caaaatcgta 135420  
taaagttata taattatcgc gttcagattg ggatcttttc aaatagacta aaaaccccat 135480  
tctctagta agtatcttat gtatatgttt gtaaaatata ttcattgggtg gaatatgctc 135540  
taccgcagtt agccattcct cattgacagc ggtagatgta ttagacaaaa ctattccaat 135600  
gtttaacaag ggccatttta cgagattatt aaatccttgt ttgataaatg tagccaatga 135660  
gggttcgagt tcaacgacga ttgaattctc tcccgcgga tgcgtcatga tgaacgacgg 135720  
gatgttggtc gattgatttg gaattctttt tgcacttttt gtttatatta aatattttta 135780  
aatttatagc ggatagcaat tcatgtacca cggataatgt agacgcgtat tgcgcatega 135840  
tatctttatt attagataaa tttatcaata aatgtgagaa gtttgccctg ttaaggtctt 135900  
ccatttaaat attatataaa catttgtgtt tgaacttat tgcgtcttta tggaaatagt 135960  
ttttactagt aaagctgcaa ttacacactt tgcctgtaaa acataaaat aaacaccagc 136020  
ttttatcaat cgttccaaaa agtcgacggc ggacattttt aacatggcat ctatttttaa 136080  
tacacttagg tttttgga aaacatcatt ttataattgt aacgattcaa taactaaaga 136140  
aaagattaag attaaacata agggatgtc atttgtat ttataagcaa agcattctac 136200  
cggtgttaaa tacttgtctg gaggaggtat atatcatgat gatttggttg tattggggaa 136260  
ggtaacaatt aataatctaa agatgatgct attttacatg gatttatcat atcatggagt 136320  
gacaagtagt ggagcaattt acaaattggg atcgtctatc gatagacttt ctctaaatag 136380  
gactattgtt acaaaagtta ataattatga tgatacattt tttgacgacg atgattgatc 136440  
gctattgcac aattttgttt ttgtacttte taatatagtg tttaggttct ttttcatatg 136500  
agaatatgta tttactaaaa tatctatggt taacttttgt tctatgacgt ccttatcggc 136560  
ggtatcggt catatacgta attcaccttc acaaaatagc gagtcttcga taataatagc 136620  
caatcgatta ttggatctag ctgtctgtat catattcaac atgtttaata tatcctttcg 136680  
tttcccttt acaggcacg atcgtagcat attttccgcg tctgatatgg aaatgttaaa 136740  
actacaaaaa tgcgtaatgt tagcccgctc taatattggt acgtgtctat aagtttggca 136800  
tagtagaata atagacgtgt ttaaatgcct tccaaagttt aagaattcta ttagagtatt 136860  
gcattttgat agtttatcgc ctacatcatc aaaaataagt aaaaagtgtg ctgatttttt 136920  
atgattttgt gcgacagcaa tacatttttc tatgttactt ttagttcgta tcagattata 136980  
ttctagatgt tcttgactac taacgaaatt aatatgattt ggccaaatgt atccatcata 137040  
atctgggtta taaacgggtg taaacaagaa tatatgttta tattttttta ctagtgtaga 137100  
aaacagagat agtaaataga tagtttttcc agatccagat cctcctgtta aaaccattct 137160  
aaacggcatt ttttaataaat tttctcttga aaattgtttt tcttggaac aattcataat 137220  
tatattttaca gttactaaat taatttgata ataaatcaaa atatggaaaa ctaaggttgt 137280  
tagtagggag gagaacaaag aaggcacatc gtgatataaa taacatttat tatcatgatg 137340  
acaccagaaa acgacgaaga gcagacatct gtgttctccg ctactgttta cggagacaaa 137400  
attcagggaa agaataaacg caaacgcgtg attggctcat gtattagaat atctatggtt 137460  
atttcactac tatctatgat taccatgtcc gcgtttctca tagtgcgcct aaatcaatgc 137520  
atgtctgcta acgaggtgc tattactgac gccgtgttg ccgttgctgc tgcateatct 137580

|             |             |             |             |            |             |        |
|-------------|-------------|-------------|-------------|------------|-------------|--------|
| actcatagaa  | agggttgcgtc | tagcactaca  | caatatgata  | acaaagaaag | ctgtaatggt  | 137640 |
| ttatattacc  | aggggttcttg | ttatatatta  | cattcagact  | accagttatt | ctcggatgct  | 137700 |
| aaagcaaatt  | gcactgcgga  | atcatcaaca  | ctaccaata   | aatccgatgt | cttgactacc  | 137760 |
| tggctcattg  | attatgttga  | ggatacatgg  | ggatctgatg  | gtaatccaat | tacaaaaact  | 137820 |
| acatccgatt  | atcaagattc  | tgatgtatca  | caagaagtta  | gaaagtattt | ttgtgttaaa  | 137880 |
| acaatgaact  | aatatattt   | tttgtacatt  | aataaatgaa  | atcgcttaat | agacaaaactg | 137940 |
| taagtagggt  | taagaagtgt  | tcgggtgccg  | ccgctataat  | gatgatactc | tcaaccatta  | 138000 |
| ttagtggcat  | aggaacattt  | ctgcattaca  | aagaagaact  | gatgcctagt | gcttgcgcca  | 138060 |
| atggatggat  | acaatacgat  | aaacattggt  | atttagatac  | taacattaaa | atgtctacag  | 138120 |
| ataatgcggt  | ttatcagtg   | cgtaaatatc  | gagctagatt  | gcctagacct | gatactagac  | 138180 |
| atctgagagt  | attgtttagt  | attttttata  | aagattattg  | ggtaagttaa | aaaaagacca  | 138240 |
| atgataaatg  | gttagatatt  | aataatgata  | aagatataga  | tattagtaaa | ttaacaaatt  | 138300 |
| ttaaacaact  | aaacagtagc  | acggatgctg  | aagcgtgtta  | tatatacaag | tctggaaaac  | 138360 |
| tgggttaaaac | agtatgtaaa  | agtactcaat  | ctgtactatg  | tgttaaaaaa | ttctacaagt  | 138420 |
| gacaacaaaa  | aatgaattaa  | taataagtcg  | ttaacgtacg  | ccgccatgga | cgccgcgttt  | 138480 |
| gttattactc  | caatgggtgt  | gttgactata  | acagatacat  | tgtatgatga | tctcgatctc  | 138540 |
| tcaatcatgg  | acttttatag  | accatacatt  | ataggttaaca | taaaaactgt | ccaaatagat  | 138600 |
| gtacgggata  | taaaaatattc | cgacatgcaa  | aaatgctact  | ttagctataa | gggttaaaata | 138660 |
| gttctcagg   | attctaatga  | tttggctaga  | ttcaacattt  | atagcatttg | tgccgcatac  | 138720 |
| agatcaaaaa  | ataccatcat  | catagcatgc  | gactatgata  | tcatgttaga | tatagaagat  | 138780 |
| aaacatcage  | catttttatct | attcccatct  | attgatgttt  | ttaacgctac | aatcatagaa  | 138840 |
| gcgtataacc  | tgtatacagc  | tggagattat  | catctaatac  | tcaatccttc | agataaatctg | 138900 |
| aaaatgaaat  | tgtcgtttta  | ttcttcattc  | tgcatatcag  | acggcaatgg | atggatcata  | 138960 |
| attgatggga  | aatgcaatag  | taatttttta  | tcataaaaag  | tgtaaagtaa | ataataaaaac | 139020 |
| aataaaattt  | gaactagtag  | tacgtatatt  | gagcaatcag  | aaatgatgct | ggtaacctctt | 139080 |
| atcacggtga  | ccgtagtgtc  | gggaacaata  | ttagtatgtt  | atatattata | tattttagag  | 139140 |
| aaaaagatac  | gtactgtcta  | taatgacaat  | aaaattatca  | tgacaaaatt | aaaaaagata  | 139200 |
| aagagttcta  | attccagcaa  | atctagttaa  | tcaactgata  | gcgaatcaga | ctgggaggat  | 139260 |
| cactgtagtg  | ctatggaaca  | aaacaatgac  | gtagataata  | tttctaggaa | tgagatattg  | 139320 |
| gacgatgata  | gcttcgctgg  | tagtttaata  | tgggataacg  | aatccaatgt | tatggcgctt  | 139380 |
| agcacagaac  | acattttacga | tagtggtgct  | ggaagcacgc  | tgctaataaa | taatgatcgt  | 139440 |
| aatgaacaga  | ctattttatca | gaacactaca  | gtagtaatta  | atgaaacgga | gactgttgaa  | 139500 |
| gtacttaatg  | aagataccaa  | acagaatcct  | aactattcat  | ccaatccttt | cgtaaaattt  | 139560 |
| aataaaacca  | gtattttag   | caagtcaaat  | ccgtttatta  | cagaacttaa | caataaattt  | 139620 |
| agtgagaata  | atccgttttag | acgagcacat  | agcgatgatt  | atcttaataa | gcaagaacaa  | 139680 |
| gatcatgaac  | acgatgatat  | agaatcatcg  | gtcgtatcat  | tggtgtgatt | agtttctctt  | 139740 |
| ttataaaatt  | gaagtaatat  | ttagtattat  | tgctgccgtc  | acgttgtaca | aatggagata  | 139800 |
| ttccctgtat  | tcggcatttc  | taaaatttagc | aattttattg  | ctaataatga | ctgtagatat  | 139860 |
| tatatagata  | cagaacatca  | aaaaattata  | tctgatgaga  | tcaatagaca | gatggatgaa  | 139920 |
| acgggtacttc | ttaccaacat  | cttaagcgta  | gaagttgtaa  | atgacaatga | gatgtaccat  | 139980 |
| cttattcttc  | atagattatc  | gacgattata  | ctctgtatta  | gttctgtcgg | aggatgtgtt  | 140040 |
| atctctatag  | ataatgacat  | caatggcaaa  | aataattctaa | cctttcccat | tgatcatgct  | 140100 |
| gtaatcatat  | ccccactgag  | taaatgtgtc  | gtagttagca  | agggtcctac | aaccatattg  | 140160 |
| gttggttaaag | cggatatacc  | tagcaaacga  | ttggtaacat  | cgttttacaa | cgacatacta  | 140220 |
| tatgtaaaca  | atctgtcact  | gattaattat  | ttgccgttgt  | ctgtattcat | tattagacga  | 140280 |
| gtcaccgact  | atttggtatg  | acacatatgc  | gatcagatat  | ttgctaataa | taagtgggat  | 140340 |
| tcccttataa  | ccatcgacga  | taagcaatat  | cctattccat  | caaactgtat | aggatgtgcc  | 140400 |
| tctgccaaagt | acataaattc  | tagcatcgag  | caagatactt  | taatccatgt | ttgtaacctc  | 140460 |
| gagcatccgt  | tgcactcagt  | atacaaaaaa  | atgcagtcgt  | acaattctct | acctatcaag  | 140520 |
| gaacaaatat  | tgtacggtag  | aattgataat  | ataaatatga  | gcattagtat | ttctgtggat  | 140580 |
| taatagattt  | ctagtatggg  | gatcattaat  | catctctaata | ctctaaatac | ctcataaaac  | 140640 |
| gaaaaaaaag  | ctattatcaa  | atactgtacg  | gaatggattc  | attctctctt | ctttttatga  | 140700 |
| aactctgttg  | tatatctact  | gataaaaactg | gaagcaaaaa  | atctgataga | aagaataaga  | 140760 |
| ataagatcaa  | ggattatatg  | gaacacgatt  | attataaaat  | aacaatagtt | cctgggtcct  | 140820 |
| cttocacgto  | tactagctcg  | tgggtattata | cacatgccta  | gtaatagtct | ctttgcgttg  | 140880 |

acggaaagca gactagaaat aacaggctaa aatgttcaga caccataata gttcccaacc 140940  
cagataataa cagagttcca tcaacacatt cctttaaact caatcccaa cccaaaaccg 141000  
ttaaaatgta tccggccaat tgatagtaga taatgaggtg tacagcgcat gataatttac 141060  
acagtaacca aaatgaaaat actttagtaa ttataagaaa tatagacggg aatgtcatca 141120  
tcaacaatcc gataatatgc ctgagagtaa acattgacgg ataaaaaaa aatgctccgc 141180  
ataactctat catggcaata acacaaccaa atacttgtaa gattcctaaa ttagtagaaa 141240  
atacaacgaa tatcgatgta taagtgatct cgagaaataa taagaataaa gtaatgcccg 141300  
taaagataaa catcaacatt gtttggtaat cattaaacca attagtatga agttgaacta 141360  
atctcacagt agattttatt ccagtgttat cctcgcatgt ataagtacct ggtaagatat 141420  
ctttatattc cataatcaat gagacatcac tatctgataa cgaatgaagt ctagcactag 141480  
tatgccattt acttaatat gtcgtcttgg aagttttatt ataagttaaa atatcatggt 141540  
tatccaattt ccatctaata tactttgtcg gattatctat agtacacgga ataagtatgg 141600  
tatcattaca tgcgttatat tctatggtct ttgtagttgt tataacaacc aacgtataga 141660  
ggatatcaaa cgatattcta actcttgaca ttttttattt atttaaaatg atacctttgt 141720  
tattttattt attctatttt gctaaccggt ttgaatggca taagtttgaa acgagtgaag 141780  
aaataatttc tacttactta ttagacgacg tattatacac ggggtgtaat ggggcggtat 141840  
acacattttc aaataataaa ctaaacaaaa ctgggtttaac taataataat tatataacaa 141900  
catctataaa agtagaggat gcggaataagg atacattagt atgcggaacc aataacggaa 141960  
atcccaaagt ttggaaaata gacggttcag acgacccaaa acatagaggt agaggatagc 142020  
ctccttatca aaatagcaaa gtaacgataa tcagtcacaa cggatgtgta ctatctgaca 142080  
taaacatata aaaagaagga attaaacgat ggagaagatt tgacggacca tgtggttatg 142140  
atctattcac ggcggataac gtaattccaa aagatgggtt acgaggagca ttcgtcgata 142200  
aagacggtac ttatgacaaa gtttacattc ttttactga tactatcggc tcaaagagaa 142260  
ttgtcaaaat tccgtatata gcacaaatgt gctaaaacga cgaagggtgg ccacatcat 142320  
tgtctagtea tagatggtcg acgtttctca aagtcgaatt agaagtgat atcgacggaa 142380  
gaagttatag acaaaattatt cattctagaa ctataaaaa acgataatgat acgatactat 142440  
atgtattctt cgatagtcct tattccaagt ccgcattatg tactattctt atgaatacca 142500  
ttaaacaatc tttttctacg tcaaaattgg aaggatatac aaagcaattg ccgtctccag 142560  
ctcctggtaa atgtttacca gctggaaaag ttgttccaca taccacgttt gaagtcatag 142620  
aacaatataa tgtactagat gatattataa agcctttatc taaccaacct atcttcgaag 142680  
gaccgtctgg tgttaaatgg ttcgatataa aggagaagga aaatgaacat cgggaatata 142740  
gaatatactt cataaaagaa aattctatat attcgttcga taaaaatct aaacaaactc 142800  
gtagctcgca agtcgatgcg cgactatttt cagtaatggg aactgcgaaa ccgttattta 142860  
tagcagatat agggatagga gtaggaaatgc cacaatgaa aaaaataactt aaatgtaat 142920  
cttaatcgag tacaccacac gacaatgaac aaacataaga cagattatgc tggttatgct 142980  
tgctcggtaa tatgcggtct aattgtcgga attattttta cagcgacact attaaaagtt 143040  
gtagaacgta aattagttca tacaccatta atagataaaa cgataaaaaga tgcataatatt 143100  
agagaagatt gtccactga ctggataagc tataataata aatgtatoca tttatctact 143160  
gatcgaaaaa cctgggagga aggacgtaat gcatgcaaag ctctaaattc aaattcggat 143220  
ctaattaaga tagagactcc aaacgagtta agttttttta gaagccttag acgaggctat 143280  
tggttaggag aatccgaaat attaaaccag acaaccccat ataattttat agctaagaat 143340  
gccacgaaga atggaactaa aaaacggaaa tatattttgt gcacaacgaa tactcccaa 143400  
ctgcattcgt gttacactat ataacaatta cactacattt ttatcatacc actacttcgg 143460  
ttagatggtt tagaaaaaaa taaatatcgc cgtaccgttc ttgtttttat aaaaaataca 143520  
attaacaatt atcaaatatt ttctttaata ttttaccgtg ttgaccattc ttgggtgtaa 143580  
aataatctct tagtggtgga atggaatgct gtttaatgtt tccacactca tegtatattt 143640  
tgacgtatgt agtcacatcg ttacgcaat agtcagactg tagttctatc atgcttctta 143700  
catcagaagg aggaacagtt ttaaagtctc ttgggtttta tctattaccg ttagttttca 143760  
tgaaatcctt tgttttatcc acttcacatt ttaaataaat gtccactata cattcttttg 143820  
ttaattttac tagatcgtea tgggtcatag aatttatagg ttccgtagtc catggatcca 143880  
aaactagcaa ctctcgctat acggtatcgc gattagtgt taaccaact gtatgaaaat 143940  
taagaaaaaa gtttaataaa tcaacagaaa tatttaattc tccgtttgat acagatgcgc 144000  
catattttat gatttcggat tcacacgttg tttgtctgag gtgttcgtct agtgttgctt 144060  
ctacgtaaac ttcgattccc atatatctct tattgtcaga atcgcatacc gatttatcat 144120  
catacactgt ttgaaaacta aatggtatag acatcaaaat aataaataat aacgagtaca 144180

ttctgcaata ttgttatcgt aattggaaaa atagtgttcg agtgagttgg attatgtgag 144240  
tattggattg tatattttat tttatatattt gtaataagaa taaaatgcta atgtcaagtt 144300  
tattccaata gatgtccttat taaaaacata tataataaat aacaatggct gaatggcata 144360  
aaattatcga ggatatctca aaaaataata agttcgagga tgcgcgcac gttgattaca 144420  
agactacaaa gaatgttcta gctgctattc ctaacagAAC atttgccaag attaataccg 144480  
gtgaaattat tctctctatc actaatcgta atattctaaa acctcttatt ggtcagaaat 144540  
attgtattgt atatactaac tctctaattg atgagaacac gtatgctatg gagtgtctta 144600  
ctgggtacgc cctgtatct ccgacgtgta tagcgagaac tcataaccgca cttaattttt 144660  
tgatgggtaa gccacaaca tccagacgtg acgtgtatag aacgtgtaga gatcacgcta 144720  
cccggttagc tgcaactggt aattaaaata aaaagtaata ttcatatgta gtgtcaattt 144780  
taaatgatga tgatgaaatg gataatatcc atattgacga tgtcaataat gccggtattg 144840  
gcatacagtt catcgatttt tagatttcat tcagaggatg tggaaattatg ttatgggcat 144900  
ttgtattttg ataggatcta taatgtagta aatataaaat ataataccgca tattccatat 144960  
agatataaatt ttattaatcg cacgttaacc gtagatgaac tagacgataa tgtctttttt 145020  
acacatgggt attttttaaa acacaaatat gggtcactta atcctagttt gattgtctca 145080  
ttatcaggaa acttaaaata taatgatata caatgctcag taaatgtatc gtgtctcatt 145140  
aaaaatttgg caacgagtca atctactata ttaacatcta aacataagac ttattctcta 145200  
catcggtcca cgtgtattac tataatagga taccgattcta ttatatggta taaagatata 145260  
aatgacaagt ataatagacat ctatgatttt actgcaatat gtatgctaatt agcgctctaca 145320  
ttgatagtga ccatatacgt gtttaaaaaa ataaaaatga actcttaatt atgctatgct 145380  
attagaaatg gataaaatca aaattacggt tgattcaaaa attggtaatg ttgttaccat 145440  
atcgataaac ttggaaaaga taactattga tgtcacacct aaaaagaaaa aagaaaagga 145500  
tgtattatta gcgcaatcag ttgctgtcga agaggcaaaa gatgtcaagg tagaagaaaa 145560  
aaatattatc gatattgaag atgacgatga tatggatgta gaaagcgcat aatacgatct 145620  
ataaaaataa gtatataaat actttttatt tactgtactc ttactgtgta gtggtgatac 145680  
cctactcgat tattttttta aaaaaaaat acttattctg attcttctaa ccatttccgt 145740  
gttcgttcga atgccacatc gacgtcaaag ataggggaggt agttaaaatc tagttctgca 145800  
ttgttggtac acaccttaaa tgtagtgttg gatattctca acgtatagtt gttgagtagt 145860  
gatggttttc taaatagaat tctcttcata tcattcttgc acgctacat ttttagcatc 145920  
catcttgga accttaactt tcgagggttat tggttgtgga tcttctacaa tatctatgac 145980  
tctgatttct tgaacatcat ctgcactaat taacagtttt actatatacc tgcctagaaa 146040  
tccggcacca ccagtaaccg cgtacacggc cattgctgcc actcataata tcagaactact 146100  
tattctattt tactaaataa tggctgtttg tataatagac cagcataata tcagaggagt 146160  
tatttacttt gaaccagtc taaagtttta taaagtttta ggatcagtta ttggattaaa 146220  
atccggaacg tatagtttga taattcatcg ttacggagat attagtcaag gatgtgattc 146280  
cataggcagt ccagaaatat ttatcggtta catctttgta aacagatatg gtgtagcata 146340  
tggtttattta gatacagatg taaatatatc tacaattatt ggaaaggcgt tatctatttc 146400  
aaaaaatgat cagagattag cgtgtggagt tattgggtatt tcttacataa atgaaaagat 146460  
aatacatttt cttacaatta acgagaatgg cgtttgatat atcagttaat gcgtctaaaa 146520  
caataaatgc attagtttac ttttctactc agcaaaataa attagtata cgtaatgaag 146580  
ttaatgatac acactacact gtcgaatttg atagggacaa agtagttgac acgtttattt 146640  
catataatag acataatgac accatagaga taagagggtt gcttcagag gaaactaata 146700  
ttggttgccg gggttaatac cgggttagta tgacttactt gtataataag tatagtttta 146760  
aactgatttt agcagaatat ttggctatta aacagtatgg agacattgat ctattatttt 146820  
cattgatgac actagatgat ttggctatta aacagtatgg agacattgat ctattatttt 146880  
atgagaaact taaagtagac tccgattcgg gactatttga ctttgtcaac tttgtaaagg 146940  
atatgatatg ttgtgattct agaatagtag tagctctatc tagtctagta tctaaacatt 147000  
gggaattgac aaataaaaaa tataggtgta tggcattagc cgaacatata tctgatagta 147060  
ttccaatatc tgagctatct agactacgat acaactctat taagtatcta cgcggacaca 147120  
ctgagagcat agaggataaa ttgtattatt ttgaagacga tgattcgtct acatgttctg 147180  
ccgtaaccga cagggaacag gatgtataat tttttttata gcgtgaagga tatgataaaa 147240  
aatataattg ttgtattttat cccattccaa tcaccttata tgattctgta acacaataaa 147300  
ggagtcttat agatgtatag aggtcagata ctggtttgat aaactgttta ttccacataa 147360  
gtatgtttga ctttatgggt agacccgcat actttaacaa atcactgaaa attggagtta 147420  
ggtattgacc tctcagaatc agttgccggt ctggaacatt aaatgtattt tttatgatat 147480

actccaacgc atttatgtgg gcatacaaca agtcattact aatggaatat tccaagagtt 147540  
ttagttgtct agtattttaac aagagaagag atttcaacag actgtttatg aactcgaatg 147600  
ccgcctcatt gtcgcttata ttgatgatgt cgaattctcc caatatcatc accgatgagt 147660  
agctcatctt gttatcgagg tccaagtttt ctaaagatgt cattaaaccc tcgatcatga 147720  
atggatttat catcatcggt tttatgttgg acatgagctt agtccgtttg tccacatcta 147780  
tagaagatga tttctgaatt atttcatata tctctctctt taactccagg aacttgtcag 147840  
gatggctctac tttaatatgt tctcgtctaa gagatgaaaa tctttggatg gttgcacgcg 147900  
acttttctct aaaggatgac gttgccccag atcctctctt aaatgaatcc atcttatcct 147960  
tggacaagat ggacagtcct ttttccttag atggtttaat atttttgtta cccatgatct 148020  
ataaaggtag acctaactcg ctcgatgac catatattta ttttcagttt tattatacgc 148080  
ataaattgta aaaaatatgt taggtttaca aaaaatgtctc gtggggcatt aatcggtttt 148140  
gaaggattgg acaaatctgg aaaaacaaca caatgtatga acatcatgga atctataccg 148200  
gcaaacacga taaaatatct taactttcct cagagatcca ctgtcactgg aaaaatgata 148260  
gatgactatc taactcgtaa aaaaacctat aatgatcata tagttaatct attattttgt 148320  
gcaaattagat gggagtttgc atcttttata caagaacaac tagaacaggg aattacttta 148380  
atagttgata gatacgcat tcttgagta gcgtatgccg ccgctaaagg cgcgtcaatg 148440  
actctcagta agagttatga atctggattg cctaaccocg acttagttat attcttggaa 148500  
tctggtagca aagaaattaa tagaaacgtc ggcgaggaaa tttatgaaga tgttacattc 148560  
caacaaaagg tattacaaga atataaaaa atgattgaag aaggagatat tcattggcaa 148620  
attatttctt ctgaattcga ggaagatgta aagaaggagt tgattaagaa tatagttata 148680  
gaggctatac acacggttac tggaccagt gggcaactgt ggatgtaata gtgaaattac 148740  
attttttata aatagatgtt agtacagtgt tataaatgga tgaagcatat tactctggca 148800  
acttggaaac agtactcga tacgtgtccg atatgcatac cgaactcga tcaatatctc 148860  
aattagttat tgccaagata gaaactatag ataattgatat attaaacaag gacattgtaa 148920  
atttttatcat gtgtagatca aacttgata atccatttat ctctttccta gatactgtat 148980  
atactattaa aaataactag ttataagttt gaatccgtca attttgattc caaaattgaa 149040  
tggactgggg atggtctata caatatatcc cttaaaaatt atggcatcaa gacgtggcaa 149100  
acaatgtata caaatgtacc agaaggaaaca taacacatat ccgcatttcc aaagaatgat 149160  
ttcgtatctt tctgggttaa atttgaacaa ggcgattata aagtggaga gtattgtacg 149220  
ggactatgcg tcgaagtaaa aattggacca ccgactgtaa cattaactga atacgacgac 149280  
catatcaatt tgtacatcga gcatccgtat gctactagag gtacgaaaaa gattcctatt 149340  
tacaaacgcg gtgacatgtg tgatatctac ttgttgata cggctaactt cacattcgga 149400  
gattctaaag aaccagtacc atatgatatc gatgactacg attgcacgtc tacagggttg 149460  
agcatagact ttgtcacaac agaaaaagt tgcgtgacag cacagggagc cacagaaggg 149520  
tttctcga aaattactcc atggagtctc aaagtatgtc tgacaccta aaagagtgt 149580  
tatacatgcg caattagatc caaagaagat gtcccaatt tcaaggacaa aatggccaga 149640  
gttatcaaga gaaaatttaa tacacagtct caatcttatt taactaaatt tctcggtagc 149700  
acatcaaatt atgttaccac ttttcttagc atgcttaact tgactaaata ttcataatta 149760  
ttttttatta atgatacaaa aacgaaataa aactgcata tatcactgg ttaacgcctt 149820  
tataggctct aaccattttc aagatgaggt ccctgattat agtccttctg ttccctcta 149880  
tcatctactc catgtctatt agacgatgtg agaagactga agaggaaaca tggggattga 149940  
aaataggggt gtgtataatt gccaaagatt tctatcccg aagaactgat tgcagtgttc 150000  
atctcccaac tgcaagtga ggattgataa ctgaaggcaa tggattcagg gatatacga 150060  
acaccgataa attataaaaa aagcaatgtg tccgtgttt ccgttaataa tactattttc 150120  
gtaactggcg gattattcat aaataactct aatagcacga tctgggacat ttataaagac 150180  
aaacaatgg cgattataga aatggctagg gtatatcacg gcatcgactc gacatttgg 150240  
atgttatatt ttgccggagg tctatccgtt accgaacaat atggttaatt agagaaaaa 150300  
aacgagatat cttgttacaa tctagaacg ataagtgggt ttgatatttc atactattt 150360  
tataagatat ccatatcatc attgtgtaaa ctaaaataacg tcttctatgt atttagtaag 150420  
gacattggat atgtggaaaa gtatgatggt gcattggaagt tagtacatga tctctcccc 150480  
gctataaagg cattatcaac ttctccttat tgattgaaaa tgaaaatata aatagttttt 150540  
atgtatagca gtattaccct atagttttat tgcttactac taacatggat acagatgtta 150600  
caaatgtaga agatatcata aatgaaatag atagagagaa agaagaaata ctaaaaaatg 150660  
tagaaattga aaataataaa aacattaaca agaatcatcc caatgaatat attagagaag 150720  
cactcgttat taataecagt agtaatagtg attccattga taaagaagtt atagaatgta 150780

tcagtcacga tgtaggaata tagatcatat ctactaattt ttataatcga tacaaaacat 150840  
aaaaaacacac tcgttattac atagcaggca tggaaatcctt caagtattgt tttgataacg 150900  
atggcaagaa atggattatc ggaaatactt tatattctgg taattcaata ctctataagg 150960  
tcagaaaaaa tttcactagt tcggtctaca attacgtaat gaagatagat cacaaatcac 151020  
acaagccatt gttgtctgaa atacgattct atatatctgt attggatcct ttgactatcg 151080  
acaactggac acgggaacgt ggtataaagt atttggctat tccagatctg tatggaattg 151140  
gagaaaccga tgattatatg ttcttcggtta taaagaattc gggaagagta ttcgccccaa 151200  
aggatactga atcagtcctc gaagcatgag tcaactatgat aaacacgtta gagttttatac 151260  
actctcgagg atttaccat ggaaaaatag aaccgaggaa tatactgatt agaaataaac 151320  
gtctttcact aattgactat tctagaacta acaactata caagagtgga aactcacata 151380  
tagattacaa cgaggacatg ataacttcag gaaatatcaa ttatatgtgt gttagacaate 151440  
atcttgagac aacagtttca agacgaggag atttagaaat gttgggatag tgcatgtag 151500  
aatggttcgg tggcaaacct ccattggaaa acgaaagtag tataaaagta ataaaacaaa 151560  
aaaaagaata taaaaaattt atagctactt tctttgagga ctgttttcct gaaggaaatg 151620  
aacctctgga attagttaga tatatagaat tagtatcac gttagattat tctcaaacctc 151680  
ctaattatga cagactacgt aaactgttta tacaagattg aaattatatt ctttttttta 151740  
tagagtgtgg tagtggtacg gatattctaat attaatatta gactatctct atcgcgctac 151800  
acgaccaata tcgattacta tggatatctt ctatgaaagg agagaatgta tttatttctc 151860  
cagcgtcaat ctcgtcagta ttgacaatac tgtattatgg agctaattgga tccactgctg 151920  
aacagctatc aaaatatgta gaaacggagg agaacacgga taaggttagc gctcagaata 151980  
tctcattcaa atccatgaat aaagtatatg ggcgatattc tgccgtgttt aaagattcct 152040  
ttttgagaaa aattggcgat aagtttcaa ctgttgactt cactgattgt cgcactatag 152100  
atgcaatcaa caagtgtgta gatattcttta ctgaggggaa aatcaatcca ctattggatg 152160  
aaccattgtc tctagcaat tagtgccgta tactttaaag caaaatgggt gacgccattc 152220  
gaaaaggaaat ttaccagtga ttatcccttt tacgtatctc cgaaggaaat ggtagacgta 152280  
agtatgatgt ctatgtacgg caaggcattt aatcacgcac ctgtaaaaga atcattcggc 152340  
aacttttcaa tcatagaact gccatatgtt ggagatacta gtatgatggt cattcttcca 152400  
gacaagattg atggattaga atccatagaa caaatctaa cagatacaaa ttttaagaaa 152460  
tggtgtgact ttatggatgc tatgtttata gatgttcaaa ttcccaagtt taaggtaaca 152520  
ggctcgtata atctggtgga taetctagta aagtcaggac tgacagaggt gttcggttca 152580  
actggagatt atagcaatat gtgtaattta gatgtgagtg tgcacgctat gatccacaaa 152640  
acgtatatag atgtcaatga agagtataca gaagcagctg cagcaacttc tgtactagtg 152700  
gcagactgtg catcaacaat tacaatagag ttctgtgcag atcatcctt catctatgtg 152760  
attaggcatg ttgatggaaa aattcttttc attggtatag attgctctcc gacaactaat 152820  
tgtaaacat tttttttaa aaaaacaatg ggtgatggat acacttgatg gtataatgat 152880  
gaatgaacgc gatgtttctg taagcgttgg caccggaata ctattcatgg aaatgttttt 152940  
cgtttacaat aaaaatagta tcaacaatca actaatgtat gatataatta atagcgtatc 153000  
tataagtgt gctaattata gatatagaag ctgcttttaa cgacgatggt atatacatcc 153060  
gtagaaatat gattaacaag ttgtacggat acgcatctct aactactatt ggcacgatcg 153120  
ctggagggtg ttgttattat ctggtgatgc atctagttag tttgtataaa taattatttc 153180  
aatatactag ttaaaatttt aagattttta atgtataaaa aactaataac gtttttattt 153240  
gtaaataggc cattagcatc ctattcgaat aatgagtaca ctccgtttta taaactgagt 153300  
gtaaaactct atatagatgg agtagataat atagaaaatt catatactga tgataataat 153360  
gaattggtgt taaattttta agagtacaca atttctatta ttacagagtc atgcgacgtc 153420  
ggatttgatt ccatagatat agatgttata aacgactata aaattattga tatgtatacc 153480  
attgactcgt ctactattca acgcagaggt cacacgtgta gaatatctac caaattatca 153540  
tgccattatg ataagtaccc ttatattcac aaatatgatg gtgatgagcg acaatattct 153600  
attactgcag agggaaaaat ctataaaagga ataaaatatg aaataagtat gatcaacgat 153660  
gatactctat tgagaaaaca tactcttaaa attggatcta cttatatatt tgatcgctcat 153720  
ggacatagta atacatatta ttcaaaatat gattttttaa aattttaaa atattatcac 153780  
ttcagtgaca gtagtcaaat aacaaacaac accatgagat atattataat tctcgcagtt 153840  
ttgttcatta atagtataca cgctaaaata actagtata agtttgaac cgteaatttt 153900  
gattccaaaa ttgaatggac tggggatggg ctatacaata tatcccttaa aaattatggc 153960  
atcaagacgt ggcaacaat gtatacaaat gtaccagaag gaacatacga catatccgca 154020  
tttccaaaga atgatttcgt atctttctgg gttaaatttg aacaaggcga ttataaagtg 154080

gaagagtatt gtacgggact atgcgctcgaa gtaaaaattg gaccaccgac tgtaacattg 154140  
actgaatacgc acgaccatat caatttgtac atcgagcatc cgtatgctac tagaggtagc 154200  
aaaaagattc ctattttacaa acgcggtgac atgtgtgata tctacttggt gtatacggct 154260  
aacttcacat tccgagattc taaagaacca gtaccatatg atatcgatga ctacgattgc 154320  
acgtctacag gttgcagcat agactttgtc acaacagaaa aagtgtgcgt gacagcacag 154380  
ggagccacag aagggtttct cgaaaaaatt actccatgga gttcgaaagt atgtctgaca 154440  
cctaaaaaga gtgtatatac atgcgcaatt agatccaaag aagatgttcc caatttcaag 154500  
gacaaaatgg ccagagttat caagagaaaa tttaatacac agtctcaatc ttatttaact 154560  
aaattttctcg gtagcacatc aaatgatgtt accacttttc ttagcatgct taacttgact 154620  
aaatattcat aactaatttt tattaatgat acaaaaacga aataaaactg catattatac 154680  
actggttaac gcccttatag gctctaacca ttttcaagat gaggtccctg attatagtc 154740  
ttctgttccc ctccatgtct attagacgat gtgagaagac tgaagaggaa acatggggat 154800  
tgaaaatagg gttgtgtata attgccaaag atttttatcc cgaaagaact gattgcagtg 154860  
ttcatctccc aactgcaagt gaaggattga taactgaagg caatggattc agggatatac 154920  
gaaacaccga taaattataa aaaaagcaat gtgtccgctg tttccgttaa taatactatt 154980  
ttcgttaactg gccgattatt cataaataac tctaatagca cgatcgtgga catttataaa 155040  
gacaaacaat ggtcgattat agaaatggct aggggtatct acggcatcga ctcgacattt 155100  
ggaatgttat attttgcgg aggtctatcc gttaccgaac aatatggtaa tttatagaaa 155160  
aacaacgaga tatcttggtt caatcctaga acgaataagt ggtttgatat ttcataact 155220  
atttataaga tatccatctc atcattgtgt aaactaaata acgtcttcta tgtatttagt 155280  
aaggacattg gatattgtga aaagtatgat ggtgcattga agttagtaca tgactcgtctc 155340  
cccgtataaa gccgattatc aacttctcct tattgattga aaatgaaaat ataaatagtt 155400  
tttatgtata gcagattatc cctatagttt tattgcttac tactaacatg gatacagatg 155460  
ttacaaatgt agaagatatc ataaatgaaa tagatagaga gaaagaagaa atactaaaaa 155520  
atgtagaaat tgaaaataat aaaaacatta acaagaatca tcccaatgaa tatattagag 155580  
aagcactcgt tattaatacc agtagtaata gtgattccat tgataaagaa gttatagaat 155640  
gtatcagtcg cgatgttaga atatagatca tatctactaa tttttataat cgatacaaaa 155700  
cataaaaaac aactcgttat tacatagcag gcattggaatc cttcaagtat tgttttgata 155760  
acgatggcaa gaaatggatt atcggaata ctttatattc tggttaattc atactctata 155820  
aggtcagaaa aaatttccact agttcgttct acaattacgt aatgaagata gatcacaat 155880  
cacacaagcc attgttgtct gaaatcagat tctatatatc tgtattggat cctttgacta 155940  
tcgacaactg gacacgggaa cgtggtataa agtatttggc tattccagat ctgtatggaa 156000  
ttggagaaac cgatggatta tatgttcttc gttataaaga attcgggaag agtatcgcc 156060  
ccaaaggata ctgaatcagt cttcgaagca tgcgtcacta tgataaacac gtttagagttt 156120  
atacactctc gaggattttac ccatggaaaa atagaaccga ggaatataat attaaaactt 156180  
accacgtaaa actttaaatt taaaatgata tttcattgac agatagatca cacattatga 156240  
actttcaagg acttgtgtta actgacaatt gcaaaaatca atgggtcgtt ggaccattaa 156300  
taggaaaagg tggatttggg agtatttata ctactaatga caataattat gttagataaaa 156360  
tagagcccaa agctaaccga tcattattta ccgaacaggc attttatact agagtactta 156420  
aaccatccgt tatcgaagaa tggaaaaaat ctacacaat atagcagcgt ggtcttatca 156480  
cgtgcaagcc atttgggtct tacaatcca ttaatgtgga atatcgattc ttggtaatta 156540  
atagattagg tgcagatcta gatgcggtga tcagagccaa taataataga ctaccaaaaa 156600  
ggtcgggtgat gttgatcgga atcgaaatct taaataccat acaatttatg cagcagcaag 156660  
gatattctca cggagatatt aaagcgagta atatagtcct agatcaaata gataagaata 156720  
aatttatctc agtggattac ggattgggtt cttaaattcat gtctaattgc gaacatgttc 156780  
catttataag aaatccaaat aaaatggata acgggtactc agaatttaca cctatagatt 156840  
cgcataaagg atacgttgta tctagacgtg gagatctaga aacacttgga tattgtatga 156900  
ttagatgggt gggagggtatc ttaccatgga ctaagatctc tgaaacaaag aattgtgcat 156960  
tagtaagtgc cacaaaacag aaatatgtta acaatactgc gactttgtta atgaccagtt 157020  
tgcaatatgc acctagagaa ttgctgcaat atattaccat ggtaaaactc ttgacatatt 157080  
ttgaggaacc caattacgac aagtttcggc acatattaat gcagggtgta tattattaag 157140  
tgtggtgttt ggtcgataaa aattaaaaaa taacttaatt tattattgat ctcggtgtga 157200  
caaccgaaat catggcgatg ttttacgcac acgctctcgg tgggtacgac gagaatcttc 157260  
atgcctttcc tggaaatca tegactgttg ccaatgatgt caggaaatat tctgttgtgt 157320  
cagtttataa taacaagtat gacattgtaa aagacaaata tatgtggtgt tacagtcagg 157380

tgaacaagag atatatattgga gcaactgctgc ctatgttttga gtgcaatgaa tatctacaaa 157440  
ttggaaatcc gatccatgat caagaaggaa atcaaatctc tatcatcaca tatcgccaca 157500  
aaaactacta tgctctaagc ggaatcgggt acgagagtct agacttgtgt ttggaaggag 157560  
tagggattca tcatcacgta cttgaaacag gaaacgctgt atatggaaaa gttcaacatg 157620  
attattctac tatcaaagag aaggccaaag aatgagtgac acttagtcca ggacctatca 157680  
tcgattacca cgtctggata ggagattgta tctgtcaagt tactgctgtg gacgtacatg 157740  
gaaaggaaat tatgaaaatg agattcaaaa aggggtgcggg gcttccgac ccaaactctgg 157800  
taaaagttaa acttggggag aatgatacag aaaatctttc ttctactata tcggcgacac 157860  
catcgaggta accacctctc tggaagacag cgtgaataat gtactcatga aacgttttga 157920  
aactatacgc catatgtggt ctgttgata tgatcatttt gatattgtga atggtaaaga 157980  
atgctgttat gtgcatacgc atttgtctaa tcaaaatctt ataccgagta ctgtaaaaaac 158040  
aaatttgtac atgaagacta tgggatcatg cattcaaag gattccatgg aagctctaga 158100  
gtatcttagc gaactgaagg aatcagggtg atggagtcct agaccagaaa tgcaggaatt 158160  
tgaatatcca gatggagtgg aagacactga atcaattgag agattggtag aggagtctct 158220  
caatagatca gaacttcagg ctggtgaatc agtcaaatct ggtaattcta ttaattgttaa 158280  
acatacatct gtttcagcta agcaactaag aacacgtata cggcagcagc ttcttctat 158340  
actctcatct tttaccaaca caaagggtgg atatttgttc attggagtgg ataataatac 158400  
acacaaagta tttggattca cgggtgggtta cgactacctc agactgatag agaataatg 158460  
agaaaagcat atcaaaagac tttgtgttgt gtatttctgt gagaagaaaag aggacatcaa 158520  
gtacgcgtgt cgattcacta aggtatataa acctggggat gaggctacct cgacatacgt 158580  
gtgcgctatc aaagtggaaa gatgctgtgt tgctgtgttt gcagattggc cagaatcatg 158640  
gtatatggat actaatggta tcaagaagta ttctccagat gaattgggtgt cacataataa 158700  
attttaatta atgtaactat agagaacaaa taataagggt gtaatatcat atagacaata 158760  
actaacaatt aattagtaac tgttatctct tttttaatta accaactaac tatataccta 158820  
ttaatacatc gtaattatag ttcttaacat ctattaatca ttaattcgct tctttaattt 158880  
tttataaact aacattgtta attgaaaagg gataacatgt tacagaatat aaattatata 158940  
tggatttttt taaaaaggaa atacttgaat ggagtatata tttatctctt cattatatag 159000  
cacgcgtgtt tcccaatttt tccacatccc atataatata ggattataat ctcgttcgaa 159060  
catacagaaa agtggataaa acaatagttg atttttatc taggttgcca aatttatctc 159120  
atattttaga atatggggaa aatattctat atattttatc tatggatgat gctaatacga 159180  
atattataat tttttttcta gatagagtat taaatattaa taagaacggg tcattttatac 159240  
acaatctcgg gttatcatca tccattaata taaaagaata tgtatatcaa ttagttaata 159300  
atgatcatcc agataatagg ataagactaa tgcttgaaaa tggacgtaga acaagacatt 159360  
ttttgtccta tatatcagat acagttaata tctatatatg tattttaata aatcatggat 159420  
tttatataga tgcggaagac agttacggtt gtacattatt acatagatgt atatatcact 159480  
ataagaaatc agaatacaga tcatacaatg aattaattaa gatattgtta aataatggat 159540  
cagatgtaga taaaaaagat acgtacggaa acacaccttt tatcctatta tgtaaacacg 159600  
atatcaacaa cgtggaattg tttgagatat gtttagagaa tgctaataata gactctgtag 159660  
actttaatag atatacacct cttcattatg tctcatgtcg taataaatat gattttgtag 159720  
agttattaat ttctaaagga gcaaatgtta atgcgcgtaa taaattcgga actactccat 159780  
tttattgtgg aattatacac ggtatctcgc ttataaaact atatttggaa tcagacacag 159840  
agttagaaat agataatgaa catatagttc gtcatttaat aatttttgat gctgttgaat 159900  
cttttagatta tctattatcc agaggagtta ttgatattaa ctatcgtagt atatacaacg 159960  
aaacactctat ttacgacgct gtcagttata atgcgtataa tacgttggct tatctattaa 160020  
acaaaaatgg tgattttgag acgattacta ctagtggatg tacatgtatt tcggaagcag 160080  
tcgcaacaaa caacaaaata ataatggaag tactattgtc taaaacgaeca tctttgaaaa 160140  
ttatgatata gtctatgata gcaattacta aacataaaca gcataatgca gattttattga 160200  
aaatgtgtat aaaatatact gcgtgtatga ccgattatga tactcttata gatgtacagt 160260  
cgctacagca atataaatgg tatattttta gatgtttcga tgaaatagat atcatgaaga 160320  
gatgttatat aaaaaataaa actgtattec aattagtttt ttgtatcaaa gacattaata 160380  
ctttaatgag atacggtaaa catccttctt tegtgaatg cactagtctc gacgtatac 160440  
gaagtcgtgt acgtaatatc atagcatcta ttagatatcg tcagagatta attagtctat 160500  
tatccaagaa gctggatcct ggagataaat ggtcgtgttt tcttaacgaa ataaaaata 160560  
aaatattgga aaactttaac gataacgaac tatccacata tctaaaaatc ttataaacat 160620  
tattaaaata taaaatctaa gtaggataaa atcacactac atcattgttt ccttttagtg 160680

ctcgacagtg tatactatatt ttaacgctca taaataaaaa tgaaaacgat ttccggttggt 160740  
acgttggttat gcgtactacc tgctgttggt tattcaacat gtactgtacc cactatgaat 160800  
aacgctaaat taacgtctac cgaaacatcg tttaatgata accagaaagt tacggtttaca 160860  
tgtgatcagg gatatcattc tttggatcca aatgctgtct gtgaaacaga taaatggaaa 160920  
tacgaaaaatc catgcaaaaa aatgtgcaca gtttctgatt atgtctctga actatataat 160980  
aaaccgctat acgaagtga ttccaccatg acactaagtt gcaacggcga aacaaaaat 161040  
tttcggttgcg aagaaaaaaa tggaaatact tcttggaaatg atactgttac gtgtccta 161100  
gcggaatgtc aacctcttca attagaacac ggatcgtgtc aaccagttaa agaaaaatac 161160  
tcatttgggg aatatatgac tatcaactgt gatgttggt atgaggttat tgggtgcttcg 161220  
tacataagtt gtacagctaa ttcttggaaat gttattccat catgtcaaca aaaatgtgat 161280  
atgccgtctc tatctaaccg attaatctcc ggatctacat tttctatcgg tggcggtata 161340  
catcttagtt gtaaaagtgg ttttacctca acgggggtctc catcatccac atgtatcgac 161400  
ggtaaatgga atcccatact cccaatatgt gtacgaacta acgaaaaatt tgatccagtg 161460  
gatgatggtc ccgacgatga gacagatttg agcaaaactc cgaaagacgt tgtacaatat 161520  
gaacaagaaa tagaatcggt agaagcaact tcatatataa tcatagtggc gttgacaatt 161580  
atgggcgtca tttttttaat ctccgttata gtattagttt gttcctgtga caaaaaat 161640  
gaccaatata agttccataa attgctaccg taaatataaa tccgttaaaa taattaataa 161700  
tttaataaca aacaagtatc aaaagattaa agacttatag ctagaatcaa ttgagatgtc 161760  
ttcttcagtg gatgttgata tctacgatgc cgttagagca ttttactca ggcactatta 161820  
taacaagaga tttattgtgt atggaagaag taacgccata ttacataata tatacaggct 161880  
atttacaaga tgcgcggtta taccggtcga tgatatagta cgtactatgc caaatgaatc 161940  
acgtgttaaa caatgggtga tggatacact taatgggtata atgatgaatg aacgcgatgt 162000  
ttctgtaagc gttggcaccg gaatactatt catggaaatg tttttcgatt acaataaaaa 162060  
tagtatcaac aatcaactaa tgtatgatat aattaatagc gtatctataa ttctagctaa 162120  
tgagagatat agaagcgctt ttaacgacga tggatatatac atccgtagaa atatgattaa 162180  
caagttgtac ggatacgcac ctctaactac tattggcacc atcgctggag gtgtttgtta 162240  
ttatctgttg atgcattctag ttatgttgta taaataatta tttcaatata ctagttaaaa 162300  
ttttaagatt ttaaatgtat aaaaaactaa taacgttttt atttgaataa ggtgcattag 162360  
catcctattc gaataatgag taactccgt ttaataaaact gagtgtaaaa ctctatatag 162420  
atggagttaga taatatagaa aattcatata agctatgata taatgaattg gtgttaaat 162480  
ttaaagagta cacaatttct attattacag agtcgatgca cgtcggattt gattccatag 162540  
atatagatgt tataaacgac tataaaatta ttgatatgta taccattgac tctgtacta 162600  
ttcaacgcag aggtcacacg tgtagaatat ctaccaaatt atcatgccat tatgataagt 162660  
acccttatat tcacaaatat gatgggtgat agcgacaata ttctattact gcagagggaa 162720  
aatgctataa aggaataaaa tatgaaataa gtatgatcaa cgatgatact ctattgagaa 162780  
aacatactct taaaattgga tctacttata tatttgatcg tcatggacat agtaatacat 162840  
attattcaaa atattgattt taaaaattta aaatatatta tcacttcagt gacagtagtc 162900  
aaataacaaa caacaccatg agatatatta agttttgttc attaatagta 162960  
tacatgctaa aataactagt tataagtgtg aatccgtcaa ttttgattcc aaaattgaa 163020  
ggactgggga tggctctatac aatatatccc ttaaaaaatta tggcatcaag acgtggcaaa 163080  
caatgtatac aaatgtacca gaaggaacat acgacatatc cgcatttcca aagaatgatt 163140  
tcgtatcttt ctgggttaaa tttgaacaag gcgattataa agtggaaagag tattgtacgg 163200  
gactatgcgt cgaagtataa attggaccac cgactgtaac attgactgaa tacgacgacc 163260  
ataaacagaa aaagtgtgcg tgacagcaca gggagccaca gaaggggttc tcgaaaaaat 163320  
tactccatgg agttcgaaag tatgtctgac acctaaaaag agtgtatata catgcgcaat 163380  
tagatccaaa gaagatgttc ccaatttcaa ggacaaaatg gccagagtta tcaagagaaa 163440  
atttaataca cagtctcaat cttattttaa taaatttctc ggtagcacat caaatgatgt 163500  
taccactttt cttagcatgc ttaacttgac taaatattca taactaattt ttattaatga 163560  
tacaaaaacg aaataaaact gcatattata cactgggtta cgccttata ggctctaacc 163620  
attttcaaga tgaggtccct gattatagtc cttctgttcc cctctatcat ctactccatg 163680  
tctattagac gatgtgagaa gactgaagag gaaacatggg gattgaaaat aggggttggt 163740  
ataattgcc aagatttcta tcccgaaga actgattgca gtgttcatct cccaactgca 163800  
agtgaaggat tgataactga aggcaatgga ttacaggata tacgaaacac cgataaatta 163860  
taaaaaaagc aatgtgtccg ctgtttccgt taataatact attttcgtaa ctggcggatt 163920  
attcataaat aactctaata gcacgatcgt ggttaacaat atggaaaaac ttgacattta 163980

|             |             |             |             |             |             |        |
|-------------|-------------|-------------|-------------|-------------|-------------|--------|
| taaagacaaa  | caatgggtcga | ttatagaaat  | gocctatggct | agggtatatc  | acggcattga  | 164040 |
| ctcgacattt  | ggaatgttat  | attttgcggg  | agggtctatcc | gttaccgaac  | aatatggtaa  | 164100 |
| tttagagaaa  | aacaacgaga  | tatcttggtta | caatcctaga  | acgaataagt  | ggtttgatat  | 164160 |
| ttcatatact  | atttataaga  | tatccatatac | atcatttgtgt | aaactaaata  | acgtcttcta  | 164220 |
| tgtatttagt  | aaggacattg  | gatattgtgga | aaagtatgat  | ggtgcatgga  | agttagtaca  | 164280 |
| tgatcgtctc  | cccgtataaa  | aggcattatc  | aacttctcct  | tattgattga  | aaatataaat  | 164340 |
| agtttttatg  | tatagcagta  | ttaccctata  | gttttattgc  | ttactactaa  | catggataca  | 164400 |
| gatgttacaa  | atgtagaaga  | tatcataaat  | gaaatagata  | gagagaaaaga | agaaatacta  | 164460 |
| aaaaatgtag  | aaattgaaaa  | taataaaaaac | attaacaaga  | atcatcccaa  | tgaatatatt  | 164520 |
| agagaagcac  | tcgttattaa  | taccagtagt  | aatagtgtat  | ccattgataa  | agaagttata  | 164580 |
| gaatgtatca  | gtcacgatgt  | aggaatatag  | atcatatcta  | ctaattttta  | taatcgatac  | 164640 |
| aaaacataaa  | aaacaactcg  | ttattacata  | gcaggcatgg  | aatccttcaa  | gtattgtttt  | 164700 |
| gataacgatg  | gcaagaaatg  | gattatcgga  | aatactttat  | attctggtaa  | ttcaatactc  | 164760 |
| tataagggtca | gaaaaaattt  | cactagtctg  | ttctacaatt  | acgtaatgaa  | gatagatcac  | 164820 |
| aaatcacaca  | agccattgtt  | gtctgaaata  | cgattctata  | tatctgtatt  | ggatcctttg  | 164880 |
| actatcgaca  | actggacacg  | ggaacgtggg  | ataaagtatt  | tggctattcc  | agatctgtat  | 164940 |
| ggaattggag  | aaaccgatga  | ttatatgttc  | ttcgttataa  | agaattcggg  | aagagtattc  | 165000 |
| gccccaaagg  | atactgaatc  | agtcttcgaa  | gcattgcgtca | ctatgataaa  | cacgttagag  | 165060 |
| tttatacact  | ctcgaggatt  | tacccatgga  | aaaatagaac  | cgaggaatat  | actgattaga  | 165120 |
| aataaacgto  | tttactaat   | tgactattct  | agaactaaca  | aactatacaa  | gagtggaaac  | 165180 |
| tcacatatag  | attacaacga  | ggacatgata  | acttcaggaa  | atatcaatta  | tatgtgtgta  | 165240 |
| gacaatcatc  | ttggagcaac  | agtttcaaga  | cgaggagatt  | tagaaatgtt  | gggatattgc  | 165300 |
| atgatagaat  | ggttcggtag  | caaacttcca  | tggaaaaacg  | aaagttagtat | aaaagtaata  | 165360 |
| aaacaaaaaa  | aagaatataa  | aaaatttata  | gctactttct  | ttgaggactg  | ttttcctgaa  | 165420 |
| ggaaatgaac  | ctctggaatt  | agttagatat  | atagaattag  | tatacacgtt  | agattattct  | 165480 |
| caaactccta  | attatgacag  | actacgtaaa  | ctgttttatac | aagattgaaa  | ttatattctt  | 165540 |
| ttttttatag  | agtgtggtag  | tgttacggat  | atctaattat  | aatatttagac | tatctctatc  | 165600 |
| gcgctacacg  | accaatatcg  | attactatgg  | atatcttcta  | tgaaggaga   | gaatgtattt  | 165660 |
| atttctccag  | cgtcaatctc  | gtcagtattg  | acaatactgt  | attatggagc  | taatggatcc  | 165720 |
| actgctgaac  | agctatcaaa  | atatgtagaa  | acggaggaga  | acacggataa  | ggttagcgct  | 165780 |
| cagaatatct  | cattcaaatc  | catgaataaa  | gtatactggc  | gatattctgc  | cgtgtttaa   | 165840 |
| gattcctttt  | tgagaaaaat  | tggcgataag  | tttcaaacgt  | ttgacttcac  | tgattgtcgc  | 165900 |
| actatagatg  | caatcaacaa  | gtgtgtagat  | atctttactg  | aggggaaaat  | caatccacta  | 165960 |
| ttggatgaac  | cattgtctcc  | tagcaattag  | tgccttatac  | tttaaagcaa  | aatggttgac  | 166020 |
| gccattcgaa  | aaggaaattt  | ccagtgatta  | tcccttttac  | gtatctccga  | cggaaatggg  | 166080 |
| agacgtaagt  | atgatgtcta  | tgtacggcaa  | ggcattttaat | cacgcctctg  | taaaagaatc  | 166140 |
| attcggcaac  | ttttcaatca  | tagaactgcc  | atatgttggg  | gatactagta  | tgatggatcat | 166200 |
| tcttcagac   | aagattgatg  | gattagaatc  | catagaacaa  | aatctaacag  | atacaaat    | 166260 |
| taagaaatgg  | tgtgacttta  | tggatgctat  | gtttatagat  | gttcacattc  | ccaagtttaa  | 166320 |
| ggtaacaggc  | tcgtataatc  | tgggtggatac | tctagtaaag  | tcaggactga  | cagaggtgtt  | 166380 |
| cggttcaact  | ggagattata  | gcaatatgtg  | taatttagat  | gtgagtgtcg  | acgctatgat  | 166440 |
| ccacaaaaacg | tatatagatg  | tcaatgaaga  | gtatacagaa  | gcagctgcag  | caacttctgt  | 166500 |
| actagtggca  | gactgtgcat  | caacaattac  | aaatgagttc  | tgtgcagatc  | atccgttcat  | 166560 |
| ctatgtgatt  | aggcatgttg  | atggaaaaat  | tcttttcgtt  | ggtagatatt  | gctctccgac  | 166620 |
| aactaattgt  | taaccatttt  | ttttaaaaaa  | aatagaaaaa  | acatgtggta  | ttagtgagag  | 166680 |
| tcgttgttct  | tccaattgca  | attggtaaga  | tgacggccaa  | cttttagtacc | cacgtctttt  | 166740 |
| caccacagca  | ctgtggatgt  | gacagactga  | ccagtattga  | tgacgtcaaa  | caatgtttga  | 166800 |
| ctgaatatat  | ttattgggtcg | tcctatgcac  | accgcaacag  | gcaatgcgct  | ggacaattgt  | 166860 |
| attccacact  | cctctctttt  | agagatgatg  | cggaaattagt | gttcacgcac  | attcgcgagc  | 166920 |
| tggtaaaaaa  | tatgcggtgg  | gatgatgtca  | aagattgtac  | agaaatcatc  | cgttgttata  | 166980 |
| taccggatga  | gcaaaaaacc  | atcagagaga  | tttcggccat  | catcggactt  | tgtgcatatg  | 167040 |
| ctgctactta  | ctggggagggt | gaagaccatc  | ccactagtaa  | cagtctgaac  | gcattgtttg  | 167100 |
| tgatgcttga  | gatgctaaat  | tacgtggatt  | ataacatcat  | attccggcgt  | atgaattgat  | 167160 |
| gagttgtaca  | tcttgacatt  | ttctttcttc  | tcttctccct  | ttcttctctt  | ctcccttctt  | 167220 |
| ccctcttctc  | cctttccag   | aaacaaactt  | ttttaccac   | tataaaataa  | aatgagtata  | 167280 |

ctacctgtta tatttctttc tatatttttt tattcttcat tegtccagac ttttaacgcg 167340  
tctgaatgta tcgacaaagg gcaatatatt gcattcattca tggagttaga aaacgagcca 167400  
gtaatcttac catgtcctca aataaatacg ctatcatccg gatataatat attagatatt 167460  
ttatgggaaa aacgaggagc ggataatgat agaattatac cgatagataa tggtagcaat 167520  
atgctaattc tgaacccgac acaatcagac tctggatttt atatatgcat taccacgaac 167580  
gaaacctact gtgacatgat gtctgttaa tttgacaatcg tgtctgtctc agaatcaaat 167640  
atagatttta tctcgtatcc acaaatagta aatgagagat ctactggcga aatggtatgt 167700  
cccaatatta atgcattttat tgctagttaac gtaaacgcag atattatatg gagcggacat 167760  
cgacgcctta gaaataagag acttaaacaa cggacacctg gaattattac catagaagat 167820  
gttagaaaaa atgatgctgg ttattataca tgtgttttag aatatatata cgggtggcaaa 167880  
acatataacg taaccagaat tgtaaaatta gaggtacggg ataaaaaat accttctact 167940  
atgcaattac cagatggcat tgtaacttca ataggtagta atttgactat tgcactgttg 168000  
agacctccca caacggatgc agacgtcttt tggataagta atggtatgta ttacgaagaa 168060  
gatgatgggg acggaacggg tagaataagt gtagcaata aaatctatat gaccgataag 168120  
agacgtgta ttacatccc gttaaaccatt aatcctgtca aggaagaaga tgctacaacg 168180  
tttacggtta tggcgtttac tattcctagc atcagcaaaa cagttactgt tagtataacg 168240  
tgaatgtatg ttgttacatt tccatgtcaa ttgagtttat aagaattttt atacattatc 168300  
ttccaacaaa caattgacga acgtattgct atgattaact cccacgatac fatgcatatt 168360  
attaatcatt aacttgacga ctatacctag tgctattttg acatactcat gttcttctgt 168420  
aattgocgta tctatattat taaagtacgt aaatctagct atagttttat tatttaattt 168480  
tagataatat accgtctcct tattttttaa aattgccaca tcttttatta aatcatgaat 168540  
gggaatttct atgtcatcgt tagtatattg tgaacaacaa gagcagatat ctataggaaa 168600  
gggtggaatg cgatacattg atctatgtag ttttaaaaca cagcgaact ttgaagaatt 168660  
tatataaatc attccatoga tacatccttc tatgttgaga tgtatatatc caggaattcg 168720  
tttattaata tcgggaaatg tataaactaa aacattgccc gaaagcggtg cctctatctg 168780  
cgttatatcc gttcttaact taaaaaatgt aaccaatacc tttgcatgac ttgttttgtt 168840  
cggcaacggt agtttaact tgacgaatgg attaattaca atagcatgat ccgcgcactc 168900  
attaagtttt ttacttttaa cgcccttgta tgtttttaca gagactttat cttaaatttct 168960  
agtgtttgta tgtgttataa atataacggg atatagaacc gaatcaccta ccttagatac 169020  
ccaattacat tttatcagat ccagataata aacaaatttt gtgcacctaa ctaattctat 169080  
attgttatat attttacaat tgggtatgat atcatgtaat aacttggagt ctaacgcgca 169140  
tcgtcgtacg tttatacaat tgtgatttag tgtagtatat ctacacatgt atttttccgc 169200  
actatagtat tctggactag tgataaaact atcgttatat ctatcttcaa tgaactcatc 169260  
gagatattgc tctctgtcat attcatacac ctgcataaac tttctagaca tcttacaatc 169320  
cgtgttattt taggatcata ttacatatt tacgggtata tcaaagatgt tagattagtt 169380  
aatgggaatc gtctataata atgaatatta aacaattata tgaggacttt taccacaaag 169440  
catcataaaa atgagtcgtc gtctgattta tgtttttaa atcaaccgca aatcaactca 169500  
taaaatacaa gagaatgaaa tatatacata ttttagtcat tgcaatatag accatacttc 169560  
tacagaactt gattttgtag ttaaaaacta tgatctaaac agacgacaac ctgtaactgg 169620  
gtatactgca ctacactgct atttgtataa taattacttt acaaacgatg tactgaagat 169680  
attattaaat catggagtgg atgtaacgat gaaaaccagt agcggacgta tgctgtttta 169740  
tatattgctt actagatgtt gtaatatctt acatgatgta gtgatagata tgatagacaa 169800  
agataaaaaac cacttattac atagagacta ttccaacctt ttactagagt atataaaatc 169860  
tcgttacatg ttattaaagg aagaggatat cgatgagaac atagtatcca ctttattaga 169920  
taaggggaatc gatcctaact ttaaacaga cggatatata gcgttacatt attattattt 169980  
gtgtctcgca caggtttata aaccagggtg gtgtagaaaa ccgataacga taaaaaaggc 170040  
caagcgaatt atttctttgt ttatacaaca tggagctaat ctaaacgcgt tagataaattg 170100  
tggtataaca ccattccatt tgtatcttag tattgaaatg tgtaataata ttcatatgac 170160  
taaaatgctg ttgactttta atccgaattt cgaatatgtt aataatcatg gattaacgcc 170220  
tatactatgt tatataactt ccgactacat acaacacgat attcttgtta tgtaataca 170280  
tcactatgaa acaaatgttg gagaaatgcc gatagatgag cgtcgtatga tcgtattcga 170340  
gtttatcaaa acatattcta cacgtccggc agattcgata acttatttga tgaatagggt 170400  
taaaaatata aatatttata cccgctatga aggaagaca ttattacacg tagcatgtga 170460  
atataataat acacacgtaa tagattatct tatacgtatc aacggagata taaatgcgtt 170520  
aaccgacaat aacaaacacg ctacacaact cattatagat aacaaagaaa attcccccata 170580

taccattaat tgtttactgt atatacttag atatatgtga gataagaatg tgataagatc 170640  
gttgggtgat caacttccat ctctacctat cttcgatata aaatcatttg agaaattcat 170700  
atcctactgt atacttttag atgacacatt ttacgatagg cacgttaaga atcgcgattc 170760  
taaaacgtat cgatacgcat tttcaaaata catgtcgttt gataaaatcg atggtataat 170820  
aactaaatgt cagcagcaaa caatgttact caaactgtcc actgttctag acactacact 170880  
atatgcagtt ttaagatgtc ataattcgag aaagttaaga agatacctca acgagttaaa 170940  
aaaatataat aacgataagt ccttttaaat atatttctaatt attatgaatg agagatacct 171000  
taatgtatat tataaagata tgtacgtgtc aaaggatatat gataaaactat ttcctgtttt 171060  
cacagataaa aattgtctac taacattact accttcagaa attatatacg aaatattata 171120  
catgctgaca attaacgac tttataatat atcgtatcca cctaccaaaag tatagttgta 171180  
tttttctcat gcgatgtgtg taaaaaaact gatattatat aaatatttta gtgocgtata 171240  
ataaagatga cgatgaaaat gatggtacat atatatttcg tatcattatt gttattgcta 171300  
ttccacagtt acgcataga catcgaaaat gaaatccag aattcttcaa taaaatgaga 171360  
gatactctac cagctaaaga ctctaaatgg ttgaatccag catgtatgtt cggaggcaca 171420  
atgaatgata tagccgctct aggagagcca ttcagcgcaa agtgcctcc tattgaagac 171480  
agtcttttat cgcacagata taaagactat gtggttaaat gggagaggct agaaaagaat 171540  
agacggcgac aggtttctaa taaacgtgtt aaacatgggtg atttatggat agccaactat 171600  
acatctaaat tcagtaaccg taggtatttg tgcaccgtaa ctacaaagaa tgggtgactgt 171660  
gttcagggtg tagttagatc tcatattaaa aaacctcctt catgcattcc aaaaacatat 171720  
gaactaggta ctcatgataa gtatggcata gacttatact gtggaattct ttacgcaaaa 171780  
cattataata atataacttg gtataaagat aataaggaaa ttaatatcga cgacattaag 171840  
tattcacaaa cgggaaagga attaatatt cataatccag agttagaaga tagcggaga 171900  
tagcactgtt acgttcatta cgacgacgtt agaatcaaga atgatatcgt agtatcaaga 171960  
tgtaaaatc ttacggttat accgtcaca gaccacaggt ttaaactaat actagatccg 172020  
aaaatcaacg taacgatagg agaacctgcc aatataacat gcactgctgt gteaagctca 172080  
ttattgatcg acgatgtact gattgaatgg gaaaatccat ccggatggct tataggattc 172140  
gattttgatg tatactctgt tttaactagt agaggcggtg tcaccgagggc gacctgtgac 172200  
tttgaaaatg ttactgaaga atatataggt aatacatata aatgtcgtgg acacaactat 172260  
tattttgaaa aaacctttac aactacagta gtattggagt aaatatataa tgcattttta 172320  
tatacattac tgaattatta ttactgaatt attattactg aattattatt aattatatcg 172380  
tattttgtgt atagaatgga tgaagatagc cgactatcta ggtatttgta tctcaccgat 172440  
agagaacata taaatgtaga ctctattaaa cagttgtgta aaatcaga tcctaagca 172500  
tggtatagat gtggatgtac ggctttacat gatctttt ataattatag atcagtaac 172560  
ggaaaataca agtatagata caacggttac tatcaatatt attcatctag cgattatgaa 172620  
aattataatg aatattatta tgatagaact ggtatgaaca gtgagagtga taatatatca 172680  
atcaaaacag aatatgaatt ctatgatgaa acacaagatc aaagtacaca actagtaggt 172740  
tacgacatta aactcaaaac caatgaggat gattttatgg ctatgataga tcagtgggtg 172800  
tccatgatta tatagatgaa tcaattaata aagtagtata tggagagag tctcacgtaa 172860  
gatggcgga tatatggcaa gaacataatg atggcgata cagtatagga aaggagtga 172920  
tagataatat atacgaagac aaccataccg tagacgaatt ctacaagata gacagcgat 172980  
cagatgtaga tgacgcgga cacatatctc cgataactaa aaaaccatag aatcagttga 173040  
tgataatacc tacatttcta atcttccgta taccatcaaa tacaataat tccagcaaca 173100  
ataagtattt tttatacctt taaaactgat aaataaattt tttctagtga tattttggca 173160  
agatgagaat cctatttctc atcgctttca tgtatgggtg tgttcaactca tatgttaacg 173220  
cggttgaaac caaatgtcca aatctagaca ttgtaacatc ttctggagaa tttcattgtt 173280  
caggatgtgt ggaacatatg cctgagttta gctatatgta ttgggtggca aaggatatga 173340  
aatcgacga ggataccaag tttatagaac atctgggtga tggcatcaaa gaagatgaaa 173400  
ccgttcgtac cacagatagt ggaatcgta ctctacgtaa agtccctcat gtaaccgata 173460  
ctaataaatt tgataattat aggttcactt gtgtctcac tacgatagat ggcgtttcaa 173520  
aaaagaatat ttggctgaag tagtgcgtgc tactattttt atttatgata taatctaattg 173580  
gaattaattt gaattgatat ttatccaata ctaagatta tattagaatc aaattaatct 173640  
tttatacgag aaaaaataac gacatacgtc gtcaacaaat taaactttt atttattagt 173700  
taactagctt atagaacttg ctcatgttta tgtttctaaa acgggtacgg catataggac 173760  
aattatccga cgcaccggtt tctcttcgtg ttctatgcca tatattgatg catgttatgc 173820  
aaaatatatg agtacacgaa tccaataaac caaagtatct atcgttttga gtaaacact 173880

tcataagcaaa ttccacattc tttttcttta ottactctat acacgtcttc gtattttattt 173940  
agtattttga tgatatccaa ctcagaaatg gttgttgat tattgggtgt ataggtatta 174000  
ttagctatgt accaatttac caaccctctt aatattgatt gataatcaca tcgggttatcc 174060  
aatcaataac cacattaata actaaattgt agtgatatata tagaccatat atgtttctat 174120  
ttttttgaca gttacgtata gtttcagtaa gttttgattg ttgtattcct gtatctctag 174180  
ataagttagt catatagtc cttccggcga tacgtttttt ccaagcccg aattgattag 174240  
ccaaatgtgt atttattttt gtgatattga tataatattt cggataatgc atactgttag 174300  
tcttatatca tttggttcat ctatgtattg taatattggt acatgatcta tagatgatgt 174360  
attgattttg gcaggatcga attccatatt cgcgactaaa cagtgaataa aatgtaata 174420  
ctttttaaat tttaaattag taaaactttt ttttattttt tatgattcca aaaatactga 174480  
atacaaagtc ctaaattata aatattggaga tcatactacc acaactattt attatgtata 174540  
caaggccggt gtaatagata gatatatata attctattac accggcagac aattaccgac 174600  
cggattttgt cgttaccaac ataccgtata atatgtaata tacaattcca taaccattg 174660  
acagttgtta tacatcaaaa ttgcaattct tttgattacg atgttataag aatgtagtta 174720  
attgatgtat gatgttaatg tgcctctttt cctcttataa catcgtaatc aaaaactttt 174780  
ttataatata tacctaataa tgtgtcttaa tagttctcgt gattcgtcaa acaatcattc 174840  
ttataaaata taataaagca acgtaaaaac acataaaaat aagcgtact aataagacaa 174900  
tgatatttta cgacgataaa ggtctacaga ctattaaact gtttaataat gaatttgatt 174960  
gtataaggaa tgacatcaga gaattattta aacatgtaac tgattccgat agtatacaac 175020  
ttccgatgga agacaattct gatattatag aaaatatcag aaaaatacta tatagacgat 175080  
taaaaaatgt agaattgtgt gacatcgata acacaataac ttttatgaaa tacgatccaa 175140  
atgatgataa taagcgtacg tgttctaatt ggtaccctt aactaataac tatatggaat 175200  
attgtctagt aatatatttg gaaacaccga tatgtggagg caaaataaaa ttataccacc 175260  
ctacaggaaa tataaagtcg gataaggata ttatgtttgc aaagactcta gactaagata 175320  
gacagcgtat cagatgtaga tgacgcggaa cacatatctc ctataactaa tgatgtatct 175380  
acacaaacat gggaaaagaa atcagagtta gatagataca tggaaatcgta tctctgctat 175440  
agatatagta aacattctgt atttaaggga tttcttgata aagttagaaa aaatgattta 175500  
gacatgaatg tggtaaaaga attactttct aacggtgcac ctctaacaat caaggatagc 175560  
agtaataagg atccaattgc tgtttatttt agaagaacga taatgaattt agaatgatt 175620  
gatattatta acaaacatac aactattgat gaacgaagt atatagtaca ctctatcta 175680  
aaaaattata gaaatttcga ttatccattt ttcagggaagt tagttttgac taataaacat 175740  
tgtctcaaca attattataa tataagcgac agcaaatatg gaacaccgct acatatattg 175800  
gogtctaata aaaaattaat aactcctaac tacatgaagt tattagtgt taacggaaat 175860  
gatataaacg cagcaggtga agatacacia atgcgaacca actcagaaat ggttggtgta 175920  
ttattgggtg tataggtatt attagctatg taccaattta ccaaccctct taatattgat 175980  
tgataatcac atcggttatc caattaataa ctaaaattgta gtgtatatat agaccatata 176040  
tgtttctatt tttttgacag ttacgtatag tttcagtaag ttttgattgt tgtattcctg 176100  
tatctctaga taagttagtc atatagtcct ttcggcgat acgttttttc caagcccgaa 176160  
attgattagc caaatgtgta tttatttttg tgatattgat ataattttc ggataatgca 176220  
tactgttagt cttatatcat ttggttcac tatgtattgt aatattgtta catgatctat 176280  
agatgatgta ttgatttttg caggatcgaa ttccatatcc gcgactaac agtgaaaaa 176340  
atgtaaatatc tttttaaatt tttaattagt aaaactttt tttattttt atgattccaa 176400  
aaatactgaa taaaaagtcc taaattataa atatggagat catactacca caacttatta 176460  
ttatgtatac aaggccggtg taatagatag atatatataa ttctattaca ccggcagaca 176520  
attaccgacc ggtatttgc gttaccaaca taecgtataa tatgtaatat acaattccat 176580  
aaccattga cagttgttat acatcaaat tgcaattctt ttgattacga tgttataaga 176640  
atgtagttaa ttgatgtatg atgttaattg gtcctctttc ctcttataac atcgtaatca 176700  
aaaactttt tataatata acctaataat gtgtcttaac agttctcgtg attcgtcaa 176760  
caatcattct tataaaatat aataaagcaa cgtaaaaaa cataaaaaa agcgtacta 176820  
ataagacaat ggatatttac gacgataaag gtctacagac tattaactg tttaataatg 176880  
aatttgattg tataaggaaat gacatcagag aattatttaa acatgtaact gattccgata 176940  
gtatacaact tccgatggaa gacaattctg atattataga aaatatcaga aaaatactat 177000  
atagacgatt aaaaaatgta gaattgtgtg acatcgataa cacaataact tttatgaaat 177060  
acgatccaaa tgatgataat aagcgtacgt gttctaattg ggtaccctta actaataact 177120  
atatggaata ttgtctagta atatatttgg aaacaccgat atgtggaggc aaaaataaat 177180

tataccaccc tacaggaat ataaagtcgg ataaggatat tatgtttgca aagactctag 177240  
acttttaaatc aacgaaagt ttaactggac gtaaaacaat tgccgttcta gacatatccg 177300  
tttcatataa tagatcaatg actactattc actacaacga cgacgttgat atagatatatc 177360  
atactgataa aaatggaaaa gagttatggt attgtttatat aacaatagat gatcattact 177420  
tggttgatgt ggaaactata ggagttatag tcaatagatc tggaaaatgt ctgttagtaa 177480  
ataaccatct aggtataggt atcgttaaag ataaacgtat aagcgatagt tttggagatg 177540  
tatgtatgga tacaatattt gacttttctg aagcacgaga gttattttca ttaactaatg 177600  
atgataacag gaatatagca tgggacactg ataaactaga cgatgatata gatatatgga 177660  
ctcccgtcac agaagatgat tacaattttc tttctagact agtattgtat gcaaaatctc 177720  
aatcggtacac tgtatttgac tattatgttc ttactgggtg tacggaacca cccactgtat 177780  
tcattttcaa ggtaactaga ttttacttta atatgccgaa ataaaaaatt tttgtataat 177840  
atctagaggt agaggtattg tttagataaa tacaataaac atagatacat cgcatactta 177900  
gcatttttat aaatatatat aagacataca cttttatcat ttttgtaaaa atactcataa 177960  
aaaaatttat aaaaattatg gcacaacac atcttggtata ggtagttagg ttctgcgagt 178020  
gaacctataa acagataata gacaacacat aataatgcct actaatataa gcataatacc 178080  
gggagatggg atatatgacg ttgtagtgtt tgggttttct gaacgttgat agtctactaa 178140  
tactacatgc tgacatctaa tgcctgtata accatgagag catctacaat acataccgctc 178200  
aatatctcta gegtggatac agtcacccgtg taaacaatat ccatctccct ctggaccgca 178260  
taatctgata gctggaatat ctgttgtagc gtttgtaatt tctggcaatg tctgttcgat 178320  
agcgttaacca ctatcggcga atgatctgat tatcatagca gcgaacaaca acatcagata 178380  
attttacaac atttttgatg gattctgtgt ttatgctgtt tctcagtggtg tgtttatgac 178440  
aagattggga attttatatt attaatcag taatataaac taataatata ttgttaattg 178500  
tgtaataat ataaaaataa caatacaata ttgaatgtgt tgctgttaaa aatgtatgtg 178560  
ttaatataat agaataaaat aaatgagtat gatcatttta gataacgatt gattttatca 178620  
ttaccgcttc attcttatat tctttgctta cggaacctat atttagaaac atctactaac 178680  
aattttttat gcttgcatta ttaatggtat gtaatatgat tgatttgtgta cgeaatacca 178740  
atgtgttaag tatgaatacg ggtacaaaac ataaattgaa atttaacatt atttatttat 178800  
gatatataac gttatcggtta ggtctatacc atggatatct ttaaagaact aatcttaaaa 178860  
cacctcgatg aaaatgtttt gatttctoca gtttccattt tatctacttt atcttaattc 178920  
aatcatggag cagctggttc tacagctgaa caactatcaa aatatataga gaatatgaat 178980  
gagaatacac ccgatgacaa taatgatgac atggaggtag atattccgta ttgtgcgaca 179040  
ctagctaccg caaataaaat atacggtagc gatagtatcg agttccacgc ctcccttcta 179100  
caaaaaataa aagacgattt tcaaaactgta aactttaata atgctaacca aacaaaggaa 179160  
ctaatacaac aatgggttaa gacaatgaca aatggtaaaa ttaattcctt attgactagt 179220  
ccgctatoca ttaatactcg tatgacagtt gttagcgccg tccattttta agcaatgtgg 179280  
aaatatccat tttctaaaca tcttatcat acagacaagt tttatatctc taagaatata 179340  
gttaccagcg ttgatatgat ggtgggtacc gagaataact tgcaatatgt acatatata 179400  
gaattattcg gaggattctc tattatcgat attccatagc agggaaactc tagtatggta 179460  
attatactac cggacgacat agaaggtata tataacatag aaaaaaatat aacagatgaa 179520  
aaatttaaaa aatggtgtgg tatgttatct actaaaagta tagacttgta tatgccaaag 179580  
tttaaagtgg aatgacaga accgtataat ctggtaccga ttttagaaaa tttaggactt 179640  
actaatatat tcggatatta tgcagatttt agcaagatgt gtaatgaaac tatcactgta 179700  
gaaaaatttc tacatacgac gtttatagat gttaatgagg agtatacaga agcatcggcc 179760  
gttacaggag tatttacgat taacttttct atggtatctc gtacgaaggc ctacataaac 179820  
catccattca tgtacatgat taaagacacc acaggacgta tactttttat agggaaatag 179880  
tgctatccgc aataaatata aacaaataga cttttataaa gagtcttcaa cgataagtat 179940  
atcgacatac tacttatgct gcgaaagatt ctgaacgaga acgactatct caccctcttg 180000  
gatcatatcc gactgctaa atactaaatc tccactacac tttttatcat cttatgagga 180060  
atgattgctt tcgtgaaata ggaataatta gcaccagaat agctatggat tattgtggta 180120  
gagagtgcac tattctatgt cgtctactgg atgaagatgt gacgtacaaa aaaataaaac 180180  
tagaaattga aacgtgtcac aacttatcaa aacatataga tagacgagga aacaatgccc 180240  
tacattgtta cgtctccaat aaatgcgata cagacattaa gattgttctc tcgaggagtc 180300  
gagagacttt gttagaaaca cgaaggatta actccgtag gagtatacag taagcataga 180360  
tacgtaaaat ctacagattgt gcatctactg atatccagct attcaaattc ctctaacgaa 180420  
ctcaagtcga atataaatga tttcgatctg tatteggata atatcgactt acgtctgcta 180480

|             |             |             |             |             |             |        |
|-------------|-------------|-------------|-------------|-------------|-------------|--------|
| aaatacctaa  | ttgtggataa  | acggatacgt  | ccgtccaaga  | atacgaatta  | tgcaatcaat  | 180540 |
| ggctctcggat | tggtggatat  | atacgtaacg  | acgcctaate  | cgagaccaga  | agtattgcta  | 180600 |
| tggtctctta  | aatcagaatg  | ttacagcacc  | ggttacgtat  | ttcgtacctg  | tatgtacgac  | 180660 |
| agtgatatgt  | gtaagaactc  | tcttcattac  | tatatatcgt  | ctcatagaga  | atctcaatct  | 180720 |
| ctatccaagg  | atgtaattaa  | atgtttgatc  | gataacaatg  | tttccatcca  | tggcagagac  | 180780 |
| gaaggaggat  | ctttacccat  | ccaatactac  | tggtctttct  | caaccataga  | tatagagatt  | 180840 |
| gttaaattat  | tattaataaa  | ggatgtggac  | acgtgtagag  | tatacgacgt  | cagccctata  | 180900 |
| ttagaggcgt  | attatctaaa  | caagcgattt  | agagtaacct  | catataatgt  | agacatggaa  | 180960 |
| atcgttaatc  | ttcttattga  | gagacgtcat  | actcttgctg  | acgtaatgcg  | tagtattact  | 181020 |
| tcgtacgatt  | ccagagaata  | taaccactac  | atcatcgata  | acattctaaa  | gagatttaga  | 181080 |
| caacaggatg  | tacaagccat  | gttgataaac  | tacttacatt  | acggcgatat  | ggtcgttcga  | 181140 |
| tgcatgttag  | ataacggaca  | acaactatcc  | tctgcacgac  | tactttgtta  | ataataatct  | 181200 |
| cgtcgatgta  | aacgtcgtaa  | ggtttatcgt  | ggaaaatatg  | gacacgcggc  | tgtaaatcac  | 181260 |
| gtatcgaaca  | atggccgtct  | atgtatgtac  | ggctctgatat | tatcgagatt  | taataattgc  | 181320 |
| gggtatcact  | ggtatgaaac  | catactgata  | gatgtatttg  | atatactaag  | caagtacatg  | 181380 |
| gatgatatag  | atatgatcga  | taactctact  | atattacgcg  | gtcgatgtca  | ataatataca  | 181440 |
| atttgcaaaag | cggttatttg  | aatatggagc  | gagtgctcac  | ctcgataatc  | aatacggcca  | 181500 |
| tccagaaaag  | cagttaccaa  | agagaaaaca  | aaacgaagct  | agttgattfa  | ttactgagtt  | 181560 |
| accatcccac  | tctagagact  | atgattgacg  | catttaatag  | agatatacgc  | tatctatata  | 181620 |
| ctgaaccatt  | attcgccctg  | atcagatacg  | ccttaatcct  | agatgatgat  | tttccttcta  | 181680 |
| aagtaaagta  | tgatatcgcc  | ggtcgtcata  | aggaactaaa  | gcgctataga  | gtagacatta  | 181740 |
| atagaatgaa  | gaatgtctac  | atatcaggcg  | tctccatggt  | tgatatatta  | tttaaacgaa  | 181800 |
| gcaaacgcca  | caaattgaga  | tacgcaaaga  | atccgacatc  | aaatggtaca  | aaaaagaact  | 181860 |
| aacgtccatc  | attacagaaa  | ctgtaaagaa  | caatgagagg  | atcgactcca  | tagtgacaaa  | 181920 |
| cattaataca  | gacgataact  | tgatttcgaa  | attaccatg   | gagatacttt  | attactccat  | 181980 |
| taaataattt  | atcatggagc  | gataatgtcc  | tgtttcattt  | gtttccatga  | catattacaa  | 182040 |
| aatcgattcc  | gtccaagatg  | ataaaaaacat | ttaccggcat  | cataaacacg  | gagtttattt  | 182100 |
| tatatgtctc  | gcataaacat  | tactaaaaaa  | atatattgtc  | gataacttga  | tttcgaaaatt | 182160 |
| acccatggag  | atactttatt  | actccattaa  | ataattttatc | atggagcgat  | aatgtcctgt  | 182220 |
| ttcatttgtt  | tccatgacat  | attacaaaaat | cgattccgctc | caagatgata  | aaaacattta  | 182280 |
| cgggcatcat  | aaacacggag  | tttattttat  | atgtctcgca  | taaacattac  | taaaaaatac  | 182340 |
| tattgttctg  | tttttcttct  | acatctttta  | ttatgaaaaa  | gtaaatcatt  | atgagatgga  | 182400 |
| cgagattgta  | cgcatcgctc  | gcgacagtat  | gtggtacata  | cctaacgtat  | ttatggacga  | 182460 |
| cggtaaagaat | gaagggtcacg | tttctgtcaa  | caatgtctgt  | catatgtatt  | ttacgttctt  | 182520 |
| tgatgtggat  | acatcgctctc | atctgtttta  | gctagttatt  | aaacactgcg  | atctgaataa  | 182580 |
| acgaggtaac  | tctccattac  | attgtctatac | gatgaataca  | cgatttaatc  | catctgtatt  | 182640 |
| aaagatattg  | ttacaccacg  | gcatgcgtaa  | ctttgatagc  | aaggatgacc  | actatcaatc  | 182700 |
| gataacaaga  | tctttgatat  | actaacggac  | accattgatg  | acttttagta  | atcatccgat  | 182760 |
| ctattgctgt  | ggtatcttag  | atataaattc  | aatgggagct  | taaaactatta | cgttctgtac  | 182820 |
| aaaggatccg  | accctaattg  | cgccgacgag  | gatgaactca  | cttctcttca  | ttactactgt  | 182880 |
| aaacacatat  | ccacgttcta  | cgaaagcaat  | tattacaagt  | taagtccacac | taagatgcga  | 182940 |
| gccgagaagc  | gattcatcta  | cgcgataata  | gattatggag  | caaacattaa  | cgcggttaca  | 183000 |
| cacttacctt  | caacagtata  | ccaaacatag  | tctcgtgtg   | gtgtatgctc  | ttttatctcg  | 183060 |
| aggagccgat  | acgaggatac  | gtaataatct  | tgattgtaca  | cccatcatgg  | aacgattgtg  | 183120 |
| caacaggtca  | tattctcata  | atgttactca  | attggcacga  | acaaaaggaa  | gaaggacaac  | 183180 |
| atctacttta  | tctattcata  | aaacataatc  | aaggatacac  | tctcaatata  | ctacggtatc  | 183240 |
| tattagatag  | gttcgacatt  | cagaaaagacg | aataactataa | taccgccttt  | caaaattgta  | 183300 |
| acaacaatgt  | tgccctatac  | atcggtatcg  | acatcaacct  | tccgactaaa  | gacggtattc  | 183360 |
| gacttggtgt  | ttgaaaacag  | aaacatcata  | tacaaggcgg  | atgttgtgaa  | tgacatcatc  | 183420 |
| caccacagac  | tgaaagtatc  | tctacctatg  | attaaatcgt  | tgttctacaa  | gatgtctctc  | 183480 |
| cctacgacga  | ttactacgta  | aaaaagatac  | tagcctactg  | cctattaagg  | gacgagtcac  | 183540 |
| tcgcggaact  | acatagtaaa  | ttctgtttta  | acgaggacta  | taaaagtgtg  | tttatgaaaa  | 183600 |
| atatatcatt  | cgataagata  | gattccatca  | tcgtgacata  | agtcgcctca  | aagagattcg  | 183660 |
| aatctccgac  | accgacctgt  | atacggatc   | acagctatct  | taaagccata  | cattcagaca  | 183720 |
| gtcacatttc  | atttcccatg  | tacgacgac   | tcatagaaca  | gtgccatcta  | tcgatggagc  | 183780 |

gtaaaagtaa actcgtcgac aaagcactca ataaattaga gtctaccatc ggtcaatcta 183840  
gactatcgta tttgcctccg gaaattatgc gcaatatcat cttaaagta tgggtgtacgg 183900  
aaagaacat tacaaatatt atccatgata gaaagaaaat atctatatga ttggagaagt 183960  
aggaaacagg aacaagacaa cgattactac attattaaat catgaagtcc gtattatact 184020  
cgtatatatt gtttctctca tgtataataa taaacggaag agatatagca ccgcatgcac 184080  
catccgatgg aaagtgtaaa gacaacgaat acaaacgcca taatttgtgt ccgggaacat 184140  
acgcttccag attatgcatg agcaagacta acacacgatg tacgccgtgt ggttcgggta 184200  
ccttcacatc tcgcaataat catttacccg cttgtctaaag ttgtaacgga agacgcgatc 184260  
gtgtaacacg actcacaata gaatctgtga atgctctccc ggatattatt gtcttctcaa 184320  
aggatcatcc ggaatgcaagg catgtgtttc ccaaacaaaa tgtggaatag gatacggagt 184380  
atccggagac gtcattctgtt ctccgtgtgg tctcggaaca tattctcaca ccgtctcttc 184440  
cgcagataaa tgcgaacccg taccagaaa tacgtttaac tatatcgatg tggaaattaa 184500  
cctgtatcca gtaacgaca cgtcgtgtac tccgacgacc actaccggtc tcagcgaatc 184560  
catctcaacg tcggaactaa ctattactat gaatcataaa gactgtaatc ccgtatttccg 184620  
tgatggatac ttctccgttc ttaataagggt agcgacttca ggtttcttta caggagaaag 184680  
gtgtgcactc tgaatttoga gattaaatgc aataacaaag attcttcttc caaacagtta 184740  
acgaaagcaa agaagatgac tatcatgccg cattcggaga cagtaactct agtgggagc 184800  
atctatatatc tatatagtaa taccaatact caagactacg aaactgatac aatctcttat 184860  
catgtgggta atgttctcga tgcgatagc catatgcccg gtatgtgcca tatacataaa 184920  
ctgatcacta attccaaacc caccacttt ttatagtaag tttttcacc ataaataata 184980  
aatacaataa ttaatttctc gtaaaagtag aaaatatatt ctaatttatt gcacggtaag 185040  
gaagtagaat cataaagaac agtactcaat caatagcaat tatgaaacaa tatatcgtcc 185100  
tggcatgcat gtgcctggcg gcagctgcta tgcctgccag tcttcagcaa tcatcctcat 185160  
cctctctctc gtgtacggaa gaagaaaaca aacatcatat gggaaatgat gttattatca 185220  
aagtcacaaa gcaagaccaa acaccgacca atgataagat ttgccaatcc gtaacggaaa 185280  
ttacagagtc cgagtcagat ccagatcccg aggtggaatc agaagatgat tccacatcag 185340  
tcgaggatgt agatctctct accacttatt actccatcat cgggtggagg ctgagaatga 185400  
actttggatt caccaaatgt cctcagatta aatccatctc agaatccgct gatggaaaca 185460  
cagtgaatgc tagattgtcc agcgtgtccc caggacaagg taaggactct cccgcgatca 185520  
ctcatgaaga agctcttget atgatcaaaag actgtgaagt gtctatcgac atcagatgta 185580  
gcgaagaaga gaaagacagc gacatcaaga cccatccagt actcgggtct aacatctctc 185640  
ataagaaagt gagttacgaa gatcatcatc gttcaacgat cgtcgataca aaatgcgtca 185700  
agaatctaga gtttagcgtt cgtatcggag acatgtgcaa ggaatcatc gaacttgagg 185760  
tcaaggatgg attcaagtat gtcgacggat cggcatctga aggtgcaacc gatgatactt 185820  
cactcatcga ttcaacaaaa ctcaaagcgt gtgtctgaat cgataactct attcatctga 185880  
aattggatga gtagggttaa tcgaacgatt caggcacacc acgaattaaa aaagtgtacc 185940  
ggacactata ttccggtttg caaaacaaaa atgttcttaa ctacattcac aaaaagttac 186000  
ctctcgcgac ttcttctttt tctgtctcaa tagtgtgata cgattatgac actattccta 186060  
ttcctattcc tatttctttt cagagtatca caaaaatatt aaacctcttt ctgatggctt 186120  
cataaaaaaa gttttacaaa aatattttta ttctctttct ctctttgatg gtctcataaa 186180  
aaaagtttta caaaaatatt ttattctctt ttctctcttt gatggctctc taaaaaagt 186240  
tttacaaaaa tatttttatt ctctttctct ctttgatgg ctcataaaaa aagttttaca 186300  
aaaatatttt tattctcttt ctctctttga tggctctata aaaaaagttt taaaaaata 186360  
tttttattct ctttctctct ttgatggctt cataaaaaaa gttttacaaa aatattttta 186420  
ttctctttct ctctttgatg gtctcataaa aaaagtttta caaaaatatt tttattctct 186480  
ttctctcttt gatggctctc taaaaaaagt tttaaaaaa tatttttatt ctctttctct 186540  
ctttgatggc ctcataaaaa aagttttaca aaaatatttt tattctcttt ctctctttga 186600  
tggctctcata aaaaaagttt tacaaaaata tttttattct ctttctctct ttgatggctt 186660  
cataaaaaaa gttttacaaa aatattttta ttctctttct ctctttgatg gtctcataaa 186720  
aaaagtttta caaaaatatt ttattctctt ttctctcttt gatggctctc taaaaaagt 186780  
tttacaaaaa tatttttatt ctctttctct ctttgatggc ctcataaaaa aagttttaca 186840  
aaaatatttt tatt 186854

&lt;211&gt; 1131

&lt;212&gt; DNA

&lt;213&gt; Human Herpesvirus-1

&lt;300&gt;

&lt;308&gt; GenBank No. NC\_00180

&lt;309&gt; 2004-01-13

&lt;400&gt; 35

```
atggcttcgt acccctgcc tcaacacgcg tctgcgttcg accaggctgc gcgttctcgc 60
ggccataaca accgacgtac ggcgttgccg cctcgccggc aacaaaaagc cacggaagtc 120
cgcttgagc agaaaatgcc cacgtactg cgggtttata tagacggtcc ccacgggatg 180
gggaaaacca ccaccacgca actgctgggt gccctgggtt cgcgcgacga tategtctac 240
gtaccgcgac cgatgactta ctggcgggtg ttgggggctt ccgagacaat cgcgaacatc 300
tacaccacac aacaccgcct cgaccagggt gagatatcgg cgggggacgc ggcggtggta 360
atgacaagcg ccagataaac aatgggcatg ccttatgcgg tgaccgacgc cgttctggct 420
cctcatatcg ggggggaggc tgggagctca catgccccgc ccccgccct caccctcatc 480
ttcgaccgcc atcccatcgc cgccctcctg tgctaccgg cgcgcgafa ccttatgggc 540
agcatgaccc ccaggccgt gctggcgctt gtggccctca tcccgccgac cttgcccggc 600
acaaacatcg tgttgggggc ccttcgggag gacagacaca tcgaccgcct ggccaaacgc 660
cagcgccccg gcgagcggct tgacctggct atgctggccg cgattcgcg cgtttatggg 720
ctgcttgcca atacggtgcg gtatctgcag ggcggcgggt cgtggcggga ggattgggga 780
cagctttcgg gggcgccgt gccgccccag ggtgcgagc cccagagcaa cgcgggcccc 840
cgaccccata tcggggacac gttatttacc ctgtttcggg ccccgagtt gctggcccc 900
aacggcgacc tgtataacgt gtttgccctg gctttggacg tcttgccaa acgcctcgt 960
cccatgcatg tctttatcct ggattacgac caatcgcccg ccggctgccg ggacgccctg 1020
ctgcaactta cctccgggat ggtccagacc cacgtacca cccaggetc cataccgacg 1080
atctgcgacc tggcgcgcac gtttgcccg gagatggggg aggctaactg a 1131
```

&lt;210&gt; 36

&lt;211&gt; 376

&lt;212&gt; PRT

&lt;213&gt; Human Herpesvirus-1

&lt;300&gt;

&lt;308&gt; GenBank No. NP\_04462

&lt;309&gt; 2004-01-13

&lt;400&gt; 36

```
Met Ala Ser Tyr Pro Cys His Gln His Ala Ser Ala Phe Asp Gln Ala
1          5          10          15
Ala Arg Ser Arg Gly His Asn Asn Arg Arg Thr Ala Leu Arg Pro Arg
20          25          30
Arg Gln Gln Lys Ala Thr Glu Val Arg Leu Glu Gln Lys Met Pro Thr
35          40          45
Leu Leu Arg Val Tyr Ile Asp Gly Pro His Gly Met Gly Lys Thr Thr
50          55          60
Thr Thr Gln Leu Leu Val Ala Leu Gly Ser Arg Asp Asp Ile Val Tyr
65          70          75          80
Val Pro Glu Pro Met Thr Tyr Trp Arg Val Leu Gly Ala Ser Glu Thr
85          90          95
Ile Ala Asn Ile Tyr Thr Thr Gln His Arg Leu Asp Gln Gly Glu Ile
100         105         110
Ser Ala Gly Asp Ala Ala Val Val Met Thr Ser Ala Gln Ile Thr Met
115         120         125
```

Gly Met Pro Tyr Ala Val Thr Asp Ala Val Leu Ala Pro His Ile Gly  
 130 135 140  
 Gly Glu Ala Gly Ser Ser His Ala Pro Pro Pro Ala Leu Thr Leu Ile  
 145 150 155 160  
 Phe Asp Arg His Pro Ile Ala Ala Leu Leu Cys Tyr Pro Ala Ala Arg  
 165 170 175  
 Tyr Leu Met Gly Ser Met Thr Pro Gln Ala Val Leu Ala Phe Val Ala  
 180 185 190  
 Leu Ile Pro Pro Thr Leu Pro Gly Thr Asn Ile Val Leu Gly Ala Leu  
 195 200 205  
 Pro Glu Asp Arg His Ile Asp Arg Leu Ala Lys Arg Gln Arg Pro Gly  
 210 215 220  
 Glu Arg Leu Asp Leu Ala Met Leu Ala Ala Ile Arg Arg Val Tyr Gly  
 225 230 235 240  
 Leu Leu Ala Asn Thr Val Arg Tyr Leu Gln Gly Gly Gly Ser Trp Arg  
 245 250 255  
 Glu Asp Trp Gly Gln Leu Ser Gly Ala Ala Val Pro Pro Gln Gly Ala  
 260 265 270  
 Glu Pro Gln Ser Asn Ala Gly Pro Arg Pro His Ile Gly Asp Thr Leu  
 275 280 285  
 Phe Thr Leu Phe Arg Ala Pro Glu Leu Leu Ala Pro Asn Gly Asp Leu  
 290 295 300  
 Tyr Asn Val Phe Ala Trp Ala Leu Asp Val Leu Ala Lys Arg Leu Arg  
 305 310 315 320  
 Pro Met His Val Phe Ile Leu Asp Tyr Asp Gln Ser Pro Ala Gly Cys  
 325 330 335  
 Arg Asp Ala Leu Leu Gln Leu Thr Ser Gly Met Val Gln Thr His Val  
 340 345 350  
 Thr Thr Pro Gly Ser Ile Pro Thr Ile Cys Asp Leu Ala Arg Thr Phe  
 355 360 365  
 Ala Arg Glu Met Gly Glu Ala Asn  
 370 375