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(54) Title: REOVIRUSES HAVING MODIFIED SEQUENCES

(57) Abstract: The invention provides for modified reovirus nucleic acid sequences and modified reovirus polypeptide sequences as well as reoviruses containing such modified nucleic acid or polypeptide sequences. The invention also provides for pharmaceutical compositions that include reoviruses having a modified sequence as well as methods of making and using such reoviruses.

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REOVIRUSES HAVING MODIFIED SEQUENCES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. 119(e) to U.S. Application No. 60/894,425 filed on March 12, 2007 and U.S. Application No. 60/989,568 filed on November 21, 2007, both of which are incorporated herein by reference.

TECHNICAL FIELD

This invention relates to viruses, and more particularly to reoviruses having modified sequences.

BACKGROUND

The name reovirus derives from an acronym for respiratory and enteric orphan virus, reflecting that the initial isolates came from human respiratory and enteric tracts but were not associated with serious disease. Reoviruses have a double-stranded, segmented RNA genome. The virions measure 60-80 nm in diameter and possess two concentric capsid shells, each of which is icosahedral. The mammalian reovirus genome consists of double-stranded RNA in 10 discrete segments with a total genome size of ~23.5 kbp. The individual RNA segments vary in size.

Three serologically distinct but related types of reovirus have been recovered from mammalian species: type 1 (representative strains include, for example, Lang (T1L)), type 2 (representative strains include, for example, Jones (T2J)) and type 3 (representative strains include, for example, Dearing or Abney (T3D or T3A, respectively)). The three serotypes are easily identifiable on the basis of neutralization and hemagglutinin-inhibition assays (see, for example, Sabin, 1959, *Science*, 130:966; Fields, et al., 1996, *Fundamental Virology*, 3rd Ed., Lippincott-Raven; Rosen, 1960, *Am. J. Hyg.*, 71:242; and Stanley, 1967, *Br. Med. Bull.*, 23:150).

SUMMARY

Provided herein are reoviruses having modified nucleic acid and polypeptide sequences. Sequence modifications include, for example, modifications in one or more of the reovirus genome segments. Also provided are pharmaceutical compositions that include reoviruses having a modified sequence as well as methods of using such reoviruses.

In one aspect, the invention provides a reovirus that has a lambda-3 polypeptide having one or more amino acid modifications; a sigma-3 polypeptide having one or more amino acid modifications; a mu-1 polypeptide having one or more amino acid modifications; and/or a mu-2 polypeptide having one or more amino acid modifications. Such a reovirus can be, for example, non-naturally occurring. In another aspect, the invention provides a reovirus lambda-3 polypeptide having one or more amino acid modifications; a reovirus sigma-3 polypeptide having one or more amino acid modifications; a reovirus mu-1 polypeptide having one or more amino acid modifications; and/or a reovirus mu-2 polypeptide having one or more amino acid modifications.

By way of example, the one or more amino acid modifications in the lambda-3 polypeptide can be a Val at residue 214, an Ala at residue 267, a Thr at residue 557, a Lys at residue 755, a Met at residue 756, a Pro at residue 926, a Pro at residue 963, a Leu at residue 979, an Arg at residue 1045, a Val at residue 1071, or any combination thereof, numbered relative to GenBank Accession No. M24734.1. It is noted that, when the amino acid sequence is a Val at residue 214 or a Val at residue 1071, the amino acid sequence further includes at least one additional change in the amino acid sequence. In one embodiment, the lambda-3 polypeptide includes the sequence shown in SEQ ID NO:18.

Further by way of example, the one or more amino acid modifications in the sigma-3 polypeptide can be a Leu at residue 14, a Lys at residue 198, or any combination thereof, numbered relative to GenBank Accession No. K02739. It is noted that, when the amino acid sequence is a Leu at residue 14, the amino acid sequence further includes at least one additional change in the amino acid sequence. In one embodiment, the sigma-3 polypeptide includes the sequence shown in SEQ ID NO:14.

Further by way of example, the one or more amino acid modifications in the mu-1 polypeptide can be an Asp at residue 73 numbered relative to GenBank Accession No. M20161.1. In one embodiment, the mu-1 polypeptide includes the sequence shown in SEQ ID NO:16.

Also by way of example, the amino acid modification mu-2 polypeptide can be a Ser at residue 528 numbered relative to GenBank Accession No. AF461684.1. In one embodiment, the mu-1 polypeptide includes the sequence shown in SEQ ID NO:15.

A reovirus as described herein having one or more modifications can further include a reovirus sigma-2 polypeptide. Such a sigma-2 polypeptide can have a Cys at one or more of position 70, 127, 195, 241, 255, 294, 296, or 340, numbered relative to GenBank Accession No. NP_694684.1. In one embodiment, the sigma-2 polypeptide includes the sequence shown in SEQ ID NO:12.

In another aspect, the invention provides a reovirus that has a L1 genome segment having one or more nucleic acid modifications; a S4 genome segment having one or more nucleic acid modifications; a M1 genome segment having one or more nucleic acid modifications; and/or a M2 genome segment having one or more nucleic acid modifications. Such a reovirus can be, for example, non-naturally occurring. In another aspect, the invention provides a L1 genome segment having one or more nucleic acid modifications; a S4 genome segment having one or more nucleic acid modifications; a M1 genome segment having one or more nucleic acid modifications; and/or a M2 genome segment having one or more nucleic acid modifications.

By way of example, the one or more nucleic acid modifications in the L1 genome segment can be a T at position 660, a G at position 817, an A at position 1687, a G at position 2283, an ATG at positions 2284-2286, a C at position 2794, a C at position 2905, a C at position 2953, an A at position 3153, or a G at position 3231, numbered relative to GenBank Accession No. M24734.1. In one embodiment, the L1 genome segment includes the sequence shown in SEQ ID NO:8.

Further by way of example, the one or more nucleic acid modifications in the S4 genome segment can be an A at position 74 and an A at position 624, numbered relative to GenBank Accession No. K02739. In one embodiment, the S4 genome segment includes the sequence shown in SEQ ID NO:4.

Further by way of example, the nucleic acid modification in the M2 genome segment can be a C at position 248, numbered relative to GenBank Accession No. M20161.1. In one embodiment, the M2 genome segment includes the sequence shown in SEQ ID NO:6.

Also by way of example, the nucleic acid modification in the M1 genome segment can be a T at position 1595, numbered relative to GenBank Accession No. AF461684.1. In one embodiment, the M1 genome segment includes the sequence shown in SEQ ID NO:5.

A reovirus as described herein can include any modification or combination of modifications disclosed herein. In some embodiments, a reovirus as described herein is a reassortant. In certain embodiments, a reovirus as described herein includes genomic segments having the sequences shown in SEQ ID NOs:1-10 or the polypeptides shown in SEQ ID NOs:11, 12, and 16-21, and either or both SEQ ID NO:13 or 14. In one embodiment, a reovirus as disclosed herein is identified as IDAC Accession No. 190907-01.

A reovirus as disclosed herein generally exhibits a growth advantage over a reovirus that does not contain a corresponding modification. Representative growth advantages include, but are not limited to, an increased rate of lysis; an increased size of plaque formation; an increased rate of RNA replication; an increased rate of RNA transcription; an increased rate of translation; an increased rate of virus assembly and/or packaging; an increased number of viral progeny; an increased ability of a reovirus to be taken up by a host cell; an increased or enhanced ability to uncoat; enhanced cell lysis or inducement to cell death including apoptosis, necrosis or autophagy; an enhanced ability to infect, lyse and kill human neoplastic cells lines; decreased immunogenicity in mammalian cells; differential susceptibility to interferon sensitivity; decreased toxicity toward the host; enhanced drug interaction; enhanced radiotherapy interaction; or the ability to release effective tumor epitopes.

A reovirus as described herein can be included, along with a pharmaceutically acceptable carrier, in a pharmaceutical composition. Such pharmaceutical compositions can include, for example, one or more chemotherapeutic agents and/or one or more immunosuppressive agents.

In still another aspect, the invention provides for methods of making an improved reovirus. Such methods generally include the steps of modifying the nucleic acid sequence of the reovirus, and selecting one or more improved reoviruses. In some embodiments, the modifying step includes, for example, mutagenizing the reovirus. Representative types of mutagenesis include, without limitation, site-directed mutagenesis and chemical mutagenesis. In other embodiments, the modifying step includes culturing the reovirus in a human cell line.

An improved reovirus made according to the methods disclosed herein can be selected for an increased rate of lysis; an increased size of plaque formation; an increased rate of RNA replication; an increased rate of RNA transcription; an increased rate of translation; an increased rate of virus assembly and/or packaging; an increased number of viral progeny; an increased ability of a reovirus to be taken up by a host cell; an increased or enhanced ability to uncoat; enhanced cell lysis or inducement to cell death including apoptosis, necrosis or autophagy; an enhanced ability to infect, lyse and kill human neoplastic cells lines; decreased immunogenicity in mammalian cells; differential susceptibility to interferon sensitivity; decreased toxicity toward the host; enhanced drug interaction; enhanced radiotherapy interaction; or the ability to release effective tumor epitopes.

In yet another aspect, the invention provides methods of treating a proliferative disorder in a patient. Such methods generally include administering a modified reovirus as described herein or a pharmaceutical composition containing such a modified reovirus to the patient. Typically, the reovirus is administered in an amount effective to cause oncolysis, and can be administered more than once. Representative routes of administration include, for example, direct injection, intravenously, intravascularly, intrathecally, intramuscularly, subcutaneously, intraperitoneally, topically, orally, rectally, vaginally, nasally, or by inhalation. The methods of treating a proliferative disorder as described herein can be accompanied by one of more procedures such as surgery, chemotherapy, radiation therapy, and immunosuppressive therapy.

In another aspect, the invention provides a kit (or article of manufacture) that includes a reovirus having a modified sequence or any combination of genome segments having a modified sequence as disclosed herein. A kit also can include one or more agents as disclosed herein.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the drawings and detailed description, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1 is the nucleotide sequence of a representative S1 segment (SEQ ID NO:1), S2 segment (SEQ ID NO:2), S3 segment (SEQ ID NO:3) and S4 segment (SEQ ID NO:4).

Figure 2 is the nucleotide sequence of a representative M1 segment (SEQ ID NO:5), M2 segment (SEQ ID NO:6) and M3 segment (SEQ ID NO:7).

Figure 3 is the nucleotide sequence of a representative L1 segment (SEQ ID NO:8), L2 segment (SEQ ID NO:9) and L3 segment (SEQ ID NO:10).

Figure 4 is the amino acid sequence of a representative sigma-1 polypeptide (SEQ ID NO:11), sigma-2 polypeptide (SEQ ID NO:12), sigma-NS polypeptide (putative coding sequence 1, SEQ ID NO:13; putative coding sequence 2, SEQ ID NO:14) and sigma-3 polypeptide (SEQ ID NO:15).

Figure 5 is the amino acid sequence of a representative mu-2 polypeptide (SEQ ID NO:16), mu-1 polypeptide (SEQ ID NO:17) and mu-NS polypeptide (SEQ ID NO:18).

Figure 6 is the amino acid sequence of a representative lambda-3 polypeptide (SEQ ID NO:19), lambda-2 polypeptide (SEQ ID NO:20) and lambda-1 polypeptide (SEQ ID NO:21).

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

This disclosure describes modifications in the nucleotide and amino acid sequence of a reovirus. Such modifications are optionally selected to affect the virus's ability to replicate and/or package itself and, therefore, alter the infectivity and/or rate of replication of a reovirus.

Reovirus Having Modified Sequences and Methods of Making

Any of the genomic segments from any type 3 mammalian orthoreovirus (referred to herein simply as "reovirus") can be modified as disclosed herein. Representative type 3 mammalian orthoreoviruses include, without limitation, Dearing and Abney strains. See, for example, ATCC Accession Nos. VR-232 and VR-824. Reoviruses that can be modified as disclosed herein include naturally-occurring reoviruses (e.g., isolated from a source in nature such as from a patient) and reassortant reoviruses (see, e.g., U.S. Patent No. 7,163,678).

Representative modifications to the different genomic segments of a reovirus and their manifestations in the encoded polypeptide are shown in Table 1. The modifications shown in Table 1 show modifications (both in the number of modifications and in the non-conservative nature of many of the modifications) in the sequence of segments encoding polypeptides associated with RNA-dependent RNA polymerase, transcriptional activities and/or RNA binding. For example, many of the novel modifications disclosed herein are located in the L1 genome segment. The wild-type L1 genome segment encodes a 1,267 amino acid (142 kDa) protein designated lambda-3. Lambda-3 represents the catalytic subunit of the reovirus RNA-dependent RNA polymerase, which mediates both plus- and minus-strand RNA synthesis within reovirus particles. Further modifications were observed in the M2 genome segment. The wild-type M2 genome segment encodes a 708 amino acid (76 kDa) protein designated mu-1, which is involved in the regulation of particle-bound transcription. In addition, modifications also were observed in the S4 and M1 genomic segments, which encode sigma-3 and mu-2, respectively, and play a role in transcription or single-stranded or double-stranded RNA binding.

Thus, this disclosure provides for L1, S4, M1, M2 or any combination of such genome segments that contain one or more nucleic acid modifications in the respective genome segment. Provided herein is a reovirus L1 genome segment having one or more nucleic acid modifications; a reovirus S4 genome segment having one or more nucleic acid modifications; a reovirus M1 genome segment having one or more nucleic acid modifications; and/or a M2 genome segment having one or more nucleic acid modifications.

A reovirus L1 genome segment has, for example, any combination of one or more of the following nucleotides: a T at position 660, a G at position 817, an A at position 1687, a G at position 2283, an ATG at positions 2284-2286, a C at position 2794, a C at position 2905, a C at position 2953, an A at position 3153, or a G at position 3231 (numbered relative to GenBank Accession No. M24734.1). A reovirus S4 genome segment has, for example, any combination of one or more of the following nucleotides: an A at position 74 or an A at position 624 (numbered relative to GenBank Accession No. K02739). A reovirus M1 genome segment has, for example, a T nucleotide at position 1595 (numbered relative to GenBank Accession No. AF461684.1). A reovirus M2 genome segment has, for example, a C nucleotide at position 248 (numbered relative to GenBank Accession No. M20161.1). The indicated nucleotide at the indicated position represents modifications when compared to other corresponding sequences available in public databases (e.g., GenBank Accession Nos. M24734.1, K02739, AF461684.1, and M20161.1).

A reovirus lambda-3 polypeptide has, for example, any combination of one or more amino acid residues: a Val at residue 214, an Ala at residue 267, a Thr at residue 557, a Lys at residue 755, a Met at residue 756, a Pro at residue 926, a Pro at residue 963, a Leu at residue 979, an Arg at residue 1045, or a Val at residue 1071 (numbered relative to GenBank Accession No. M24734.1). It is noted that, when the polypeptide sequence comprises a Val at residue 214 or a Val at residue 1071, the polypeptide sequence further comprises at least one additional change in the amino acid sequence. A reovirus sigma-3 polypeptide has, for example, any combination of one or more amino acid residues: a Leu at residue 14 or a Lys at residue 198 (numbered relative to GenBank Accession No. K02739). It is noted that, when the polypeptide sequence comprises a Leu at residue 14, the polypeptide sequence further comprises at least one additional change in the amino acid sequence. A reovirus mu-1 polypeptide has, for example, an Asp at residue 73 (numbered relative to GenBank Accession No. AF461684.1). A reovirus mu-2 polypeptide has, for example, a Ser at residue 528 (numbered relative to GenBank Accession No. M20161.1). The indicated amino acid at the indicated position represents modifications when compared to other corresponding sequences in public databases (e.g., GenBank Accession Nos. M24734.1, K02739, AF461684.1, and M20161.1).

As used herein, a "non-naturally occurring" reovirus is a reovirus that has at least one nucleic acid or amino acid modification as compared to wild type sequences derived from, for example, a field isolate (e.g., a patient). "Non-naturally occurring" reovirus refers to a virus which has been manipulated or modified in the laboratory. Such manipulated or modified reoviruses include laboratory strains or mutagenized versions. These versions are distinguishable, in nucleic acid and/or amino acid sequence, from, for example, Dearing and Abney strains (e.g., ATCC VR-824 and VF-232, respectively). Representative modifications to one or more of the genome segments, the encoded polypeptide, or both are disclosed herein. In addition to a genome segment or polypeptide containing one or more of the modifications described herein, a reovirus optionally contains an S2 genome segment, which encodes the sigma-2 polypeptide. A sigma-2 polypeptide, for example, has a Cys at one or more or all of the following positions: 70, 127, 195, 241, 255, 294, 296 or 340 (numbered relative to GenBank Accession No. NP_694684.1).

A modification generally occurs at the nucleic acid level, which may or may not manifest itself in the encoded polypeptide. Modifications to a nucleic acid include, without limitation, single or multiple nucleotide transitions (purine to purine or pyrimidine to pyrimidine) or transversions (purine to pyrimidine or *vice versa*) and single- or multiple-nucleotide deletions or insertions. A modification in a nucleic acid can result in one or more conservative or non-conservative amino acid substitutions in the encoded polypeptide, a shift in the reading frame of translation ("frame-shift) resulting in an entirely different polypeptide encoded from that point on, a premature stop codon resulting in a truncated polypeptide ("truncation"), or a modification in a reovirus nucleic acid may not change the encoded polypeptide at all ("silent" or "nonsense"). See, for example, Johnson & Overington, 1993, *J. Mol. Biol.*, 233:716-38; Henikoff & Henikoff, 1992, *Proc. Natl. Acad. Sci. USA*, 89:10915-19; and U.S. Patent No. 4,554,101 for disclosure on conservative and non-conservative amino acid substitutions.

Nucleic acids from reovirus particles are isolated, for example, using standard methodologies, which are commercially available. See also, for example, Schiff et al., "Orthoreoviruses and Their Replication," Ch 52, in *Fields Virology*, Knipe & Howley, eds., 2006, Lippincott Williams & Wilkins. As used herein, "isolated" nucleic acids refer to nucleic acids that are substantially separated from other nucleic acids with which they are usually associated. Thus, an "isolated" nucleic acid includes, without limitation, reoviral nucleic acid that is essentially free of non-reoviral (e.g., host cell) nucleic acid, or a reoviral genomic segment that is essentially free of nucleic acid corresponding to other genomic segments. In addition, an isolated nucleic acid includes an engineered nucleic acid such as recombinant or synthetic nucleic acids.

Modifications are generated in the nucleic acid of a reovirus using any number of methods known in the art. For example, site directed mutagenesis can be used to modify a reovirus nucleic acid sequence. One of the most common methods of site-directed mutagenesis is oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, an oligonucleotide encoding the desired change(s) in sequence is annealed to one strand of the DNA of interest and serves as a primer for initiation of DNA synthesis. In this manner, the oligonucleotide containing the sequence change is incorporated into the newly synthesized strand. See, for example, Kunkel, 1985, *Proc. Natl. Acad. Sci. USA*, 82:488; Kunkel et al., 1987, *Meth. Enzymol.*, 154:367; Lewis & Thompson, 1990, *Nucl. Acids Res.*, 18:3439; Bohnsack, 1996, *Meth. Mol. Biol.*, 57:1; Deng & Nickoloff, 1992, *Anal. Biochem.*, 200:81; and Shimada, 1996, *Meth. Mol. Biol.*, 57:157.

Other methods are routinely used in the art to introduce a modification into a sequence. For example, modified nucleic acids are generated using PCR or chemical synthesis, or polypeptides having the desired change in amino acid sequence can be chemically synthesized. See, for example, Bang & Kent, 2005, *Proc. Natl. Acad. Sci. USA*, 102:5014-9 and references therein. Selection on a cell type on which reovirus is not usually grown (e.g., human cells) and/or chemical mutagenesis (see, for example, Rudd & Lemay, 2005, *J. Gen. Virology*, 86:1489-97) also can be used to generate modifications in the nucleic acid of a reovirus. For example, the modifications shown in Table 1 were generated by culturing reovirus on human cells (e.g., human embryonic kidney (HEK) 293 cells), which are not typically used in the art of culturing reovirus. In contrast, cells that are commonly used to culture reovirus are described in, for example, Tyler, "Mammalian Reoviruses," Ch 53, page 1731-2, in *Fields Virology*, Knipe & Howley, eds., 2006, Lippincott Williams & Wilkins. The modifications described herein represent an adaptation by the reovirus to human cells. There was also a selection step at each of these plaque purification steps by selection the largest plaque (triple plaque purification), thus a growth or virulence advantage in these cells.

After one or more modifications have been introduced into a reovirus nucleic acid or polypeptide, virus particles are reconstituted using methods known in the art. See, for example, Schiff et al., "Orthoreoviruses and Their Replication," Ch 52, in *Fields Virology*, Knipe & Howley, eds., 2006, Lippincott Williams & Wilkins; Smith et al., 1969, Virology, 39(4):791-810; and U.S. Patent Nos. 7,186,542; 7,049,127; 6,808,916; and 6,528,305. Reoviruses having one or more modifications in their sequence are cultured in, for example, mouse L929 cells or neoplastic cells (e.g., MCF7 (ATCC Accession No. HTB-22), SKBR3 (ATCC Accession No. HTB-30), or MDA MB 468 (ATCC Accession No. HTB 132) cells), and selected based on any number of characteristics that may indicate, for example, a growth advantage over a reovirus that does not contain one or more modifications. Reoviruses are selected following culturing in a cell line (neoplastic or otherwise) and/or following infection of an animal model system.

Such characteristics include, without limitation, an increased rate of lysis; an increased size of plaque formation; an increased rate of RNA replication; an increased rate of RNA transcription; an increased rate of translation; an increased rate of virus assembly and/or packaging; an increased number of viral progeny; an increased ability of a reovirus to be taken up by a host cell; an increased or enhanced ability to uncoat; enhanced cell lysis or inducement to cell death including apoptosis, necrosis or autophagy; an enhanced ability to infect, lyse and kill human neoplastic cells lines; decreased immunogenicity in mammalian cells; differential susceptibility to interferon sensitivity; decreased toxicity toward the host; enhanced drug interaction; enhanced radiotherapy interaction; or the ability to release effective tumor epitopes. Additionally, reoviruses having a modified sequence are selected, for example, for the ability to lytically infect a mammalian cell having an active Ras pathway. See, for example, U.S. Patent No. 7,052,832.

Reovirus particles are obtained using any number of methods known in the art. For example, reoviruses are cultured in L929 mouse fibroblast cells or human cells (e.g., HEK 293), and the viral particles purified using standard methodology. See, for example, Schiff et al., "Orthoreoviruses and Their Replication," Ch 52, in *Fields Virology*, Knipe & Howley, eds., 2006, Lippincott Williams & Wilkins; Smith et al., 1969, Virology, 39(4):791-810; and U.S. Patent Nos. 7,186,542; 7,049,127; 6,808,916; and 6,528,305. As used herein, "purified" viral particles refers to virus particles that have been substantially separated from cellular components that naturally accompany it. Typically, virus particles are considered "purified" when they are at least 70% (e.g., at least 75%, 80%, 85%, 90%, 95%, or 99%) by dry weight, free from the proteins and other cellular components with which the viruses are naturally associated.

A reovirus having the nucleic acid sequence shown in Figures 1, 2 and 3 (SEQ ID NOs: 1-10) and the amino acid sequence shown in Figures 4, 5, and 6 (SEQ ID NOs:11-20), which contain the nucleotide and amino acid modifications shown in Table 1, was deposited with the International Depositary Authority of Canada (IDAC, National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington St., Winnipeg, Manitoba Canada R3E 3R2) on September 19, 2007, and assigned Accession No. 190907-01. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit is exemplary and was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required for patentability (e.g., under 35 U.S.C. §112).

Methods of Using Reoviruses Having Modified Sequences

As described previously (see, for example, U.S. Patent Nos. 6,110,461; 6,136,307; 6,261,555; 6,344,195; 6,576,234; and 6,811,775), reoviruses use a host cell's Ras pathway machinery to downregulate double-stranded RNA-activated protein kinase (PKR) and thus replicate in the cell. Based upon these discoveries, methods have been developed for using reovirus to treat proliferative disorders in mammals. Representative mammals include mice, dogs, cats, sheep, goats, cows, horses, pigs, non-human primates, and humans. As used herein, a "patient" includes any mammal with a proliferative disorder.

A "proliferative disorder" is any cellular disorder in which the cells proliferate more rapidly than normal tissue growth. Thus a "proliferating cell" is a cell that is proliferating more rapidly than normal cells. A proliferative disorder includes, but is not limited to, neoplasms, which are also referred to as tumors. A neoplasm includes, but is not limited to, pancreatic cancer, breast cancer, brain cancer (e.g., glioblastoma), lung cancer, prostate cancer, colorectal cancer, thyroid cancer, renal cancer, adrenal cancer, liver cancer, neurofibromatosis, and leukemia. A neoplasm includes a solid neoplasm (e.g. sarcoma or carcinoma) or a cancerous growth affecting the hematopoietic system (e.g., lymphoma or leukemia). Other proliferative disorders include, but are not limited to neurofibromatosis.

Generally, in proliferative disorders for which reovirus is used as a treatment, at least some of the proliferating cells have a mutation in which the Ras gene (or an element of the Ras signaling pathway) is activated, either directly (e.g., by an activating mutation in Ras) or indirectly (e.g., by activation of an upstream or downstream element in the Ras pathway). Activation of an upstream element in the Ras pathway includes, for example, transformation with epidermal growth factor receptor (EGFR) or Sos. See, for example, Wiessmuller & Wittinghofer, 1994, *Cellular Signaling*, 6(3):247-267; and Barbacid, 1987, *Ann. Rev. Biochem.*, 56, 779-827. Activation of a downstream element in the Ras pathway includes, for example, a mutation within B-Raf. See, for example, Brose et al., 2002, *Cancer Res.*, 62:6997-7000. In addition, reovirus is useful for treating proliferative disorders caused by mutations or dysregulation of PKR. See, for example, Strong et al., 1998, *EMBO J.*, 17:3351-662.

A reovirus having a modified sequence as disclosed herein is administered to a mammal that has a proliferative disorder. As used herein, administration refers to delivery of a reovirus such that the reovirus contacts the proliferating cells. The route by which a reovirus is administered depends on the type of disorder and the location of the proliferating cells. A wide variety of administration routes can be employed. For example, for a solid neoplasm that is accessible, a reovirus is administered by direct injection. For a hematopoietic neoplasm, for example, a reovirus is administered intravenously or intravascularly. For certain neoplasms, e.g., those not easily accessible within the body such as metastases or brain tumors, a reovirus is administered in a manner such that it is transported systemically through the body of the mammal to thereby reach the neoplasm (e.g., intrathecally, intravenously, intramuscularly, subcutaneously, or intra-peritoneally). A reovirus also is administered locally including, for example, topically (e.g., for melanoma), orally (e.g., for oral or esophageal neoplasm), rectally (e.g., for colorectal neoplasm), vaginally (e.g., for cervical or vaginal neoplasm), nasally or by inhalation (e.g., for lung neoplasm). A reovirus is optionally administered by more than one route and/or to more than one location in an individual.

Targeted administration may be used to administer a reovirus. For example, dendritic cells containing a reovirus may be administered to a subject. See, for example, US Publication No. 2008/0014183. In another example of targeted delivery, carrier cells may be used to target cells of a proliferative disorder and prevent immune recognition of a reovirus which they carry. See, for example, Qiao et al., 2008, *Nature Med.*, 14:37-44; and WO 2008/009115.

A reovirus having a modified sequence as disclosed herein is administered in an amount that is sufficient to treat the proliferative disorder (e.g., an "effective amount"). A proliferative disorder is "treated" when administration of a reovirus having a modified sequence to proliferating cells affects one or more symptoms or clinical signs of the disorder including, e.g., increasing lysis (e.g., "oncolysis") of the cells, reducing the number of proliferating cells, reducing the size or progression of a neoplasm, reducing pain associated with the neoplasm, as compared to the signs or symptoms in the absence of the treatment. As used herein, the term "oncolysis" means at least 10% of the proliferating cells are lysed (e.g., at least 20%, 30%, 40%, 50%, or 75% of the cells are lysed). The percentage of lysis can be determined, for example, by measuring the reduction in the size of a neoplasm or in the number of proliferating cells in a mammal, or by measuring the amount of lysis of cells *in vitro* (e.g., from a biopsy of the proliferating cells).

An effective amount of a reovirus having a modified sequence is determined on an individual basis and is based, at least in part, on the particular reovirus used; the individual's size, age, gender; and the size and other characteristics of the proliferating cells. For example, for treatment of a human, approximately 10^3 to 10^{12} plaque forming units (PFU) of a reovirus is used, depending on the type, size and number of proliferating cells or neoplasms present. The effective amount can be from about 1.0 PFU/kg body weight to about 10^{15} PFU /kg body weight (e.g., from about 10^2 PFU /kg body weight to about 10^{13} PFU /kg body weight). A reovirus is administered in a single dose or in multiple doses (e.g., two, three, four, six, or more doses). Multiple doses are administered concurrently or consecutively (e.g., over a period of days or weeks). Treatment with a reovirus having a modified sequence lasts from several days to several months or until diminution of the disease is achieved.

It is contemplated that a reovirus having a modified sequence as disclosed herein is optionally administered in conjunction with surgery or removal of proliferating cells (e.g., a neoplasm). It also is contemplated that a reovirus having a modified sequence is optionally administered in conjunction with or in addition to radiation therapy. It is further contemplated that a reovirus having a modified sequence is optionally administered in conjunction with or in addition to known anticancer compounds, chemotherapeutic agents, and/or immunosuppressive agents. Such agents, include, but are not limited to, 5-fluorouracil, mitomycin C, methotrexate, hydroxyurea, gemcitabine, cyclophosphamide, dacarbazine, mitoxantrone, anthracyclins (Epirubicin, Irinotecan, and Doxurubicin), antibodies to receptors such as herceptin, topoisomerase inhibitors such as etoposide or camptothecin, pregnasome, platinum compounds such as carboplatin and cisplatin, taxanes such as taxol and taxotere, hormone therapies such as tamoxifen and anti-estrogens, interleukins, interferons, aromatase inhibitors, progestational agents, LHRH analogs, mTOR inhibitors (e.g., rapamycin and derivatives thereof; see, for example, Homicsko et al., 2005, *Cancer Res.*, 65:6882-90; and Rao et al., 2004, *Curr. Cancer Drug Targets*, 4:621-35), and combinations thereof.

It is further contemplated that a reovirus having a modified sequence is administered in conjunction with an agent that can increase endothelial permeability and/or decrease interstitial fluid pressure. Such agents include, for example, TNF-α. See, for example, Sacchi et al., 2006, *Clin. Cancer Res.*, 12:175-182. It is contemplated that a reovirus having a modified sequence can be administered in conjunction with any combination of the therapies and agents described herein.

Pharmaceutical Compositions

Pharmaceutical compositions that include one or more reoviruses, at least one of which has a modified sequence as described herein, are provided. See, for example, U.S. Patent No.

6,576,234. In addition to one or more reoviruses, at least one of which has a modified sequence, a pharmaceutical composition typically includes a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier includes a solid, semi-solid, or liquid material that acts as a vehicle, carrier or medium for the reovirus. Thus, for example, compositions containing a reovirus having a modified sequence are in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

Some examples of suitable carriers include phosphate-buffered saline or another physiologically acceptable buffer, lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. A pharmaceutical composition additionally can include, without limitation, lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. Pharmaceutical compositions of the invention can be formulated to provide quick, sustained or delayed release of a reovirus having a modified sequence after administration by employing procedures known in the art. In addition to the representative formulations described below, other suitable formulations for use in a pharmaceutical composition are found in *Remington: The Science and Practice of Pharmacy* (2003, Gennaro & Gennaro, eds., Lippincott Williams & Wilkens).

For preparing solid compositions such as tablets, a reovirus having a modified sequence is mixed with a pharmaceutical carrier to form a solid composition. Optionally, tablets or pills are coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, a tablet or pill comprises an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components, for example, are separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials are used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

Liquid formulations that include a reovirus having a modified sequence for oral administration or for injection generally include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. These liquid or solid compositions optionally contain suitable pharmaceutically acceptable excipients as described herein. Such compositions are administered, for example, by the oral or nasal respiratory route for local or systemic effect. Compositions in pharmaceutically acceptable solvents are nebulized by use of inert gases. Nebulized solutions are inhaled, for example, directly from the nebulizing device, from an attached face mask tent, or from an intermittent positive pressure breathing machine. Solution, suspension, or powder compositions are administered, orally or nasally, for example, from devices which deliver the formulation in an appropriate manner.

Another formulation that is employed in the methods taught herein employs transdermal delivery devices ("patches"). Such transdermal patches are used to provide continuous or discontinuous infusion of a reovirus having a modified sequence. The construction and use of transdermal patches for the delivery of pharmaceutical agents are performed according to methods known in the art. See, for example, U.S. Patent No. 5,023,252. Such patches are constructed for continuous, pulsatile, or on-demand delivery of a reovirus having a modified sequence.

A reovirus having a modified sequence is optionally chemically or biochemically pretreated (e.g., by treatment with a protease such as chymotrypsin or trypsin) prior to administration (e.g., prior to inclusion in the pharmaceutical composition). Pretreatment with a protease removes the outer coat or capsid of the virus and can be used to increase the infectivity of the virus. Additionally or alternatively, a reovirus having a modified sequence is coated in a liposome or micelle to reduce or prevent an immune response in a mammal that has developed immunity toward a reovirus. Such reoviruses are referred to as "immunoprotected reoviruses." See, for example, U.S. Patent Nos. 6,565,831 and 7,014,847.

A reovirus having a modified sequence or a pharmaceutical composition comprising such a reovirus can be packaged into a kit. It is contemplated that a kit optionally includes one or more chemotherapeutic agents and/or immunosuppressive agents (e.g., anti-antireovirus antibodies). A pharmaceutical composition, for example, is formulated in a unit dosage form. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of a reovirus having a modified sequence calculated to produce the desired therapeutic effect in association with a suitable pharmaceutically acceptable carrier.

In accordance with the present invention, there may be employed conventional molecular biology, microbiology, biochemical, and recombinant DNA techniques within the skill of the art.

Such techniques are explained fully in the literature. The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1—Sequencing and Analysis

Cultures for production of reovirus were initiated from a suspension-adapted HEK 293 S Master Cell Bank (MCB). HEK 293 cells were maintained in Serum Free Medium (HEK 293 SFM II) supplemented with L-glutamine. HEK 293 cells were expanded and seeded into three 15 L spinner flasks, and further expanded until there was 12 L in each of the three flasks. Infection of the HEK 293 cells by reovirus was performed by direct inoculation of the virus into the cell culture. The virus was harvested when the viability of the HEK 293 S cells had decreased by 20-50% post-infection. The virus material from all three spinners was pooled in a single sterile container and agitated to create a homogeneous mixture. Three liters of the pooled cell suspension was removed, transferred to conical tubes, and centrifuged at ~3000 rpm for 15 minutes. The cells were then resuspended with 100 mL of clarified conditioned medium, snap frozen in an alcohol-dry ice bath three times, and then filled into sterile, labeled cryovials for use as a seed stock.

A viral stock was prepared by performing a three-time plaque purification. Adherent HEK 293 S cells were plated onto 6-well tissue culture plates, infected with the seed stock described above, and two of the largest plaques were picked. These two plaques were separately amplified and harvested and two of the largest plaques were picked again. This procedure was repeated again for a total of three times. Of the two plaques, one was selected as seed stock for subsequent expansions.

Cultures were initiated from the same suspension adapted HEK 293 S MCB described above and were maintained in the same HEK 293 Serum Free Medium (HEK 293 SFM II) supplemented with L-glutamine. Cells were expanded from T-flasks up to multiple 3 L spinner flasks. The infection was performed by first diluting the plaque-purified virus in HEK 293 SFM media and then adding 8 to 12 mL of the diluted virus into the cell culture. The virus was harvested when the viability of the HEK 293 S cells had decreased by 20-50% following infection, and microscopic examination of each of the spinners confirmed the lack of microbial contamination and that a cytopathic effect (CPE) was present in the cells. CPE was indicated by cells having a swollen and granular appearance. The material from the spinners was pooled in a single sterile container, agitated to create a homogeneous mixture, and a bulk harvest sample removed. The remaining pooled cell suspension was transferred to conical tubes and centrifuged at ~3000 rpm for 15 minutes. The cells were then resuspended with 400 mL of clarified conditioned medium, and the concentrated cell suspension was snap-frozen in an alcohol-dry ice bath three times to lyse the cells and then filled into sterile, labeled cryovials for use in sequencing reactions.

Both RNA strands were sequenced from both directions, and the sequence of each of the 10 genomic segments was assembled from the overlapping contigs. The assembled sequence of each genomic segment was used in a BLAST search of the NCBI database (ncbi.nlm.nih.gov on the World Wide Web). Alignments with three or four different reovirus sequences found in the NCBI database were examined and the alignment having the highest amount of homology was used for further analysis. The polymorphisms or modifications compared to other reported sequences are shown in Table 1. Those modifications that are unique to the selected reovirus strain are indicated with an asterisk in Table 1.

Table 1. Modifications Identified

Genomic Segment	**Position (nucleotide; amino acid)**	**Published Sequence (nucleotide; amino acid)**	**Novel Polymorphism (nucleotide; amino acid)**
S1		GenBank Accession No. M10262.1	SEQ ID NO:1
	499; 163	A; Thr	T; Ser
S3		GenBank Accession No. X01627.1	SEQ ID NO:3
	1057; 344	T; *Leu*	C; *Leu*
S4		GenBank Accesion No. K02739	SEQ ID NO:4
*	74; 14	G; *Leu*	A; *Leu*
*	624; 198	G; Glu	A; Lys
	719; 229	G; Glu	T; Asp
M1		GenBank Accession No. AF461684.1	SEQ ID NO:5
	1129; 372	G; Met	T; Ile
*	1595; 528	G; Ala	T; Ser
M2		GenBank Accession No. M20161.1	SEQ ID NO:6
*	248; 73	A; Glu	C; Asp
	302; 91	G; *Ala*	C; *Ala*
	303; 92	C; *Leu*	T; *Leu*
	305; 92	T; *Leu*	G; *Leu*
	709-10; 227	CG; Thr	GC; Ser
	1173; 382	T; *Leu*	C; *Leu*
L1		GenBank Accession No. M24734.1	SEQ ID NO:8
*	660; 214	A; *Val*	T; *Val*
*	817; 267	T; Ser	G; Ala
*	1687; 557	C; Pro	A; Thr
*	2283; 755	C; Asn	G; Lys
*	2284-6; 756	GAT; Asp	ATG; Met
*	2794; 926	A; Thr	C; Pro

*	2905; 963	T; Ser	C; Pro
*	2953; 979	A; Met	C; Leu
*	3153; 1045	C; Ser	A; Arg
*	3231; 1071	T; *Val*	G; *Val*
L2		GenBank Accession No. J03488.1	SEQ ID NO:9
	1838-40; 609	TTT; Phe	GGG; Gly
	3703; 1230	A; *Leu*	G; *Leu*
L3		GenBank Accession No. AF129822	SEQ ID NO:10
	1512; 500	T; Ile	G; Ser
	2569; 852	G; Gln	T; His

* designates unique modifications; italicized residues indicate silent modifications; position numbers are with respect to the indicated GenBank Accession No.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

Disclosed are materials, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed method and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular modification of a reovirus or treatment regime is disclosed and discussed and a number of modifications that can be made to the reovirus or regime are discussed, each and every combination and permutation of the reovirus and the regime are specifically contemplated unless specifically indicated to the contrary. Likewise, any subset or combination of these is also specifically contemplated and disclosed.

WHAT IS CLAIMED IS:

1. A reovirus comprising a lambda-3 polypeptide having one or more amino acid modifications, wherein the one or more amino acid modifications are selected from the group consisting of a Val at residue 214, an Ala at residue 267, a Thr at residue 557, a Lys at residue 755, a Met at residue 756, a Pro at residue 926, a Pro at residue 963, a Leu at residue 979, an Arg at residue 1045, a Val at residue 1071, or any combination thereof, numbered relative to SEQ ID NO:23 (GenBank Accession No. M24734.1), wherein when the amino acid sequence comprises a Val at residue 214 or a Val at residue 1071, the amino acid sequence further comprises at least one additional modification in the amino acid sequence.

2. The reovirus of claim 1, wherein the lambda-3 polypeptide comprises SEQ ID NO:18.

3. A reovirus comprising a sigma-3 polypeptide having one or more amino acid modifications, wherein the one or more amino acid modifications are selected from the group consisting of a Leu at residue 14, a Lys at residue 198, or any combination thereof, numbered relative to SEQ ID NO:25 (GenBank Accession No. K02739), wherein when the amino acid sequence comprises a Leu at residue 14, the amino acid sequence further comprises at least one additional modification in the amino acid sequence.

4. The reovirus of claim 3, wherein the sigma-3 polypeptide comprises SEQ ID NO:14.

5. A reovirus comprising a mu-1 polypeptide having at least one amino acid modification, wherein the at least one amino acid modification comprises an Asp at residue 73 numbered relative to SEQ ID NO:27 (GenBank Accession No. M20161.1).

6. The reovirus of claim 5, wherein the mu-1 polypeptide comprises SEQ ID NO:16.

7. A reovirus comprising a mu-2 polypeptide having at least one amino acid modification, wherein the at least one amino acid modification comprises a Ser at residue 528 numbered relative to SEQ ID NO:29 (GenBank Accession No. AF461684.1).

8. The reovirus of claim 7, wherein the mu-1 polypeptide comprises SEQ ID NO:15.

9. The reovirus of claim 1, 3, 5 or 7, where the reovirus further comprises a reovirus sigma-2 polypeptide.

10. The reovirus of claim 9, wherein the sigma-2 polypeptide comprises a Cys at one or more of position 70, 127, 195, 241, 255, 294, 296, or 340, numbered relative to SEQ ID NO:30 (GenBank Accession No. NP_694684.1).

11. The reovirus of claim 10, wherein the sigma-2 polypeptide comprises SEQ ID NO:12.

12. The reovirus of any of claims 1-11, wherein the reovirus is a reassortant.

13. A reovirus comprising a L1 genome segment having one or more nucleic acid modifications, wherein the one or more nucleic acid modifications are selected from the group consisting of a T at position 660, a G at position 817, an A at position 1687, a G at position 2283, an ATG at positions 2284-2286, a C at position 2794, a C at position 2905, a C at position 2953, an A at position 3153, a G at position 3231, numbered relative to SEQ ID NO:22 (GenBank Accession No. M24734.1).

14. The reovirus of claim 13, wherein the L1 genome segment comprises SEQ ID NO:8.

15. A reovirus comprising a S4 genome segment having one or more nucleic acid modifications, wherein the one or more nucleic acid modifications in the S4 genome segment are selected from the group consisting of an A at position 74 and an A at position 624, numbered relative to SEQ ID NO:24 (GenBank Accession No. K02739).

16. The reovirus of claim 15, wherein the S4 genome segment comprises SEQ ID NO:4.

17. A reovirus comprising a M2 genome segment having at least one nucleic acid modification, wherein the at least one nucleic acid modification comprises a C at position 248, numbered relative to (SEQ ID NO:26 (GenBank Accession No. M20161.1).

18. The reovirus of claim 17, wherein the M2 genome segment comprises SEQ ID NO:6.

19. A reovirus comprising a M1 genome segment having at least one nucleic acid modification, wherein the at least one nucleic acid modification comprises a T at position 1595, numbered relative to SEQ ID NO:28 (GenBank Accession No. AF461684.1).

20. The reovirus of claim 19, wherein the M1 genome segment comprises SEQ ID NO:5.

21. The reovirus of any of claims 13-20, wherein the reovirus is a reassortant.

22. A polypeptide comprising an amino acid sequence of a reovirus lambda-3 polypeptide, wherein the sequence of the reovirus lambda-3 polypeptide comprises one or more amino acid modifications, wherein the one or more amino acid modifications are selected from the group consisting of a Val at residue 214, an Ala at residue 267, a Thr at residue 557, a Lys at residue 755, a Met at residue 756, a Pro at residue 926, a Pro at residue 963, a Leu at residue 979, an Arg at residue 1045, a Val at residue 1071, or any combination thereof, numbered relative to SEQ ID NO:23 (GenBank Accession No. M24734.1), wherein when the amino acid sequence comprises a Val at residue 214 or a Val at residue 1071, the amino acid sequence further comprises at least one additional change in the amino acid sequence.

23. The polypeptide of claim 22, wherein the lambda-3 polypeptide comprises SEQ ID NO:18.

24. A polypeptide comprising an amino acid sequence of a reovirus sigma-3 polypeptide, wherein the sequence of the reovirus sigma-3 polypeptide comprises one or more amino acid modifications, wherein the one or more amino acid modifications are selected from the group consisting of a Leu at residue 14, a Lys at residue 198, or any combination thereof, numbered relative to SEQ ID NO:25 (GenBank Accession No. K02739), wherein when the amino acid sequence comprises a Leu at residue 14, the amino acid sequence further comprises at least one additional change in the amino acid sequence.

25. The polypeptide of claim 24, wherein the sigma-3 polypeptide comprises SEQ ID NO:14.

26. A polypeptide comprising an amino acid sequence of a reovirus mu-1 polypeptide, wherein the sequence of the reovirus mu-1 polypeptide comprises at least one amino acid

modification, wherein the at least one amino acid modification comprises an Asp at residue 73 numbered relative to SEQ ID NO:27 (GenBank Accession No. M20161.1).

27. The polypeptide of claim 26, wherein the mu-1 polypeptide comprises SEQ ID NO:16.

28. A polypeptide comprising an amino acid sequence of a reovirus mu-2 polypeptide, wherein the sequence of the reovirus mu-2 polypeptide comprises at least one amino acid modification, wherein the at least one amino acid modification comprises a Ser at residue 528 numbered relative to SEQ ID NO:29 (GenBank Accession No. AF461684.1).

29. The polypeptide of claim 28, wherein the mu-1 polypeptide comprises SEQ ID NO:15.

30. A reovirus comprising one or more of the polypeptides of claims 22-29.

31. The reovirus of claim 30, wherein the reovirus further comprises a sigma-2 polypeptide comprising a Cys at one or more of position 70, 127, 195, 241, 255, 294, 296, or 340, numbered relative to SEQ ID NO:30 (GenBank Accession No. NP_694684.1).

32. The reovirus of claim 31, wherein the sigma-2 polypeptide comprises SEQ ID NO:12.

33. The reovirus of any of claims 30-32, wherein the reovirus is a reassortant.

34. A nucleic acid molecule comprising a nucleic acid sequence of a reovirus L1 genome segment, wherein the nucleic acid sequence of the reovirus L1 genome segment comprises one or more nucleic acid modifications, wherein the one or more nucleic acid modifications are selected from the group consisting of a T at position 660, a G at position 817, an A at position 1687, a G at position 2283, an ATG at positions 2284-2286, a C at position 2794, a C at position 2905, a C at position 2953, an A at position 3153, a G at position 3231, numbered relative to SEQ ID NO:22 (GenBank Accession No. M24734.1).

35. The nucleic acid molecule of claim 34, wherein the L1 genome segment comprises SEQ ID NO:8.

36. A nucleic acid molecule comprising a nucleic acid sequence of a reovirus S4 genome segment, wherein the nucleic acid sequence of the reovirus S4 genome segment comprises one or more nucleic acid modifications, wherein the one or more nucleic acid modifications are selected from the group consisting of an A at position 74 and an A at position 624, numbered relative to SEQ ID NO:24 (GenBank Accession No. K02739).

37. The nucleic acid molecule of claim 36, wherein the S4 genome segment comprises SEQ ID NO:4.

38. A nucleic acid molecule comprising a nucleic acid sequence of a reovirus M1 genome segment, wherein the nucleic acid sequence of the reovirus M1 genome segment comprises at least one nucleic acid modification, wherein the at least one nucleic acid modification comprises a T at position 1595, numbered relative to SEQ ID NO:26 (GenBank Accession No. AF461684.1).

39. The nucleic acid molecule of claim 38, wherein the M1 genome segment comprises SEQ ID NO:5.

40. A nucleic acid molecule comprising a nucleic acid sequence of a reovirus M2 genome segment, wherein the nucleic acid sequence of the reovirus M2 genome segment comprises at least one nucleic acid modification, wherein the at least one nucleic acid modification comprises a C at position 248, numbered relative to SEQ ID NO:28 (GenBank Accession No. M20161.1).

41. The nucleic acid of claim 40, wherein the M2 genome segment comprises SEQ ID NO:6.

42. A reovirus comprising one or more of the nucleic acid molecules of claim 34-41.

43. The reovirus of claim 42, wherein the reovirus is a reassortant.

44. A reovirus, wherein the reovirus comprises genomic segments having the sequences as set forth in SEQ ID NOs:1-10.

45. A reovirus, wherein the reovirus comprises polypeptides as set forth in SEQ ID NOs:11, 12, and 16-21, and at least one of SEQ ID NO:13 or 14.

46. The reovirus of claim 44 or 45 having IDAC Accession No. 190907-01.

47. The reovirus of any of claims 1-21, 30-33, 42-46, wherein the reovirus exhibits a growth advantage over a reovirus that does not contain a modification.

48. The reovirus of claim 47, wherein the growth advantage is selected from the group consisting of an increased rate of lysis; an increased size of plaque formation; an increased rate of RNA replication; an increased rate of RNA transcription; an increased rate of translation; an increased rate of virus assembly and/or packaging; an increased number of viral progeny; an increased ability of a reovirus to be taken up by a host cell; an increased or enhanced ability to uncoat; enhanced cell lysis or inducement to cell death including apoptosis, necrosis or autophagy; an enhanced ability to infect, lyse and kill human neoplastic cells lines; decreased immunogenicity in mammalian cells; differential susceptibility to interferon sensitivity; decreased toxicity toward the host; enhanced drug interaction; enhanced radiotherapy interaction; or the ability to release effective tumor epitopes.

49. A pharmaceutical composition comprising the reovirus of any one of claims 1-21, 30-33, or 42-48 and a pharmaceutically acceptable carrier.

50. The pharmaceutical composition of claim 49, further comprising one or more chemotherapeutic agents and/or one or more immunosuppressive agents.

51. A method of making an improved reovirus, comprising the steps of:
modifying the nucleic acid sequence of said reovirus, and
selecting one or more improved reoviruses.

52. The method of claim 51, wherein the modifying comprises mutagenizing said reovirus.

53. The method of claim 52, wherein the mutagenizing comprises site-directed mutagenesis.

54. The method of claim 52, wherein the mutagenizing comprises chemical mutagenesis.

55. The method of claim 51, wherein the modifying comprises culturing said reovirus in a human cell line.

56. The method of claim 51, wherein the improved reovirus is selected for an increased rate of lysis; an increased size of plaque formation; an increased rate of RNA replication; an increased rate of RNA transcription; an increased rate of translation; an increased rate of virus assembly and/or packaging; an increased number of viral progeny; an increased ability of a reovirus to be taken up by a host cell; an increased or enhanced ability to uncoat; enhanced cell lysis or inducement to cell death including apoptosis, necrosis or autophagy; an enhanced ability to infect, lyse and kill human neoplastic cells lines; decreased immunogenicity in mammalian cells; differential susceptibility to interferon sensitivity; decreased toxicity toward the host; enhanced drug interaction; enhanced radiotherapy interaction; or the ability to release effective tumor epitopes.

57. A method of treating a proliferative disorder in a patient, comprising:

administering the reovirus of any of claims 1-21, 30-33, or 42-48 or the pharmaceutical composition of claim 49 or 50 to said patient.

58. The method of claim 57, wherein the reovirus is administered in an amount effective to cause oncolysis.

59. The method of claim 57, further comprising at least one of the procedures selected from the group consisting of surgery, chemotherapy, radiation therapy, and immunosuppressive therapy.

60. The method of claim 57, wherein the reovirus or pharmaceutical composition is administered more than once.

61. The method of claim 57, wherein the reovirus or pharmaceutical composition is administered intravenously, intravascularly, intrathecally, intramuscularly, subcutaneously, intraperitoneally, topically, orally, rectally, vaginally, nasally, by direct injection, or by inhalation.

62. A kit comprising the reovirus of any of claims 1-21, 30-33, or 42-48.

63. The kit of claim 62, further comprising one or more agents.

AMENDED CLAIMS
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1. A reovirus comprising a lambda-3 polypeptide having one or more amino acid modifications, wherein the one or more amino acid modifications are selected from the group consisting of a Val at residue 214, an Ala at residue 267, a Thr at residue 557, a Lys at residue 755, a Met at residue 756, a Pro at residue 926, a Pro at residue 963, a Leu at residue 979, an Arg at residue 1045, a Val at residue 1071, or any combination thereof, numbered relative to GenBank Accession No. M24734.1, wherein when the amino acid sequence comprises a Val at residue 214 or a Val at residue 1071, the amino acid sequence further comprises at least one additional modification in the amino acid sequence.

2. The reovirus of claim 1, wherein the lambda-3 polypeptide comprises SEQ ID NO:19.

3. A reovirus comprising a sigma-3 polypeptide having one or more amino acid modifications, wherein the one or more amino acid modifications are selected from the group consisting of a Leu at residue 14, a Lys at residue 198, or any combination thereof, numbered relative to GenBank Accession No. K02739, wherein when the amino acid sequence comprises a Leu at residue 14, the amino acid sequence further comprises at least one additional modification in the amino acid sequence.

4. The reovirus of claim 3, wherein the sigma-3 polypeptide comprises SEQ ID NO:15.

5. A reovirus comprising a mu-1 polypeptide having at least one amino acid modification, wherein the at least one amino acid modification comprises an Asp at residue 73 numbered relative to GenBank Accession No. M20161.1.

6. The reovirus of claim 5, wherein the mu-1 polypeptide comprises SEQ ID NO:17.

7. A reovirus comprising a mu-2 polypeptide having at least one amino acid modification, wherein the at least one amino acid modification comprises a Ser at residue 528 numbered relative to GenBank Accession No. AF461684.1.

8. The reovirus of claim 7, wherein the mu-2 polypeptide comprises SEQ ID NO:16.

19. A reovirus comprising a M1 genome segment having at least one nucleic acid modification, wherein the at least one nucleic acid modification comprises a T at position 1595, numbered relative to SEQ ID NO:28 (GenBank Accession No. AF461684.1).

20. The reovirus of claim 19, wherein the M1 genome segment comprises SEQ ID NO:5.

21. The reovirus of any of claims 13-20, wherein the reovirus is a reassortant.

22. A polypeptide comprising an amino acid sequence of a reovirus lambda-3 polypeptide, wherein the sequence of the reovirus lambda-3 polypeptide comprises one or more amino acid modifications, wherein the one or more amino acid modifications are selected from the group consisting of a Val at residue 214, an Ala at residue 267, a Thr at residue 557, a Lys at residue 755, a Met at residue 756, a Pro at residue 926, a Pro at residue 963, a Leu at residue 979, an Arg at residue 1045, a Val at residue 1071, or any combination thereof, numbered relative to GenBank Accession No. M24734.1, wherein when the amino acid sequence comprises a Val at residue 214 or a Val at residue 1071, the amino acid sequence further comprises at least one additional change in the amino acid sequence.

23. The polypeptide of claim 22, wherein the lambda-3 polypeptide comprises SEQ ID NO:19.

24. A polypeptide comprising an amino acid sequence of a reovirus sigma-3 polypeptide, wherein the sequence of the reovirus sigma-3 polypeptide comprises one or more amino acid modifications, wherein the one or more amino acid modifications are selected from the group consisting of a Leu at residue 14, a Lys at residue 198, or any combination thereof, numbered relative to GenBank Accession No. K02739, wherein when the amino acid sequence comprises a Leu at residue 14, the amino acid sequence further comprises at least one additional change in the amino acid sequence.

25. The polypeptide of claim 24, wherein the sigma-3 polypeptide comprises SEQ ID NO:15.

26. A polypeptide comprising an amino acid sequence of a reovirus mu-1 polypeptide, wherein the sequence of the reovirus mu-1 polypeptide comprises at least one amino acid

modification, wherein the at least one amino acid modification comprises an Asp at residue 73 numbered relative to GenBank Accession No. M20161.1.

27. The polypeptide of claim 26, wherein the mu-1 polypeptide comprises SEQ ID NO:17.

28. A polypeptide comprising an amino acid sequence of a reovirus mu-2 polypeptide, wherein the sequence of the reovirus mu-2 polypeptide comprises at least one amino acid modification, wherein the at least one amino acid modification comprises a Ser at residue 528 numbered relative to GenBank Accession No. AF461684.1.

29. The polypeptide of claim 28, wherein the mu-2 polypeptide comprises SEQ ID NO:16.

30. A reovirus comprising one or more of the polypeptides of claims 22-29.

31. The reovirus of claim 30, wherein the reovirus further comprises a sigma-2 polypeptide comprising a Cys at one or more of position 70, 127, 195, 241, 255, 294, 296, or 340, numbered relative to GenBank Accession No. NP_694684.1.

32. The reovirus of claim 31, wherein the sigma-2 polypeptide comprises SEQ ID NO:12.

33. The reovirus of any of claims 30-32, wherein the reovirus is a reassortant.

34. A nucleic acid molecule comprising a nucleic acid sequence of a reovirus L1 genome segment, wherein the nucleic acid sequence of the reovirus L1 genome segment comprises one or more nucleic acid modifications, wherein the one or more nucleic acid modifications are selected from the group consisting of a T at position 660, a G at position 817, an A at position 1687, a G at position 2283, an ATG at positions 2284-2286, a C at position 2794, a C at position 2905, a C at position 2953, an A at position 3153, a G at position 3231, numbered relative to GenBank Accession No: M24734.1.

35. The nucleic acid molecule of claim 34, wherein the L1 genome segment comprises SEQ ID NO:8.

STATEMENT UNDER ARTICLE 19 (1)

The amendments shown in the attached documents simply serve to correct typographical errors in the sequence identifiers that were assigned to some of the disclosed polypeptide sequences. Evidence that these amendments are correcting simple typographical errors can be found in the 'Brief Description of the Drawings' section, which indicates the correct sequence identifier for each of the disclosed polypeptides.

In addition, the attached documents contain an amendment to correct typographical errors regarding the mu-2 polypeptide. In claims 8 and 29, the mu-2 polypeptide was incorrectly referred to as the mu-1 polypeptide. Evidence that this was a simple typographical error can be found in claims 7 and 28, from which claims 8 and 29 depend, which all correctly refer to the mu-2 polypeptide.

Applicants believe that they have complied fully with the requirements of the International Bureau under Article 19 and Rule 46 under the Patent Cooperation Treaty. In the event that the International Bureau has any questions or requires any further action or information, a telephone call to Applicant's agent at the telephone number listed below is requested.

Figure 1-1

S1:
GCTATTGGTCGGATGGATCCTCGCCTACGTGAAGAAGTAGTACGGCTGATAATCGCATTAACGAGTGATAA
TGGAGCATCACTGTCAAAAGGGCTTGAATCAAGGGTCTCGGCGCTCGAGAAGACGTCTCAAATACACTCTG
ATACTATCCTCCGGATCACCCAGGGACTCGATGATGCAAACAAACGAATCATCGCTCTTGAGCAAAGTCGG
GATGACTTGGTTGCATCAGTCAGTGATGCTCAACTTGCAATCTCCAGATTGGAAAGCTCTATCGGAGCCCT
CCAAACAGTTGTCAATGGACTTGATTCGAGTGTTACCCAGTTGGGTGCTCGAGTGGGACAACTTGAGACAG
GACTTGCAGAGCTACGCGTTGATCACGACAATCTCGTTGCGAGAGTGGATACTGCAGAACGTAACATTGGA
TCATTGACCACTGAGCTATCAACTCTGACGTTACGAGTAACATCCATACAAGCGGATTTCGAATCTAGGAT
ATCCACGTTAGAGCGCACGGCGGTCACTAGCGCGGGAGCTCCCCTCTCAATCCGTAATAACCGTATGACCA
TGGGATTAAATGATGGACTCACGTTGTCAGGGAATAATCTCGCCATCCGATTGCCAGGAAATACGGGTCTG
AATATTCAAAATGGTGGACTTCAGTTTCGATTTAATACTGATCAATTCCAGATAGTTAATAATAACTTGAC
TCTCAAGACGACTGTGTTTGATTCTATCAACTCAAGGATAGGCGCAACTGAGCAAAGTTACGTGGCGTCGG
CAGTGACTCCCTTGAGATTAAACAGTAGCACGAAGGTGCTGGATATGCTAATAGACAGTTCAACACTTGAA
ATTAATTCTAGTGGACAGCTAACTGTTAGATCGACATCCCCGAATTTGAGGTATCCGATAGCTGATGTTAG
CGGCGGTATCGGAATGAGTCCAAATTATAGGTTTAGGCAGAGCATGTGGATAGGAATTGTCTCCTATTCTG
GTAGTGGGCTGAATTGGAGGGTACAGGTGAACTCCGACATTTTTATTGTAGATGATTACATACATATATGT
CTTCCAGCTTTTGACGGTTTCTCTATAGCTGACGGTGGAGATCTATCGTTGAACTTTGTTACCGGATTGTT
ACCACCGTTACTTACAGGAGACACTGAGCCCGCTTTTCATAATGACGTGGTCACATATGGAGCACAGACTG
TAGCTATAGGGTTGTCGTCGGGTGGTGCGCCTCAGTATATGAGTAAGAATCTGTGGGTGGAGCAGTGGCAG
GATGGAGTACTTCGGTTACGTGTTGAGGGGGGTGGCTCAATTACGCACTCAAACAGTAAGTGGCCTGCCAT
GACCGTTTCGTACCCGCGTAGTTTCACGTGAGGATCAGACCACCCCGCGGCACTGGGGCATTTCATC
(SEQ ID NO:1)

S2:
GCTATTCGCTGGTCAGTTATGGCTCGCGCTGCGTTCCTATTCAAGACTGTTGGGTTTGGTGGTCTGCAAAA
TGTGCCAATTAACGACGAACTATCTTCACATCTACTCCGAGCTGGTAATTCACCATGGCAGTTAACACAGT
TTTTAGACTGGATAAGCCTTGGGAGGGGTTTAGCTACATCGGCTCTCGTTCCGACGGCTGGGTCAAGATAC
TATCAAATGAGTTGCCTTCTAAGTGGCACTCTCCAGATTCCGTTCCGTCCTAACCACCGATGGGGAGACAT
TAGGTTCTTACGCTTAGTGTGGTCAGCTCCTACTCTCGATGGATTAGTCGTAGCTCCACCACAAGTTTTGG
CTCAGCCCGCTTTGCAAGCACAGGCAGATCGAGTGTACGACTGCGATGATTATCCATTTCTAGCGCGTGAT
CCAAGATTCAAACATCGGGTGTATCAGCAATTGAGTGCTGTAACTCTACTTAACTTGACAGGTTTTGGCCC
GATTTCCTACGTTCGAGTGGATGAAGATATGTGGAGTGGAGATGTGAACCAGCTTCTCATGAACTATTTCG
GGCACACGTTTGCAGAGATTGCATACACATTGTGTCAAGCCTCGGCTAATAGGCCTTGGGAATATGACGGT
ACATATGCTAGGATGACTCAGATTGTGTTATCCTTGTTCTGGCTATCGTATGTCGGTGTAATTCATCAGCA
GAATACGTATCGGACATTCTATTTTCAGTGTAATCGGCGAGGTGACGCCGCTGAGGTGTGGATTCTTTCTT
GTTCGTTGAACCATTCCGCACAAATTAGACCGGGTAATCGTAGCTTATTCGTTATGCCAACTAGCCCAGAT
TGGAACATGGACGTCAATTTGATCCTGAGTTCAACGTTGACGGGGTGTTTGTGTTCGGGTTCACAGCTGCC
ACTGATTGACAATAATTCAGTACCTGCAGTGTCGCGTAACATCCATGGCTGGACTGGTAGAGCTGGTAACC
AATTGCATGGGTTCCAGGTGAGACGAATGGTGACTGAATTTTGTGACAGGTTGAGACGCGATGGTGTCATG
ACCCAAGCTCAGCAGAATCAAGTTGAAGCGTTGGCAGATCAGACTCAACAGTTTAAGAGGGACAAGCTCGA
AACGTGGGCGAGAGAAGACGATCAATATAATCAGGCTCATCCCAACTCCACAATGTTCCGTACGAAACCAT
TTACGAATGCGCAATGGGGACGAGGTAATACGGGGGCGACTAGTGCCGCGATTGCAGCCCTTATCTGATCG
TCTTGGAGTGAGGGGGTCCCCCCACACACCTCACGACTGACCACACATTCATC (SEQ ID NO:2)

S3:
GCTAAAGTCACGCCTGTCGTCGTCACTATGGCTTCCTCACTCAGAGCTGCGATCTCCAAGATCAAGAGGGA
TGACGTCGGTCAGCAAGTTTGTCCTAATTATGTCATGCTGCGGTCCTCTGTCACAACAAAGGTGGTACGAA
ATGTGGTTGAGTATCAAATTCGTACGGGCGGATTCTTTTCGTGCTTAGCTATGCTAAGGCCACTCCAGTAC
GCTAAGCGTGAGCGTTTGCTTGGTCAGAGGAATCTGGAACGTATATCGACTAGGGATATCCTTCAGACTCG
TGATTTACACTCACTATGTATGCCAACTCCTGATGCGCCAATGTCTAATCATCAAGCATCCACCATGAGAG
AGCTGATTTGCAGTTACTTCAAGGTCGATCATGCGGATGGGTTGAAATATATACCCATGGATGAGAGATAC

Figure 1-2

TCTCCGTCATCACTTGCCAGATTGTTTACCATGGGCATGGCTGGGCTGCACATTACCACTGAGCCATCTTA
TAAGCGTGTTCCGATTATGCACTTAGCTGCGGACTTGGACTGTATGACGCTGGCTCTACCTTACATGATTA
CGCTTGATGGTGATACTGTGGTTCCTGTCGCTCCAACACTGTCAGCGGAACAGCTTCTGGACGACGGACTC
AAAGGATTAGCATGCATGGATATCTCCTATGGATGTGAGGTGGACGCGAATAGCCGGCCGGCTGGTGATCA
GAGTATGGACTCTTCACGCTGCATCAACGAGTTGTATTGCGAGGAGACAGCAGAAGCCATCTGTGTGCTTA
AGACATGCCTTGTGTTAAATTGCATGCAGTTTAAACTTGAGATGGATGACCTAGCACATAACGCTGCTGAG
CTGGACAAGATACAGATGATGATACCCTTCAGTGAGCGTGTTTTTAGGATGGCCTCGTCCTTTGCGACTAT
TGATGCCCAGTGTTTTAGGTTTTGCGTGATGATGAAGGATAAAAATCTGAAAATAGATATGCGTGAAACGA
CGAGACTGTGGACTCGTTCAGCATCAGATGATTCTGTGGCCACGTCATCTTTAAGTATTTCCCTGGACCGG
GGTCGATGGGTGGCGGCTGACGCCAGTGATGCTAGACTGCTGGTTTTTCCGATTCGCGTGTAATGGGTGAG
TGAGCTGATGTGGTCGCCAAGACATGTGCCGGTGTCTTGGTGGTGGGTGACGCCTAATCATC (SEQ ID
NO:3)

S4:
GCTATTTTTGCCTCTTCCCAGACGTTGTCGCAATGGAGGTGTGCTTGCCCAACGGTCATCAGGTCGTGGAC
TTAATTAACAACGCTTTTGAAGGTCGTGTATCAATCTACAGCGCGCAAGAGGGATGGGACAAAACAATCTC
AGCACAGCCAGATATGATGGTATGTGGTGGCGCCGTCGTTTGCATGCATTGTCTAGGTGTTGTTGGATCTC
TACAACGCAAGCTGAAGCATTTGCCTCACCATAGATGTAATCAACAGATCCGTCATCAGGATTACGTCGAT
GTACAGTTCGCAGACCGTGTTACTGCTCACTGGAAGCGGGGTATGCTGTCCTTCGTTGCGCAGATGCACGA
GATGATGAATGACGTGTCGCCAGATGACCTGGATCGTGTGCGTACTGAGGGAGGTTCACTAGTGGAGCTGA
ACCGGCTTCAGGTTGACCCAAATTCAATGTTTAGATCAATACACTCAAGTTGGACAGATCCTTTGCAGGTG
GTGGACGACCTTGACACTAAGCTGGATCAGTACTGGACAGCCTTAAACCTGATGATCGACTCATCCGACTT
GATACCCAACTTTATGATGAGAGACCCATCACACGCGTTCAATGGTGTGAAACTGAAGGGAGATGCTCGTC
AAACCCAATTCTCCAGGACTTTTGATTCGAGATCGAGTTTGGAATGGGGTGTGATGGTTTATGATTACTCT
GAGCTGGATCATGATCCATCGAAGGGCCGTGCTTACAGAAAGGAATTGGTGACGCCAGCTCGAGATTTCGG
TCACTTTGGATTATCCCATTATTCTAGGGCGACTACCCCAATCCTTGGAAAGATGCCGGCCGTATTCTCAG
GAATGTTGACTGGGAACTGTAAAATGTATCCATTCATTAAAGGAACGGCTAAGCTGAAGACAGTGCGCAAG
CTAGTGGAGGCAGTCAATCATGCTTGGGGTGTCGAGAAGATTAGATATGCTCTTGGGCCAGGTGGCATGAC
GGGATGGTACAATAGGACTATGCAACAGGCCCCCATTGTGCTAACTCCTGCTGCTCTCACAATGTTCCCAG
ATACCATCAAGTTTGGGGATTTGAATTATCCAGTGATGATTGGCGATCCGATGATTCTTGGCTAAACACCC
CCATCTTCACAGCGCCGGGCTTGACCAACCTGGTGTGACGTGGGACAGGCTTCATTCATC (SEQ ID
NO:4)

Figure 2-1

M1:

GCTATTCGCGGTCATGGCTTACATCGCAGTTCCTGCGGTGGTGGATTCACGTTCGAGTGAGGCTATTGGAC
TGCTAGAATCGTTTGGAGTAGACGCTGGGGCTGACGCGAATGACGTTTCATATCAAGATCATGACTATGTG
TTGGATCAGTTACAGTACATGTTAGATGGATATGAGGCTGGTGACGTTATCGATGCACTCGTCCACAAGAA
TTGGTTACATCACTCTGTCTATTGCTTGTTGCCACCCAAAAGTCAACTATTAGAGTATTGGAAAAGTAATC
CTTCAGCGATACCGGACAACGTTGATCGTCGGCTTCGTAAACGACTAATGCTAAAGAAAGATCTCAGGAAA
GATGATGAATACAATCAGCTAGCGCGTGCTTTCAAGATATCGGATGTCTACGCACCTCTCATCTCATCCAC
GACGTCACCGATGACAATGATACAGAACTTGAATCGAGGCGAGATCGTGTACACCACGACGGACAGGGTAA
TAGGGGCTAGAATCTTGTTATATGCTCCTAGAAAGTACTATGCGTCAACTCTGTCATTTACTATGACTAAG
TGCATCATTCCGTTTGGTAAAGAGGTGGGTCGTGTTCCTCACTCTCGATTTAATGTTGGCACATTTCCGTC
AATTGCTACCCCGAAATGTTTTGTCATGAGTGGGGTTGATATTGAGTCCATCCCAAATGAATTTATCAAGT
TGTTTTACCAGCGCGTCAAGAGTGTTCACGCTAACATACTAAATGACATATCTCCTCAGATCGTCTCTGAC
ATGATAAACAGAAAGCGTCTGCGCGTTCATACTCCATCAGATCGTCGAGCCGCGCAGTTGATGCATTTGCC
TTACCATGTTAAACGAGGAGCGTCTCACGTCGACGTTTACAAGGTGGATGTTGTAGACATGTTGTTCGAGG
TAGTGGATGTGGCCGATGGGTTGCGCAACGTATCTAGGAAACTAACTATGCATACCGTTCCTGTATGTATT
CTTGAAATGTTGGGTATTGAGATTGCGGACTATTGCATTCGTCAAGAGGATGGAATGCTCACAGATTGGTT
CCTACTTTTAACCATGCTATCTGATGGCTTGACTGATAGAAGGACGCATTGTCAATACTTGATTAATCCGT
CAAGTGTGCCTCCTGATGTGATACTTAACATCTCAATTACTGGATTTATAAATAGACATACAATCGATGTC
ATGCCTGACATATATGACTTCGTTAAACCCATTGGCGCTGTGCTGCCTAAGGGATCATTTAAATCAACAAT
TATGAGAGTTCTTGATTCAATATCAATATTAGGAATCCAAATCATGCCGCGCGCGCATGTAGTTGACTCAG
ATGAGGTGGGCGAGCAAATGGAGCCTACGTTTGAGCAGGCGGTTATGGAGATATACAAAGGGATTGCTGGC
GTTGACTCGCTGGATGATCTCATCAAGTGGGTGTTGAACTCGGATCTCATTCCGCATGATGACAGGCTTGG
TCAATTATTTCAAGCGTTTTTGCCTCTCGCAAAGGACTTATTAGCTCCAATGGCCAGAAAGTTTTATGATA
ACTCAATGAGTGAGGGTAGATTGCTAACATTCTCTCATGCCGACAGTGAGTTGCTGAACGCAAATTATTTT
GGTCATTTATTGCGACTAAAAATACCATATATTACAGAGGTTAATCTGATGATTCGCAAGAATCGTGAGGG
TGGAGAGCTATTTCAGCTTGTGTTATCTTATCTATATAAAATGTATGCTACTAGCGCGCAGCCTAAATGGT
TTGGATCATTATTGCGATTGTTAATATGTCCCTGGTTACATATGGAGAAATTAATAGGAGAAGCAGACCCG
GCATCTACGTCGGCTGAAATTGGGTGGCATATCCCTCGTGAACAGCTGATGCAAGATGGATGGTGTGGATG
TGAAGACGGATTCATTCCCTATGTTAGCATACGTGCGCCAAGACTGGTTATAGAGGAGTTGATGGAGAAGA
ACTGGGGCCAATATCATGCCCAAGTTATTGTCACTGATCAGCTTGTCGTAGGCGAACCGCGGAGGGTATCT
GCTAAGGCTGTGATCAAGGGTAACCACTTACCAGTTAAGTTAGTTTCACGATTTGCATGTTTCACATTGAC
GGCGAAGTATGAGATGAGGCTTTCGTGCGGCCATAGCACTGGACGTGGAGCTGCATACAGTGCGAGACTAG
CTTTCCGATCTGACTTGGCGTGATCCGTGACATGCGTAGTGTGACACCTGCTCCTAGGTCAATGGGGGTAG
GGGGCGGGCTAAGACTACGTACGCGCTTCATC (SEQ ID NO:5)

M2:

GGCTAATCTGCTGACCGTTACTCTGCAAAGATGGGGAACGCTTCCTCTATCGTTCAGACGATCAACGTCAC
TGGAGATGGCAATGTATTTAAACCATCAGCTGAAACTTCATCTACCGCTGTACCATCGTTAAGCTTATCAC
CTGGAATGCTGAATCCCGGAGGGGTACCATGGATTGCTGTTGGAGATGAGACATCTGTGACTTCACCAGGC
GCATTACGTCGAATGACGTCAAAGGACATCCCGGACACGGCAATAATCAACACAGACAATTCATCAGGCGC
CGTGCCAAGCGAATCAGCCTTGGTGCCCTACATCGATGAGCCGCTGGTAGTGGTTACAGAGCATGCTATTA
CCAACTTCACCAAAGCTGAGATGGCACTTGAATTCAATCGTGAGTTCCTTGACAAGATGCGTGTGCTGTCA
GTGTCACCAAAATATTCGGATCTTCTGACCTATGTTGACTGCTACGTCGGTGTGTCTGCTCGTCAGGCTTT
AAACAATTTTCAGAAACAAGTGCCTGTGATTACACCTACTAGGCAGACGATGTATGTCGACTCGATACAAG
CGGCCTTGAAAGCTTTAGAAAAGTGGGAGATTGATCTGAGAGTGGCTCAAACGTTGCTGCCTACGAACGTT
CCGATTGGAGAAGTCTCTTGTCCAATGCAGTCGGTAGTGAAACTGCTGGATGATCAGCTGCCAGATGACAG
CCTGATACGGAGGTATCCCAAGGAAGCCGCCGTCGCTTTGGCTAAACGAAACGGGGGAATACAATGGATGG
ACGTATCAGAAGGCACCGTGATGAACGAGGCTGTCAACGCTGTTGCAGCTAGTGCACTGGCACCTTCAGCA
TCAGCCCCACCCTTAGAAGAGAAGTCAAAGTTAACCGAACAAGCGATGGATCTCGTGACCGCGGCTGAGCC
TGAGATAATTGCCTCACTCGCGCCAGTTCCCGCACCCGTGTTTGCCATACCACCTAAACCAGCAGATTATA
ATGTGCGTACTCTGAGGATCGACGAGGCCACTTGGCTGCGAATGATTCCAAAATCAATGAACACACCTTTT
CAAATCCAGGTGACTGATAACACAGGAACTAATTGGCATCTCAATTTGAGGGGGGGACTCGTGTAGTGAA

Figure 2-2

TCTGGACCAAATCGCTCCGATGCGGTTTGTATTAGATCTAGGGGGAAAGAGTTATAAAGAGACGAGCTGGG
ATCCAAACGGCAAGAAGGTCGGATTCATCGTTTTTCAATCGAAGATACCATTCGAACTTTGGACTGCTGCT
TCACAGATCGGTCAAGCCACGGTGGTTAACTATGTCCAACTATACGCTGAAGACAGCTCATTTACCGCGCA
GTCTATCATTGCTACTACCTCTTTGGCTTATAACTATGAGCCTGAGCAGTTGAATAAGACTGACCCTGAGA
TGAATTATTATCTTTTGGCGACCTTTATAGACTCAGCCGCTATAACGCCAACGAATATGACACAGCCTGAT
GTTTGGGATGCCTTGCTGACGATGTCCCCACTATCAGCTGGCGAGGTGACAGTGAAGGGTGCGGTAGTGAG
TGAAGTAGTCCCTGCAGACTTGATAGGTAGCTACACTCCAGAATCCCTAAACGCCTCACTTCCGAATGATG
CTGCTAGATGCATGATCGATAGAGCTTCGAAGATAGCCGAAGCAATCAAGATTGATGATGATGCTGGACCA
GATGAATATTCCCCAAACTCTGTACCAATTCAAGGTCAGCTTGCTATCTCGCAACTCGAAACTGGATATGG
TGTGCGAATATTCAACCCTAAAGGGATCCTTTCTAAAATTGCATCTAGGGCAATGCAGGCTTTCATTGGTG
ACCCGAGCACAATCATCACGCAGGCGGCGCCAGTGTTATCAGACAAGAATAATTGGATTGCATTGGCACAG
GGAGTGAAAACTAGTCTGCGTACTAAAAGTCTATCAGCGGGAGTGAAGACTGCAGTGAGTAAGCTGAGCTC
ATCTGAGTCTATCCAGAATTGGACTCAAGGATTCTTGGATAAAGTGTCAGCGCATTTTCCAGCACCAAAGC
CCGATTGTCCGACTAGCGGAGATAGTGGTGAATCGTCTAATCGCCGAGTGAAGCGCGACTCATACGCAGGA
GTGGTCAAACGTGGGTACACACGTTAGGCCGCTCGCCCTGGTGACGCGGGGTTAAGGGATGCAGGCAAATC
ATC (SEQ ID NO:6)

M3:
GCTAAAGTGACCGTGGTCATGGCTTCATTCAAGGGATTCTCCGCCAACACTGTTCCAGTTTCTAAGGCCAA
GCGTGACATATCATCTCTTGCCGCTACTCCTGGACTTCGTTCACAATCCTTCACTCCGTCTGTGGATATGT
CTCAATCGCGTGAATTCCTCACAAAGGCAATTGAGCAAGGGTCCATGTCTATACCTTATCAGCATGTGAAT
GTACCGAAAGTTGATCGTAAAGTTGTTAGCCTGGTAGTGCGACCTTTCTCTTCAGGTGCTTTCTCTATCTC
TGGAGTGATTTCGCCAGCCCATGCCTATCTACTAGAGTGTCTACCCCAGCTTGAGCAGGCGATGGCTTTTG
TTGCTTCACCTGAGTCTTTCCAGGCTTCCGACGTCGCGAAGCGCTTTGCCATAAAGCCAGGTATGAGCCTC
CAGGATGCCATCACTGCCTTTATTAACTTTGTGTCCGCGATGCTGAAAATGACGGTGACTCGTCAAAACTT
TGACGTTATTGTGGCTGAGATCGAGAGGCTTGCTTCAACCAGCGTGTCCGTCAGGACTGAAGAAGCGAAGG
TTGCTGATGAGGAGCTAATGCTATTCGGGTTAGATCATAGAGGGCCACAGCAGCTGGATGTTTCTGACGCT
AAAGGGATAATGAAGGCTGCTGATATTCAGACAACTCATGATGTCCATTTGGCACCAGGCGTTGGTAATAT
TGATCCTGAAATCTATAACGAGGGGCGGTTCATGTTCATGCAGCACAAGCCACTTGCGGCGGATCAATCGT
ATTTCACCTTGGAGACTGCGGATTATTTCAAGATTTATCCAACATACGATGAACATGATGGCAGGATGGCT
GACCAAAAGCAGTCGGGATTGATACTGTGTACTAAGGACGAGGTATTGGCTGAGCAAACTATATTTAAACT
GGACGCCCCTGATGACAAGACTGTTCATCTGTTGGATCGCGATGACGACCACGTTGTTGCCAGATTTACTA
AGGTATTTATAGAGGACGTGGCTCCCGGGCATCATGCTGCTCAAAGATCGGGACAACGCTCTGTGCTTGAT
GACCTATATGCGAATACGCAAGTGATTTCCATTACTTCTGCTGCTTTAAAGTGGGTGGTCAAGCACGGCGT
ATCTGATGGAATCGTGAACAGGAAGAATGTCAAAGTGTGTGTTGGTTTTGACCCCCTGTACACCTTGTCTA
CACATAACGGGGTGTCCTTATGTGCCCTGCTGATGGACGAAAAACTCTCTGTGCTGAACAGTGCGTGTCGT
ATGACGTTACGCTCACTCATGAAGACCGGACGCGACGTTGATGCACACAGAGCTTTTCAGCGAGTCCTCTC
TCAAGGATACACATCGCTAATGTGCTACTATCATCCTTCACGGAAGTTGGCATATGGTGAGGTGCTCTTTC
TAGAACGATCCAATGACGTGACAGATGGGATCAAGCTTCAGTTGGACGCATCTAGACAGTGTCATGAATGT
CCTGTGTTGCAGCAGAAAGTGGTTGAGTTAGAGAAACAGATTATTATGCAGAAGTCAATCCAGTCAGACCC
TACCCCAGTGGCGCTGCAACCATTGTTGTCTCAGTTGCGTGAGTTGTCTAGTGAAGTTACTAGGCTACAGA
TGGAGTTGAGTCGAGCTCAGTCCCTGAATGCTCAGTTGGAGGCGGATGTCAAGTCAGCTCAATCATGTAGC
TTGGATATGTATCTGAGACACCACACTTGCATTAATGGTCATGCTAAAGAAGATGAATTGCTTGACGCTGT
GCGTGTCGCGCCGGATGTGAGGAGAGAAATCATGGAAAAGAGGAGTGAAGTGAGACAAGGTTGGTGCGAAC
GTATTTCTAAGGAAGCAGCTGCCAAATGTCAAACTGTTATTGATGACCTGACTTTGATGAATGGAAAGCAA
GCACAAGAGATAACAGAATTACGTGATTCGGCTGAAAAATATGAGAAACAGATTGCAGAGCTGGTGAGTAC
CATCACCCAAAACCAGATAACGTATCAGCAAGAGCTACAAGCCTTGGTAGCGAAAAATGTGGAATTGGACG
CGTTGAATCAGCGTCAGGCTAAGTCTTTGCGTATTACTCCCTCTCTTCTATCAGCCACTCCTATCGATTCA
GTTGATGATGTTGCTGACTTAATTGATTTCTCTGTTCCAACTGATGAGTTGTAAATAATCCGTGATGCAGT
GTTGCCCTAATCCCTTAAGCCTTCCCGACCCCCATTCATC (SEQ ID NO:7)

Figure 3-1

L1:
GCTACACGTTCCACGACAATGTCATCCATGATACTGACTCAGTTTGGACCGTTCATTGAGAGCATTTCAGG
TATCACTGATCAATCGAATGACGTGTTTGAAGATGCAGCAAAAGCATTCTCTATGTTTACTCGCAGCGATG
TCTACAAGGCGCTGGATGAAATACCTTTCTCTGATGATGCGATGCTTCCAATCCCTCCAACTATATATACG
AAACCATCTCACGATTCATATTATTACATTGATGCTCTAAACCGTGTGCGTCGCAAAACATATCAGGGCCC
TGATGACGTGTACGTACCTAATTGTTCTATTGTTGAATTGCTGGAGCCACATGAGACTCTGACATCTTATG
GGCGGTTGTCCGAGGCCATCGAGAATCGTGCCAAGGATGGGGACAGCCAAGCCAGAATCGCCACAACGTAT
GGTAGAATCGCTGAATCTCAAGCTCGACAGATTAAGGCTCCATTGGAGAAGTTTGTGTTGGCACTATTAGT
GGCCGAAGCAGGGGGGTCTTTATATGATCCAGTTTTGCAGAAGTATGATGAGATTCCAGATCTATCGCATA
ATTGCCCTTTATGGTGTTTTAGAGAGATCTGTCGTCACATATCTGGTCCATTACCAGATCGGGCACCTTAT
CTTTACTTATCTGCAGGGGTTTTCTGGTTAATGTCACCACGAATGACGTCTGCAATCCCTCCGCTACTATC
CGATCTTGTTAATTTAGCTATTTTGCAACAAACTGCGGGTTTAGATCCATCATTAGTGAAATTGGGAGTAC
AGATATGCCTTCATGCAGCAGCTAGCTCAAGTTATGCATGGTTTATCTTAAAGACTAAGTCTATTTTTCCT
CAAAACACGTTGCACAGTATGTATGAATCTCTAGAAGGGGGATACTGTCCTAATCTTGAATGGTTAGAGCC
TAGATCAGACTATAAGTTCATGTACATGGGAGTCATGCCATTGTCCGCTAAGTATGCTAGGTCGGCGCCGT
CCAATGATAAGAAAGCGCGGGAACTTGGCGAGAAATATGGACTGAGCTCAGTCGTCGGTGAGCTTCGTAAA
CGGACAAAGACGTATGTTAAACATGACTTTGCTTCAGTGAGGTACATTCGTGACGCTATGGCATGTACTAG
CGGTATTTTCTTGGTAAGAACACCCACCGAAACGGTATTGCAAGAATATACGCAGAGTCCGGAGATTAAGG
TTCCCATTCCCCAGAAAGACTGGACAGGCCCAATAGGTGAAATCAGAATTCTAAAAGATACAACAAGTTCC
ATCGCGCGTTACTTATATAGAACATGGTACTTGGCAGCGGCGAGAATGGCGGCTCAACCACGTACGTGGGA
TCCATTGTTTCAAGCGATTATGAGATCTCAATACGTGACAGCTAGGGGTGGATCTGGCGCAGCACTCCGCG
AATCTTTGTATGCAATCAATGTGTCGTTACCTGATTTCAAGGGCTTACCAGTGAAGGCAGCAACTAAGATA
TTCCAGGCGGCACAATTAGCGAACTTGCCGTTCTCCCACACATCAGTGGCTATACTAGCTGACACTTCAAT
GGGATTGCGAAATCAGGTGCAGAGGCGGCCACGATCCATTATGCCATTAAATGTGCCCCAGCAGCAGGTTT
CGGCGCCCCATACATTGACAGCGGATTACATTAACTACCACATGAATCTATCAACCACGTCTGGTAGTGCG
GTCATTGAGAAGGTGATTCCTTTAGGTGTATACGCTTCGAGCCCTCCTAACCAGTCGATCAACATTGACAT
ATCTGCGTGTGACGCTAGTATTACTTGGGATTTCTTTCTGTCAGTGATTATGGCGGCTATACACGAAGGTG
TCGCTAGTAGCTCCATTGGAAAACCATTTATGGGGGTTCCTGCATCCATTGTAAATGATGAGTCTGTCGTT
GGAGTGAGAGCTGCTAGGCCGATATCGGGAATGCAGAACATGATTCAGCATCTATCGAAACTATATAAACG
TGGATTTTCATATAGAGTAAACGATTCTTTTTCTCCAGGTAACGATTTTACTCATATGACTACCACTTTCC
CGTCAGGTTCAACAGCCACCTCTACTGAGCATACTGCTAATAATAGTACGATGATGGAAACTTTCCTGACA
GTATGGGGACCCGAACATACTGACGACCCTGACGTCTTACGTTTAATGAAGTCTTTAACTATTCAAAGGAA
TTACGTATGTCAAGGTGATGATGGATTAATGATTATCGATGGGACTACTGCTGGTAAGGTGAACAGTGAAA
CTATTCAGAAGATGCTAGAATTAATCTCAAAATATGGTGAGGAATTCGGATGGAAATATGACATAGCGTAC
GATGGGACTGCCGAATACTTAAAGCTATACTTCATATTTGGCTGTCGAATTCCAAATCTTAGTCGCCATCC
AATCGTGGGGAAAGAACGGGCGAATTCTTCAGCAGAGGAGCCATGGCCAGCAATTCTAGATCAGATTATGG
GTGTCTTCTTTAATGGTGTTCATGATGGGTTACAGTGGCAGCGGTGGATACGTTATTCATGGGCTCTATGC
TGTGCTTTCTCACGTCAAAGAACAATGATTGGTGAGAGCGTGGGTTACCTTCAATATCCTATGTGGTCTTT
TGTCTACTGGGGATTACCACTGGTTAAAGCGTTTGGGTCAGACCCATGGATATTTTCTTGGTACATGCCTA
CTGGAGATCTGGGAATGTATAGTTGGATTAGCTTGATACGCCCTCTGATGACAAGATGGATGGTGGCTAAT
GGTTACGTAACTGACAGATGCTCACCCGTATTCGGGAACGCAGATTATCGCAGGTGTTTCAATGAACTTAA
ACTATATCAAGGTTATTATATGGCACAATTGCCCAGGAATCCTAAGAAGTCTGGACGAGCGGCCCCTCGGG
AGGTAAGAGAACAATTCACTCAGGCATTATCCGACTATCTACTGCAAAATCCAGAGCTGAAGTCACGTGTG
CTACGTGGTCGTAGTGAGTGGGAGAAATATGGAGCGGGGATAATTCACAATCCTCCGTCATTATTCGATGT
GCCCCATAAATGGTATCAGGGTGCGCAAGAGGCAGCAATCGCTACGAGAGAAGAGCTGGCAGAAATGGATG
AGACATTAATGCGCGCTCGAAGGCACAGATATTCGAGCTTTTCAAAGTTATTAGAGGCGTATCTGCTCGTG
AAATGGCGAATGTGCGAGGCCCGCGAACCGTCGGTGGATTTGCGATTACCATTATGTGCGGGTATTGACCC
ATTAAACTCAGATCCTTTTCTCAAGATGGTAAGCGTTGGACCAATGCTCCAGAGTACGAGAAAGTACTTTG
CTCAGACACTATTCATGGCAAAGACGGTGTCGGGTCTTGACGTTAACGCGATTGATAGCGCGTTATTACGA
CTGCGAACATTAGGTGCTGATAAGAAAGCATTAACGGCGCAGTTATTAATGGTGGGCTTCAGGAGTCAGA
AGCGGACGCATTGGCCGGGAAGATAATGCTACAGGATGTGAATACTGTGCAATTAGCCAGAGTGGTTAACT
TAGCTGTGCCAGATACTTGGATGTCGTTAGACTTTGACTCTATGTTCAAACACCACGTCAAGCTGCTTCCC
AAAGATGGACGTCATCTAAATACTGATATTCCTCCTCGAATGGGATGGTTACGGGCCATTTTACGATTCTT

Figure 3-2

AGGTGCCGGAATGGTAATGACTGCGACTGGAGTTGCTGTCGACATCTATCTGGAGGATATACATGGCGGTG
GTCGGTCACTTGGACAGAGATTCATGACTTGGATGCGACAGGAAGGACGGTCAGCGTGAGTCTACCATGGG
TCGTGGTGCGTCAACTCATC (SEQ ID NO:8)

L2:
GCTAAATGGCGCGATGGCGAACGTTTGGGGGGTGAGACTTGCAGACTCGTTATCTTCACCCACTATTGAGA
CACGAACGCGTCAGTATACCTTACACGATCTTTGCTCAGACCTAGATGCTAATCCGGGGAGGGAACCGTGG
AAACCTCTGCGTAATCAGCGTACTAATAATATTGTGGCTGTGCAATTATTCAGACCATTGCAGGGTTTAGT
TTTAGATACCCAGCTTTATGGATTTCCAGGAGCATTTGATGACTGGGAGCGATTCATGAGAGAGAAGCTGC
GTGTGCTAAAGTATGAAGTATTGCGCATCTATCCAATCAGCAACTATAGCAATGAACATGTCAACGTCTTC
GTGGCCAATGCTTTGGTGGGCGCTTTCCTGTCGAATCAAGCTTTCTATGACCTGCTACCGTTGTTGATAAT
TAATGACACTATGATTGGTGATCTACTTGGCACGGGGGCATCGCTATCACAGTTCTTTCAATCTCATGGAG
ATGTGCTGGAAGTCGCAGCTGGTCGTAAGTATCTGCAGATGGAAAACTACTCCAACGATGACGATGATCCT
CCATTATTTGCGAAAGACCTGTCAGATTATGCTAAAGCATTCTACAGTGACACATATGAAGTGTTGGACAG
GTTCTTTTGGACGCATGACTCTTCAGCGGGGGTCTTAGTGCATTATGATAAGCCAACGAATGGTCATCACT
ATCTGCTGGGTACTTTGACTCAGATGGTCAGTGCACCTCCTTATATTATTAACGCTACTGACGCAATGTTG
CTTGAATCCTGTCTAGAACAGTTCTCAGCTAATGTGCGTGCGAGACCTGCGCAACCCGTTACACGCTTAGA
CCAATGCTATCATTTAAGATGGGGAGCACAATATGTAGGAGAAGATTCACTGACATATCGGTTGGGGGTGT
TATCCTTGCTGGCTACCAATGGATATCAATTAGCTAGACCGATTCCAAGACAGTTGACGAATCGATGGTTG
TCGAGCTTTGTGAGTCAAATTATGTCTGACGGCGTCAACGAGACTCCACTGTGGCCCCAAGAAAGGTATGT
GCAGATCGCTTATGATTCACCATCCGTTGTTGATGGGGCTACGCAATATGGCTATGTCAGGAAGAATCAAC
TCAGACTCGGCATGAGAATATCGGCGCTGCAATCGCTGAGTGATACGCCCTCGCCGGTACAGTGGCTTCCA
CAATACACCATCGACCAGGCAGCGATGGACGAAGGCGATCTGATGGTTAGTCGGCTTACGCAACTCCCGTT
ACGTCCTGATTATGGTAATATCTGGGTCGGCGATGCGCTATCCTATTATGTGGACTACAATCGGAGTCATC
GAGTCGTGCTTTCATCGGAACTTCCTCAGCTTCCGGACACATATTTTGATGGCGATGAACAGTATGGGCGC
AGCCTGTTCTCACTAGCTCGTAAGATTGGTGACCGCTCGTTAGTGAAAGATACGGCTGTCTTGAAGCACGC
TTACCAAGCCATCGATCCAAATACTGGTAAGGAGTATCTGAGATCTCGGCAATCTGTCGCATATTTTGGTG
CATCAGCGGGTCATTCTGGTGCCGACCAGCCGTTAGTCATAGAGCCCTGGATTCAAGGGAAAATCAGTGGT
GTGCCGCCACCCTCCTCAGTGCGACAGTTCGGCTATGATGTTGCCCGTGGCGCGATCGTCGATCTGGCGAG
ACCATTTCCTTCTGGAGATTATCAATTTGTCTATTCGGATGTTGACCAGGTGGTCGATGGCCATGACGATC
TGAGTATATCATCTGGACTGGTGGAGAGCCTTTTGTCTTCATGCATGCACGCCACAGCACCCGGGGGCTCA
TTTGTTGTTAAGATAAATTTTCCGACTAGACCCGTATGGCACTACATCGAACAGAAGATCTTGCCCAATAT
TACGTCATACATGTTGATCAAGCCTTTCGTCACCAACAACGTCGAATTGTTCTTCGTCGCTTTCGGTGTGC
ATCAACACTCATCACTTACTTGGACATCTGGAGTGTACTTCTTCTTGGTGGACCATTTTTATCGTTATGAG
ACTTTATCTACGATCTCACGACAATTGCCGTCTTTTGGGTATGTTGATGATGGGTCTTCCGTGACTGGTAT
CGAGACAATTAGTATTGAGAACCCTGGCTTCTCGAATATGACCCAGGCCGCTCGCATTGGTATCTCAGGAT
TGTGTGCTAATGTAGGTAACGCGCGTAAGTCCATTGCCATTTACGAATCTCATGGGGCCAGAGTATTAACT
ATCACATCAAGGAGATCTCCGGCATCAGCTAGAAGAAAGTCTAGGTTGCGATATTTGCCATTAATAGACCC
TAGGTCGTTAGAGGTACAGGCGCGCACTATTCTGCCAGCTGATCCAGTGTTATTTGAAAACGTGAGCGGAG
CGTCACCCCATGTTTGTCTGACAATGATGTACAACTTCGAAGTGTCGTCAGCGGTATATGATGGAGACGTT
GTGCTAGATCTTGGGACGGACCAGAGGCTAAAATCCTTGAACTGATACCCGCAACCTCTCCAGTCACATG
CGTGGACATACGGCCTACAGCGCAGCCTAGTGGATGTTGGAACGTTCGTACCACGTTCCTTGAGTTAGATT
ATTTGAGCGATGGATGGATCACTGGGGTGCGTGGGACATAGTTACTTGTATGTTATCTTTGGGGGCCGCT
GCCGCTGGAAAATCAATGACTTTTGACGCTGCGTTTCAGCAATTAATCAAAGTATTATCCAAGAGTACGGC
TAATGTTGTGCTGGTGCAGGTTAACTGCCCTACAGACGTGGTGAGGAGCATTAAGGGCTACCTAGAGATAG
ATTCGACTAACAAGAGGTATAGGTTCCCCAAATTTGGTCGAGACGAGCCGTACTCTGACATGGATGCGCTG
GAGAAAATATGTCGTACCGCCTGGCCAAACTGCTCAATTACCTGGGTTCCATTGTCATACGACTTGCGGTG
GACTAGACTGGCATTATTAGAGTCCACGACATTGAGTAGCGCGTCGATTAGAATTGCTGAGCTGATGTATA
AATACATGCCTATTATGAGGATTGATATTCATGGACTACCCATGGAAAAGCGAGGTAACTTCATAGTGGGG
CAGAACTGCTCATTAGTAATCCCTGGTTTTAATGCGCAGGATGTCTTTAACTGTTATTTCAATTCCGCCCT
CGCTTTCTCGACTGAAGATGTCAATGCTGCGATGATTCCCCAAGTGTCTGCGCAGTTTGATGCGACTAAGG
GTGAGTGGACGTTGGATATGGTCTTCTCCGACGCAGGAATCTATACCATGCAGGCTCTAGTGGGATCTAAT

Figure 3-3

GCTAATCCAGTCTCTTTGGGTTCCTTTGTAGTTGATTCTCCAGATGTAGATATAACTGACGCTTGGCCAGC
TCAGTTAGACTTTACGATCGCGGGAACTGATGTCGATATAACAGTTAATCCTTATTACCGTCTGATGACCT
TTGTAAGGATCGATGGACAGTGGCAGATTGCCAATCCAGACAAATTTCAATTCTTTTCGTCGGCGTCTGGG
ACGTTAGTGATGAACGTCAAATTAGATATCGCAGATAAATATCTACTATACTATATACGAGATGTCCAGTC
TCGAGATGTTGGCTTTTACATTCAGCATCCACTTCAACTTTTGAATACGATCACATTGCCAACCAACGAGG
ACCTTTTTCTGAGCGCACCTGACATGCGAGAGTGGGCAGTTAAGGAAAGCGGTAACACGATATGTATACTC
AATAGTCAAGGGTTTGTGCTACCTCAAGATTGGGATGTGTTAACAGATACCATAAGTTGGTCCCCATCGAT
ACCCACATACATTGTGCCACCGGGTGATTATACCTTGACTCCTCTGTAACTCACTGTCCCTCGTGAGCGCG
CCTAATTCATC (SEQ ID NO:9)

L3:
GCTAATCGTCAGGATGAAGCGGATTCCAAGGAAGACAAAGGGCAAATCCAGCGGAAAGGGCAATGACTCAA
CAGAGAGAGCGGACGATGGCTCGAGCCAATTAAGAGACAAGCAAAACAATAAGGCTGGCCCCGCCACTACG
GAGCCTGGCACATCCAACCGAGAGCAATACAAAGCTCGACCAGGTATTGCATCTGTGCAGAGGGCCACTGA
AAGTGCAGAAATGCCCATGAAGAATAATGACGAAGGGACGCCAGATAAGAAAGGAAATACTAAGGGCGACC
TAGTTAATGAGCATAGTGAGGCTAAAGACGAGGCGGATGAAGCGACGAAGAAGCAGGCAAAGGATACAGAC
AAAAGTAAAGCGCAAGTCACATATTCAGACACTGGTATCAATAATGCTAATGAACTGTCAAGATCTGGGAA
TGTGGATAATGAGGGTGGAAGTAATCAGAAGCCGATGTCTACCAGAATAGCTGAGGCAACGTCTGCTATAG
TGTCGAAACATCCTGCGCGTGTTGGGCTGCCACCTACCGCTAGCAGTGGTCATGGGTATCAGTGCCATGTC
TGTTCTGCAGTCCTGTTTAGTCCTTTAGACCTAGATGCCCACGTCGCCTCACATGGTTTGCATGGTAACAT
GACATTAACATCGAGTGATATCCAGCGACATATAACTGAGTTCATCAGCTCATGGCAAAATCATCCTATTG
TTCAAGTTTCGGCTGATGTCGAAAATAAGAAAACTGCTCAATTGCTTCACGCTGACACTCCTCGACTCGTC
ACTTGGGATGCTGGTTTGTGTACTTCATTCAAAATCGTCCCGATTGTGCCAGCTCAGGTGCCGCAGGATGT
ACTGGCCTATACGTTTTTCACCTCTTCATACGCTATCCAATCACCGTTTCCAGAGGCGGCAGTGTCTAGGA
TTGTGGTGCATACGAGATGGGCATCTAATGTTGACTTTGACCGAGACTCGTCTGTCATCATGGCGCCACCT
ACAGAAAACAATATCCATTTGTTTAAACAGTTACTAAATACTGAAACCCTGTCTGTAAGGGGGGCTAATCC
GCTAATGTTCAGGGCGAATGTGTTGCATATGTTGCTAGAGTTCGTATTAGATAACTTGTATCTGAACAGAC
ATACGGGATTCTCTCAAGACCACACGCCATTTACTGAGGGTGCTAATTTGCGTTCACTTCCTGGCCCCGAT
GCTGAGAAATGGTACTCGATTATGTATCCAACGCGCATGGGAACGCCGAATGTATCCAAAATATGTAATTT
CGTCGCCTCTTGTGTGCGAAATCGGGTTGGACGGTTTGATCGAGCACAGATGATGAACGGAGCTATGTCAG
AGTGGGTGGATGTCTTCGAGACTTCAGACGCGCTAACCGTCTCCATTCGAGGTCGATGGATGGCTAGACTA
GCTCGCATGAACATAAATCCAACAGAGATCGAATGGGCATTGACTGAATGTGCACAAGGATATGTGACTGT
CACAAGTCCTTACGCTCCTAGCGTAAATAGATTGATGCCCTATCGTATCTCCAACGCTGAGCGGCAAATAT
CACAGATAATCAGGATCATGAACATTGGCAATAACGCGACGGTGATACAACCTGTTCTGCAAGATATTTCG
GTACTCCTTCAACGCATATCACCACTCCAAATAGATCCAACTATTATTTCCAACACTATGTCAACAGTCTC
GGAGTCTACTACTCAGACCCTCAGCCCCGCGTCCTCAATTTTGGGTAAACTACGACCAAGCAACTCAGATT
TTTCTAGTTTTAGAGTCGCGTTGGCTGGATGGCTTTATAATGGGGTTGTGACGACGGTGATTGATGATAGT
TCATATCCAAAAGACGGCGGCAGCGTGACCTCACTTGAAAATCTGTGGGATTTCTTCATCCTTGCGCTTGC
TCTACCACTGACAACTGACCCCTGTGCACCTGTGAAAGCATTCATGACCCTAGCCAACATGATGGTTGGTT
TCGAGACAATCCCTATGGATAATCAGATCTATACTCAATCGAGACGCGCGAGTGCTTTCTCAACGCCTCAC
ACGTGGCCACGATGCTTTATGAACATCCAGTTAATTTCTCCAATCGACGCTCCCATCTTGCGACAGTGGGC
TGAAATTATTCATAGATACTGGCCTAACCCTTCACAGATTCGTTATGGTGCACCGAACGTTTTCGGCTCGG
CAAATTTGTTCACTCCACCTGAGGTGCTGTTATTGCCAATCGATCATCAACCAGCTAATGTAACAACGCCA
ACGCTGGACTTCACCAATGAGTTAACTAATTGGCGCGCTCGTGTCTGTGAGCTTATGAAGAATCTCGTTGA
TAACCAAAGATATCAACCTGGATGGACACAAAGTCTAGTCTCGTCAATGCGCGGAACGCTAGACAAATTGA
AGTTGATTAAATCGATGACACCAATGTATCTGCAACAGCTGGCTCCGGTAGAGTTAGCAGTGATAGCTCCC
ATGTTGCCTTTTCCACCTTTCCAGGTGCCATACGTCCGTCTCGATCGTGACAGAGTTCCAACAATGGTTGG
AGTAACACGACATTCACGAGATACTATTACTCAGCCGGCGCTATCGCTGTCGACAACCAATACTACTGTTG
GCGTGCCACTAGCTCTAGACGCGAGGGCTATCACCGTTGCGCTGTTGTCAGGGAAATATCCGCCGGATTTG
GTGACAAATGTATGGTACGCTGATGCCATTTACCCAATGTATGCAGACACGGAGGTGTTCTCTAATCTTCA
GAGAGACATGATTACCTGCGAGGCCGTGCAGACATTAGTGACTCTGGTGGCGCAAATATCAGAGACCCAGT
ATCCTGTAGATAGGTATCTTGATTGGATCCCATCACTGAGAGCATCGGCGGCGACGGCGGCGACATTTGCT

Figure 3-4

GAGTGGGTTAATACTTCAATGAAGACGGCGTTTGATTTGTCTGATATGCTGTTAGAGCCTCTCCTAAGCGG
TGATCCGAGGATGACTCAACTAGCGATTCAGTATCAGCAGTACAATGGCAGAACGTTTAATATCATACCTG
AAATGCCAGGTTCAGTAATTGCTGACTGCGTTCAATTAACAGCAGAAGTCTTTAATCACGAATATAACCTG
TTTGGGATTGCGCGGGGTGATATCATCATTGGCCGTGTTCAGTCGACACATTTGTGGTCACCGCTGGCTCC
TCCACCTGACCTGGTGTTTGATCGTGATACCCCTGGTGTTCACATCTTCGGACGAGATTGCCGTATATCGT
TTGGAATGAATGGCGCCGCGCCAATGATTAGAGATGAGACTGGACTGATGGTGCCTTTTGAAGGAAATTGG
ATTTTCCCACTGGCGCTTTGGCAAATGAATACACGATATTTTAATCAACAGTTCGACGCGTGGATTAAGAC
AGGAGAGTTGCGAATCCGCATTGAGATGGGCGCGTATCCATATATGTTGCATTACTATGATCCACGTCAGT
ACGCTAATGCATGGAATTTAACATCCGCCTGGCTTGAAGAAATTACGCCGACGAGCATCCCATCCGTGCCT
TTCATGGTGCCCATTTCAAGTGATCATGACATTTCCTCTGCCCCAGCTGTCCAATATATCATTTCAACTGA
ATATAATGATCGGTCTCTGTTCTGCACTAACTCATCATCTCCCCAAACCATCGCTGGACCAGACAAACACA
TTCCAGTTGAGAGATATAACATTCTGACCAACCCCGACGCTCCACCCACGCAGATACAACTGCCTGAAGTC
GTTGACTTGTACAACGTCGTCACACGCTATGCGTATGAGACTCCGCCTATTACCGCTGTTGTTATGGGTGT
TCCTTGATCCTCATCCTCCCAACAGGTGCTAGAGCATTGCGCTCAATGCTAGTTGGGCCGATTCATC
(SEQ ID NO:10)

Figure 4

σ1:
MDPRLREEVVRLIIALTSDNGASLSKGLESRVSALEKTSQIHSDTILRITQGLDDANKRIIALEQSRDDLVASVSDA
QLAISRLESSIGALQTVVNGLDSSVTQLGARVGQLETGLAELRVDHDNLVARVDTAERNIGSLTTELSTLTLRVTSI
QADFESRISTLERTAVTSAGAPLSIRNNRMTMGLNDGLTLSGNNLAIRLPGNTGLNIQNGGLQFRFNTDQFQIVNNN
LTLKTTVFDSINSRIGATEQSYVASAVTPLRLNSSTKVLDMLIDSSTLEINSSGQLTVRSTSPNLRYPIADVSGGIG
MSPNYRFRQSMWIGIVSYSGSGLNWRVQVNSDIFIVDDYIHICLPAFDGFSIADGGDLSLNFVTGLLPPLLTGDTEP
AFHNDVVTYGAQTVAIGLSSGGAPQYMSKNLWVEQWQDGVLRLRVEGGGSITHSNSKWPAMTVSYPRSFT (SEQ
ID NO:11)

σ2:
MARAAFLFKTVGFGGLQNVPINDELSSHLLRAGNSPWQLTQFLDWISLGRGLATSALVPTAGSRYYQMSCLLSGTLQ
IPFRPNHRWGDIRFLRLVWSAPTLDGLVVAPPQVLAQPALQAQADRVYDCDDYPFLARDPRFKHRVYQQLSAVTLLN
LTGFGPISYVRVDEDMWSGDVNQLLMNYFGHTFAEIAYTLCQASANRPWEYDGTYARMTQIVLSLFWLSYVGVIHQQ
NTYRTFYFQCNRRGDAAEVWILSCSLNHSAQIRPGNRSLFVMPTSPDWNMDVNLILSSTLTGCLCSGSQLPLIDNNS
VPAVSRNIHGWTGRAGNQLHGFQVRRMVTEFCDRLRRDGVMTQAQQNQVEALADQTQQFKRDKLETWAREDDQYNQA
HPNSTMFRTKPFTNAQWGRGNTGATSAAIAALI (SEQ ID NO:12)

σNS:
MASSLRAAISKIKRDDVGQQVCPNYVMLRSSVTTKVVRNVVEYQIRTGGFFSCLAMLRPLQYAKRERLLGQRNLERI
STRDILQTRDLHSLCMPTPDAPMSNHQASTMRELICSYFKVDHADGLKYIPMDERYSPSSLARLFTMGMAGLHITTE
PSYKRVPIMHLAADLDCMTLALPYMITLDGDTVVPVAPTLSAEQLLDDGLKGLACMDMDVRWTRIAGRLVIRVWTLH
AASTSCIARRQQKPSVCLRHALC (SEQ ID NO:13)

MASSLRAAISKIKRDDVGQQVCPNYVMLRSSVTTKVVRNVVEYQIRTGGFFSCLAMLRPLQYAKRERLLGQRNLERI
STRDILQTRDLHSLCMPTPDAPMSNHQASTMRELICSYFKVDHADGLKYIPMDERYSPSSLARLFTMGMAGLHITTE
PSYKRVPIMHLAADLDCMTLALPYMITLDGDTVVPVAPTLSAEQLLDDGLKGLACMDISYGCEVDANSRPAGDQSMD
SSRCINELYCEETAEAICVLKTCLVLNCMQFKLEMDDLAHNAAELDKIQMMIPFSERVFRMASSFATIDAQCFRFCV
MMKDKNLKIDMRETTRLWTRSASDDSVATSSLSISLDRGRWVAADASDARLLVFPIRV (SEQ ID NO:14)

σ3:
MEVCLPNGHQVVDLINNAFEGRVSIYSAQEGWDKTISAQPDMMVCGGAVVCMHCLGVVGSLQRKLKHLPHHRCNQQI
RHQDYVDVQFADRVTAHWKRGMLSFVAQMHEMMNDVSPDDLDRVRTEGGSLVELNRLQVDPNSMFRSIHSSWTDPLQ
VVDDLDTKLDQYWTALNLMIDSSDLIPNFMMRDPSHAFNGVKLKGDARQTQFSRTFDSRSSLEWGVMVYDYSELDHD
PSKGRAYRKELVTPARDFGHFGLSHYSRATTPILGKMPAVFSGMLTGNCKMYPFIKGTAKLKTVRKLVEAVNHAWGV
EKIRYALGPGGMTGWYNRTMQQAPIVLTPAALTMFPDTIKFGDLNYPVMIGDPMILG (SEQ ID NO:15)

Figure 5

$\mu2$:

MAYIAVPAVVDSRSSEAIGLLESFGVDAGADANDVSYQDHDYVLDQLQYMLDGYEAGDVIDALVHKNWLHHSVYCLL
PPKSQLLEYWKSNPSAIPDNVDRRLRKRLMLKKDLRKDDEYNQLARAFKISDVYAPLISSTTSPMTMIQNLNRGEIV
YTTTDRVIGARILLYAPRKYYASTLSFTMTKCIIPFGKEVGRVPHSRFNVGTFPSIATPKCFVMSGVDIESIPNEFI
KLFYQRVKSVHANILNDISPQIVSDMINRKRLRVHTPSDRRAAQLMHLPYHVKRGASHVDVYKVDVVDMLFEVVDVA
DGLRNVSRKLTMHTVPVCILEMLGIEIADYCIRQEDGMLTDWFLLLTMLSDGLTDRRTHCQYLINPSSVPPDVILNI
SITGFINRHTIDVMPDIYDFVKPIGAVLPKGSFKSTIMRVLDSISILGIQIMPRAHVVDSDEVGEQMEPTFEQAVME
IYKGIAGVDSLDDLIKWVLNSDLIPHDDRLGQLFQAFLPLAKDLLAPMARKFYDNSMSEGRLLTFSHADSELLNANY
FGHLLRLKIPYITEVNLMIRKNREGGELFQLVLSYLYKMYATSAQPKWFGSLLRLLICPWLHMEKLIGEADPASTSA
EIGWHIPREQLMQDGWCGCEDGFIPYVSIRAPRLVIEELMEKNWGQYHAQVIVTDQLVVGEPRRVSAKAVIKGNHLP
VKLVSRFACFTLTAKYEMRLSCGHSTGRGAAYSARLAFRSDLA (SEQ ID NO:16)

$\mu1$:

MGNASSIVQTINVTGDGNVFKPSAETSSTAVPSLSLSPGMLNPGGVPWIAVGDETSVTSPGALRRMTSKDIPDTAII
NTDNSSGAVPSESALVPYIDEPLVVVTEHAITNFTKAEMALEFNREFLDKMRVLSVSPKYSDLLTYVDCYVGVSARQ
ALNNFQKQVPVITPTRQTMYVDSIQAALKALEKWEIDLRVAQTLLPTNVPIGEVSCPMQSVVKLLDDQLPDDSLIRR
YPKEAAVALAKRNGGIQWMDVSEGTVMNEAVNAVAASALAPSASAPPLEEKSKLTEQAMDLVTAAEPEIIASLAPVP
APVFAIPPKPADYNVRTLRIDEATWLRMIPKSMNTPFQIQVTDNTGTNWHLNLRGGTRVVNLDQIAPMRFVLDLGGK
SYKETSWDPNGKKVGFIVFQSKIPFELWTAASQIGQATVVNYVQLYAEDSSFTAQSIIATTSLAYNYEPEQLNKTDP
EMNYYLLATFIDSAAITPTNMTQPDVWDALLTMSPLSAGEVTVKGAVVSEVVPADLIGSYTPESLNASLPNDAARCM
IDRASKIAEAIKIDDDAGPDEYSPNSVPIQGQLAISQLETGYGVRIFNPKGILSKIASRAMQAFIGDPSTIITQAAP
VLSDKNNWIALAQGVKTSLRTKSLSAGVKTAVSKLSSSESIQNWTQGFLDKVSAHFPAPKPDCPTSGDSGESSNRRV
KRDSYAGVVKRGYTR (SEQ ID NO:17)

μNS:

MASFKGFSANTVPVSKAKRDISSLAATPGLRSQSFTPSVDMSQSREFLTKAIEQGSMSIPYQHVNVPKVDRKVVSLV
VRPFSSGAFSISGVISPAHAYLLECLPQLEQAMAFVASPESFQASDVAKRFAIKPGMSLQDAITAFINFVSAMLKMT
VTRQNFDVIVAEIERLASTSVSVRTEEAKVADEELMLFGLDHRGPQQLDVSDAKGIMKAADIQTTHDVHLAPGVGNI
DPEIYNEGRFMFMQHKPLAADQSYFTLETADYFKIYPTYDEHDGRMADQKQSGLILCTKDEVLAEQTIFKLDAPDDK
TVHLLDRDDDHVVARFTKVFIEDVAPGHHAAQRSGQRSVLDDLYANTQVISITSAALKWVVKHGVSDGIVNRKNVKV
CVGFDPLYTLSTHNGVSLCALLMDEKLSVLNSACRMTLRSLMKTGRDVDAHRAFQRVLSQGYTSLMCYYHPSRKLAY
GEVLFLERSNDVTDGIKLQLDASRQCHECPVLQQKVVELEKQIIMQKSIQSDPTPVALQPLLSQLRELSSEVTRLQM
ELSRAQSLNAQLEADVKSAQSCSLDMYLRHHTCINGHAKEDELLDAVRVAPDVRREIMEKRSEVRQGWCERISKEAA
AKCQTVIDDLTLMNGKQAQEITELRDSAEKYEKQIAELVSTITQNQITYQQELQALVAKNVELDALNQRQAKSLRIT
PSLLSATPIDSVDDVADLIDFSVPTDEL (SEQ ID NO:18)

Figure 6-1

λ3:

MSSMILTQFGPFIESISGITDQSNDVFEDAAKAFSMFTRSDVYKALDEIPFSDDAMLPIPPTIYTKPSHDSYYYIDA
LNRVRRKTYQGPDDVYVPNCSIVELLEPHETLTSYGRLSEAIENRAKDGDSQARIATTYGRIAESQARQIKAPLEKF
VLALLVAEAGGSLYDPVLQKYDEIPDLSHNCPLWCFREICRHISGPLPDRAPYLYLSAGVFWLMSPRMTSAIPPLLS
DLVNLAILQQTAGLDPSLVKLGVQICLHAAASSSYAWFILKTKSIFPQNTLHSMYESLEGGYCPNLEWLEPRSDYKF
MYMGVMPLSAKYARSAPSNDKKARELGEKYGLSSVVGELRKRTKTYVKHDFASVRYIRDAMACTSGIFLVRTPTETV
LQEYTQSPEIKVPIPQKDWTGPIGEIRILKDTTSSIARYLYRTWYLAAARMAAQPRTWDPLFQAIMRSQYVTARGGS
GAALRESLYAINVSLPDFKGLPVKAATKIFQAAQLANLPFSHTSVAILADTSMGLRNQVQRRPRSIMPLNVPQQQVS
APHTLTADYINYHMNLSTTSGSAVIEKVIPLGVYASSPPNQSINIDISACDASITWDFFLSVIMAAIHEGVASSSIG
KPFMGVPASIVNDESVVGVRAARPISGMQNMIQHLSKLYKRGFSYRVNDSFSPGNDFTHMTTTFPSGSTATSTEHTA
NNSTMMETFLTVWGPEHTDDPDVLRLMKSLTIQRNYVCQGDDGLMIIDGTTAGKVNSETIQKMLELISKYGEEFGWK
YDIAYDGTAEYLKLYFIFGCRIPNLSRHPIVGKERANSSAEEPWPAILDQIMGVFFNGVHDGLQWQRWIRYSWALCC
AFSRQRTMIGESVGYLQYPMWSFVYWGLPLVKAFGSDPWIFSWYMPTGDLGMYSWISLIRPLMTRWMVANGYVTDRC
SPVFGNADYRRCFNELKLYQGYYMAQLPRNPKKSGRAAPREVREQFTQALSDYLLQNPELKSRVLRGRSEWEKYGAG
IIHNPPSLFDVPHKWYQGAQEAAIATREELAEMDETLMRARRHRYSSFSKLLEAYLLVKWRMCEAREPSVDLRLPLC
AGIDPLNSDPFLKMVSVGPMLQSTRKYFAQTLFMAKTVSGLDVNAIDSALLRLRTLGADKKALTAQLLMVGLQESEA
DALAGKIMLQDVNTVQLARVVNLAVPDTWMSLDFDSMFKHHVKLLPKDGRHLNTDIPPRMGWLRAILRFLGAGMVMT
ATGVAVDIYLEDIHGGGRSLGQRFMTWMRQEGRSA (SEQ ID NO:19)

λ2:

MANVWGVRLADSLSSPTIETRTRQYTLHDLCSDLDANPGREPWKPLRNQRTNNIVAVQLFRPLQGLVLDTQLYGFPG
AFDDWERFMREKLRVLKYEVLRIYPISNYSNEHVNVFVANALVGAFLSNQAFYDLLPLLIINDTMIGDLLGTGASLS
QFFQSHGDVLEVAAGRKYLQMENYSNDDDDPPLFAKDLSDYAKAFYSDTYEVLDRFFWTHDSSAGVLVHYDKPTNGH
HYLLGTLTQMVSAPPYIINATDAMLLESCLEQFSANVRARPAQPVTRLDQCYHLRWGAQYVGEDSLTYRLGVLSLLA
TNGYQLARPIPRQLTNRWLSSFVSQIMSDGVNETPLWPQERYVQIAYDSPSVVDGATQYGYVRKNQLRLGMRISALQ
SLSDTPSPVQWLPQYTIDQAAMDEGDLMVSRLTQLPLRPDYGNIWVGDALSYYVDYNRSHRVVLSSELPQLPDTYFD
GDEQYGRSLFSLARKIGDRSLVKDTAVLKHAYQAIDPNTGKEYLRSRQSVAYFGASAGHSGADQPLVIEPWIQGKIS
GVPPPSSVRQFGYDVARGAIVDLARPFPSGDYQFVYSDVDQVVDGHDDLSISSGLVESLLSSCMHATAPGGSFVVKI
NFPTRPVWHYIEQKILPNITSYMLIKPFVTNNVELFFVAFGVHQHSSLTWTSGVYFFLVDHFYRYETLSTISRQLPS
FGYVDDGSSVTGIETISIENPGFSNMTQAARIGISGLCANVGNARKSIAIYESHGARVLTITSRRSPASARRKSRLR
YLPLIDPRSLEVQARTILPADPVLFENVSGASPHVCLTMMYNFEVSSAVYDGDVVLDLGTGPEAKILELIPATSPVT
CVDIRPTAQPSGCWNVRTTFLELDYLSDGWITGVRGDIVTCMLSLGAAAAGKSMTFDAAFQQLIKVLSKSTANVVLV
QVNCPTDVVRSIKGYLEIDSTNKRYRFPKFGRDEPYSDMDALEKICRTAWPNCSITWVPLSYDLRWTRLALLESTTL
SSASIRIAELMYKYMPIMRIDIHGLPMEKRGNFIVGQNCSLVIPGFNAQDVFNCYFNSALAFSTEDVNAAMIPQVSA
QFDATKGEWTLDMVFSDAGIYTMQALVGSNANPVSLGSFVVDSPDVDITDAWPAQLDFTIAGTDVDITVNPYYRLMT
FVRIDGQWQIANPDKFQFFSSASGTLVMNVKLDIADKYLLYYIRDVQSRDVGFYIQHPLQLLNTITLPTNEDLFLSA
PDMREWAVKESGNTICILNSQGFVLPQDWDVLTDTISWSPSIPTYIVPPGDYTLTPL (SEQ ID NO:20)

λ1:

MKRIPRKTKGKSSGKGNDSTERADDGSSQLRDKQNNKAGPATTEPGTSNREQYKARPGIASVQRATESAEMPMKNND
EGTPDKKGNTKGDLVNEHSEAKDEADEATKKQAKDTDKSKAQVTYSDTGINNANELSRSGNVDNEGGSNQKPMSTRI
AEATSAIVSKHPARVGLPPTASSGHGYQCHVCSAVLFSPLDLDAHVASHGLHGNMTLTSSDIQRHITEFISSWQNHP
IVQVSADVENKKTAQLLHADTPRLVTWDAGLCTSFKIVPIVPAQVPQDVLAYTFFTSSYAIQSPFPEAAVSRIVVHT
RWASNVDFDRDSSVIMAPPTENNIHLFKQLLNTETLSVRGANPLMFRANVLHMLLEFVLDNLYLNRHTGFSQDHTPF
TEGANLRSLPGPDAEKWYSIMYPTRMGTPNVSKICNFVASCVRNRVGRFDRAQMMNGAMSEWVDVFETSDALTVSIR
GRWMARLARMNINPTEIEWALTECAQGYVTVTSPYAPSVNRLMPYRISNAERQISQIIRIMNIGNNATVIQPVLQDI
SVLLQRISPLQIDPTIISNTMSTVSESTTQTLSPASSILGKLRPSNSDFSSFRVALAGWLYNGVVTTVIDDSSYPKD
GGSVTSLENLWDFFILALALPLTTDPCAPVKAFMTLANMMVGFETIPMDNQIYTQSRRASAFSTPHTWPRCFMNIQL
ISPIDAPILRQWAEIIHRYWPNPSQIRYGAPNVFGSANLFTPPEVLLLPIDHQPANVTTPTLDFTNELTNWRARVCE
LMKNLVDNQRYQPGWTQSLVSSMRGTLDKLKLIKSMTPMYLQQLAPVELAVIAPMLPFPPFQVPYVRLDRDRVPTMV
GVTRHSRDTITQPALSLSTTNTTVGVPLALDARAITVALLSGKYPPDLVTNVWYADAIYPMYADTEVFSNLQRDMIT
CEAVQTLVTLVAQISETQYPVDRYLDWIPSLRASAATAATFAEWVNTSMKTAFDLSDMLLEPLLSGDPRMTQLAIQY

Figure 6-2

QQYNGRTFNIIPEMPGSVIADCVQLTAEVFNHEYNLFGIARGDIIIGRVQSTHLWSPLAPPPDLVFDRDTPGVHIFG
RDCRISFGMNGAAPMIRDETGLMVPFEGNWIFPLALWQMNTRYFNQQFDAWIKTGELRIRIEMGAYPYMLHYYDPRQ
YANAWNLTSAWLEEITPTSIPSVPFMVPISSDHDISSAPAVQYIISTEYNDRSLFCTNSSSPQTIAGPDKHIPVERY
NILTNPDAPPTQIQLPEVVDLYNVVTRYAYETPPITAVVMGVP (SEQ ID NO:21)

INTERNATIONAL SEARCH REPORT	International application No. PCT/CA2008/000483

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12N 15/46*** (2006.01) , ***A61K 35/76*** (2006.01) , ***A61P 35/00*** (2006.01) , ***C07K 14/14*** (2006.01) , ***C12N 15/86*** (2006.01) , ***C12N 7/01*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ***C12N 15/46*** (2006.01) , ***A61K 35/76*** (2006.01) , ***A61P 35/00*** (2006.01) , ***C07K 14/14*** (2006.01) , ***C12N 15/86*** (2006.01) , ***C12N 7/01*** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Delphion, Pubmed, GenBank, GenPept, Geneseq, GenePast, SwissProt, Scopus, Esp@cenet. Keywords: reovirus, orthoreovirus, mutant/mutated reovirus, Ras reovirus, proliferative disorder, strain Lang, strain Jones, strain Dearing or strain Abney, SEQ ID Nos: 1-30

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RICHARDSON, M. A. *et al.* "Nucleotide sequence of reovirus genome segment S3, encoding non-structural protein sigma NS." NUCLEIC ACIDS RESEARCH. September 1983. Vol. 11, No. 18, pages 6399-6408, ISSN: 1362-4962 whole document	4, 12, 25, 30, 33, 47, 48
Y		49, 50, 57-63
X	MCCUTCHEON, A. M. *et al.* "Mammalian Reovirus M3 Gene Sequences and Conservation of Coiled-Coil Motifs near the Carboxyl Terminus of the μNS Protein." VIROLOGY. November 1999. Vol. 264, pages 16-24, ISSN: 1096-0341 whole document	2, 12, 23, 30, 33, 47, 48
Y		32, 49, 50, 57-63
Y	DERMODY, T. S. *et al.* "The S2 Gene Nucleotide Sequences of Prototype Strains of the Three Reovirus Serotypes: Characterization of Reovirus Core Protein σ2." JOURNAL OF VIROLOGY. November 1991. Vol 65, No. 11, pages 5721-5731, ISSN: 1098-5514 whole document	32

[X] Further documents are listed in the continuation of Box C.   [X] See patent family annex.

*	Special categories of cited documents :
"A"	document defining the general state of the art which is not considered to be of particular relevance
"E"	earlier application or patent but published on or after the international filing date
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search 25 June 2008 (25-06-2008)	Date of mailing of the international search report 03 July 2008 (03-07-2008)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer Damiano Conte 819- 934-7931

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

a. type of material

[X] a sequence listing

[ ] table(s) related to the sequence listing

b. format of material

[X] on paper

[X] in electronic form

c. time of filing/furnishing

[X] contained in the international application as filed.

[X] filed together with the international application in electronic form

[ ] furnished subsequently to this Authority for the purposes of search.

2 [X] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments :

It should be noted that the numbering for SEQ ID NOs: 22, 23, 25, 29 and 30 is incorrect and thus the reported length of said sequences is also incorrect.

SEQ ID NO. 22: 3914 nucleotides	GenBank: 3854 nucleotides
SEQ ID NO. 23: 1297 amino acids	GenPept: 1267 amino acids
SEQ ID NO. 25: 370 amino acids	GenPept: 365 amino acids
SEQ ID NO. 29: 731 amino acids	GenPept: 736 amino acids
SEQ ID NO. 30: 423 amino acids	GenPept: 418 amino acids

Box No. II **Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 57-61
because they relate to subject matter not required to be searched by this Authority, namely :

Claims 57-61 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search under Rule 39.1(iv) of the PCT. However, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in claims 57-61.

2. [X] Claim Nos. : 51-56
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

Claims 51-56 encompass methods with insufficient technical features. The claims are directed to the desired result, namely, an improved reovirus. The claims lack the specific technical details as to what constitutes an improved modified reovirus or the guidance of where such modifications should be incorporated. Claim 56 only contains a "laundry list" of possible types of functional improvements to a reovirus, without any guidance or sufficient technical details to achieve said improved viruses.

3. [ ] Claim Nos. :
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest [ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WIENER, J. R. *et al.* "The sequences of the S2 genome seqments of reovirus serotype 3 and of the dsRNA-negative mutant ts447." VIROLOGY. MAY 1989. Vol. 170, No. 1, pages 340-341, ISSN: 1096-0341 whole document	47, 48
X, P	WO 2007/099401 A2 (UNIVERSITY TECHNOLOGIES INTERNATIONAL, INC.) 7 September 2007 whole document	8, 12, 29, 30, 33, 47, 48
Y	US 6110461 (ONCOLYTICS BIOTECH INC.) 29 August 2000 whole document	49, 50, 57-63

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2008/000483

Patent Document Cited in Search Report	Publication Date	Patent Family Members	Publication Date
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		CA2617600AA	07-09-2007