RECOMBINANT ADENOVIRUSES CARRYING TRANSGENES

CROSS-REFERENCE TO RELATED APPLICATION

[0001] The present application claims priority to, and the benefits of U.S. Provisional Patent Application No. 62/511,822 filed May 26, 2017, which is herein incorporated by reference in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is EPRX_002_01WO_SeqList_ST25.txt. The text file is about 128 KB, was created on May 29, 2018 and is being submitted electronically via EFS-Web.

FIELD OF THE INVENTION

[0003] The invention described herein generally relates to the fields of virology, virotherapy, and molecular biology.

BACKGROUND

[0004] The uses of virotherapy to treat diseases such as cancer encompass employing replication-selective viruses armed with therapeutic genes or transgenes. Of the variety of infectious viral species developed as virotherapy agents, adenoviruses have emerged as one of the most promising because not only are they minimally toxic to normal non-transformed cells but their genomes, comprised of multiple endogenous genes, are amenable to manipulation, which generally takes the form of deletion of endogenous genes and insertion of exogenous ones. The downside to this manipulation is that most endogenous gene deletions or exogenous gene additions slow down or attenuate the replicative and infectivity potential of the virus. (Larson et al., Oncotarget, 6(24):19976-89 (2015))

[0005] The reduced replication efficiency of viruses carrying transgenes in these regions is undesirable because, such as in the case of an oncolytic virus for the treatment of cancer, it

impairs the ability of the virus to multiply within tumors and infect neighboring cancerous cells, decreases the number of viral genome copies within infected cells and therefore likely reduces transcription of the therapeutic transgene, and increases the size of production cultures required to manufacture the virus. Therefore, a need exists for a new method to improve the ability of recombinant adenoviruses to replicate to high levels in targeted cells or tissues such as in tumors, thereby rapidly turning the targeted cells or tissues into a "factory" for the production of particular exogenous gene products.

[0006] Typically, to have oncolytic viruses express two or more separate protein or polypeptide chains requires the use of more than one virus vector or the use of linker, such as an internal ribosome entry site (IRES), between two transgenes. Both methods have significant drawbacks. Two or more virus vectors may not all express well within a single cell or tissue. As known in the art, the sequence downstream of the IRES is expressed at much lower levels than the sequence upstream. (Mizuguchi et al., Mol. Ther. 1(4):376-82 (2000)) In addition, the linker, being non-endogenous, has the potential for immunogenicity. Therefore, a need exists for more efficient viral vectors to express more than one peptide chain within a single virus.

SUMMARY OF THE INVENTION

[0007] The invention is based, in part, upon the discovery that recombinant adenoviruses with one or more nucleotide sequences inserted between two viral transcription units in the viral genome can efficiently replicate and express the nucleotide sequences in targeted cells or tissues, do not significantly impact the oncolytic activity of the virus. The vectors of this invention can be advantageously used where equal levels of two or more transgenes are desired or to express completely native chains from dual chain proteins.

[0008] In one aspect, the invention provides a recombinant adenovirus comprising a nucleotide sequence inserted in an insertion site, wherein the insertion site is located between the stop codon of a first viral transcription unit and the stop codon of a second viral transcription unit, wherein the stop codon of the first viral transcription unit is nearer to the stop codon of the second viral transcription unit than the start site of the first viral transcription unit is to the stop codon of the second viral transcription unit, wherein the stop codon of the second viral transcription is nearer to the stop codon of the first viral transcription unit than the start site of the second viral transcription unit is to the stop codon of the first viral transcription unit, and wherein there is no viral transcription unit between the first viral transcription unit and the second viral transcription unit before the nucleotide sequence is inserted.

[0009] In certain embodiments, the first viral transcription unit is adenovirus IX gene and the second viral transcription unit is adenovirus IVa2 gene. In certain embodiments, the first viral transcription unit is adenovirus fiber gene and the second viral transcription unit is ORF6 or ORF6/7 of adenovirus E4 gene. In certain embodiments, the recombinant adenovirus is a type 5 adenovirus (Ad5). In certain embodiments, the recombinant adenovirus is a type 35 adenovirus (Ad35).

[0010] In certain embodiments, a nucleotide sequence in inserted in the IX-E2 insertion site. In certain embodiments, the IX-E2 insertion site is located between the stop codon of adenovirus IX gene and the stop codon of adenovirus IVa2 gene. In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to about 4029 and 4093 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4050, nucleotides corresponding to 4050 and 4070, or nucleotides corresponding to 4070 and 4093 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to about 3899 and 3970 of the Ad35 genome (SEQ ID NO: 41). In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to 3899 and 3920, nucleotides corresponding to 3920 and 3940, or nucleotides corresponding to 3940 and 3970 of the Ad35 genome (SEQ ID NO: 41).

[0011] In certain embodiments, a nucleotide sequence is inserted in an L5-E4 insertion site. In certain embodiments, the L5-E4 insertion site is located between the stop codon of adenovirus fiber gene and the stop codon of ORF6 or ORF6/7 of the adenovirus E4 gene. In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to 32785 to 32916 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to 32785 and 32800, nucleotides corresponding to 32800 and 32820, nucleotides corresponding to 32820 and 32840, nucleotides corresponding to 32840 and 32860, nucleotides corresponding to 32860 and 32880, nucleotides corresponding to 32880 and 32900, or nucleotides corresponding to about 32901 and 32916 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to about 31799 and 31821 of the Ad35 genome (SEQ ID NO: 41). In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to 31799 and 32810, or nucleotides corresponding to 32810 and 31821 of the Ad35 genome (SEQ ID NO: 41).

[0012] In certain embodiments, the foregoing recombinant adenovirus further comprises a nucleotide sequence inserted in an E1b-19K insertion site, an E3 insertion site, or an E4 insertion site. In certain embodiments, the E1b-19K insertion site is located between the start site of Elb-19K and the start site of Elb-55K. In certain embodiments, the E1b-19k insertion site is located between the start site of Elb-19K and the stop codon of Elb-19K. In certain embodiments, the E3 insertion site is located between the stop codon of adenovirus pVIII gene and the start site of adenovirus Fiber gene.

[0013] In certain embodiments, the invention provides a recombinant adenovirus comprising a first nucleotide sequence inserted in an IX-E2 insertion site and a second nucleotide sequence inserted in an L5-E4 insertion site.

[0014] In certain embodiments, the first nucleotide sequence is inserted between nucleotides corresponding to about 4029 and 4093 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the first nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4050, nucleotides corresponding to 4050 and 4070, or nucleotides corresponding to 4070 and 4093 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the first nucleotide sequence is inserted between nucleotides corresponding to about 3899 and 3970 of the Ad35 genome (SEQ ID NO: 41). In certain embodiments, the first nucleotide sequence is inserted between nucleotides corresponding to 3899 and 3920, nucleotides corresponding to 3920 and 3940, or nucleotides corresponding to 3940 and 3970 of the Ad35 genome (SEQ ID NO: 41).

[0015] In certain embodiments, the second nucleotide sequence is inserted between nucleotides corresponding to 32785 to 32916 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the second nucleotide sequence is inserted between nucleotides corresponding to 32785 and 32800, nucleotides corresponding to 32800 and 32820, nucleotides corresponding to 32820 and 32840, nucleotides corresponding to 32840 and 32860, nucleotides corresponding to 32860 and 32880, nucleotides corresponding to 32880 and 32900, or nucleotides corresponding to about 32901 and 32916 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the second nucleotide sequence is inserted between nucleotides corresponding to about 31799 and 31821 of the Ad35 genome (SEQ ID NO: 41). In certain embodiments, the second nucleotide sequence is inserted between nucleotides corresponding to 31799 and 32810, or nucleotides corresponding to 32810 and 31821 of the Ad35 genome (SEQ ID NO: 41).

[0016] In certain embodiments, the nucleotide sequence, the first nucleotide sequence, and/or the second nucleotide sequence comprises at least one transgene. In certain embodiments, the

nucleotide sequence further comprises a promoter, wherein the transgene is operably linked to the promoter.

[0017] In certain embodiments, the recombinant adenovirus comprises, in a 5' to 3' orientation: (i) a first polyadenylation signal; (ii) a promoter; (iii) a transgene; (iv) a second polyadenylation signal; and (v) a third polyadenylation signal; wherein the transgene is operably linked to the promoter. In some embodiments, the nucleotide sequence, the first nucleotide sequence, and/or the second nucleotide sequence (comprising one or more transgenes) is inserted between the first polyadenylation signal and the third polyadenylation signal. In some embodiments, the one or more transgenes is inserted between the first polyadenylation signal and the third polyadenylation signal. In certain embodiments, wherein the second polyadenylation signal is in the opposite transcriptional direction of the third polyadenylation signal.

[0018] In certain embodiments, the nucleotide sequence is inserted in the L5-E4 insertion site, and the first polyadenylation signal is the polyadenylation signal of the fiber (L5) gene, the second polyadenylation signal is the polyadenylation signal of the transgene, and the third polyadenylation signal is the polyadenylation signal of the ORF6 or ORF6/7 of the adenovirus E4 gene. In certain embodiments, the nucleotide sequence is inserted in the IX-E2 insertion site, and the first polyadenylation signal is the polyadenylation signal of the IX gene, the second polyadenylation signal is the polyadenylation signal of the transgene, and the third polyadenylation signal is the polyadenylation signal of the adenovirus IVa2 gene.

[0019] In certain embodiments, the recombinant adenovirus comprises, in a 5' to 3' orientation: (i) a first polyadenylation signal; (ii) a second polyadenylation signal; (iii) a promoter; (iv) a transgene; (v) a third polyadenylation signal; and (vi) a fourth polyadenylation signal, and the transgene is operably linked to the promoter. In some embodiments, the nucleotide sequence, the first nucleotide sequence, and/or the second nucleotide sequence (comprising one or more transgenes) is inserted between the first polyadenylation signal and the fourth polyadenylation signal. In some embodiments, the one or more transgenes is inserted between the first polyadenylation signal and the fourth polyadenylation signal. In certain embodiments, wherein the second polyadenylation signal is in the opposite transcriptional direction of the first polyadenylation signal. In certain embodiments, wherein the fourth polyadenylation signal is in the opposite transcriptional direction of the third polyadenylation signal.

[0020] In certain embodiments, the nucleotide sequence is inserted in the L5-E4 insertion site, and the first polyadenylation signal is the polyadenylation signal of the fiber (L5) gene, the third polyadenylation signal is the polyadenylation signal of the transgene, and the fourth polyadenylation signal is the polyadenylation signal of the ORF6 or ORF6/7 of the adenovirus E4 gene. In certain embodiments, the nucleotide sequence is inserted in the IX-E2 insertion site, the first polyadenylation signal is the polyadenylation signal of the IX gene, the third polyadenylation signal is the polyadenylation signal of the transgene, and the fourth polyadenylation signal is the polyadenylation signal of the adenovirus IVa2 gene.

[0021] In certain embodiments, the promoter is a ubiquitous promoter, a tissue-specific promoter, or tumor-specific promoter.

[0022] In certain embodiments, the IX-E2 insertion site comprises a deletion of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, or 60 nucleotides. In certain embodiments, the L5-E4 insertion site comprises a deletion of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, or 130 nucleotides.

[0023] In certain embodiments, the nucleotide sequence further comprises a consensus Kozak sequence. In certain embodiments, the recombinant adenovirus comprises a partial or complete deletion of the nucleotide sequence encoding the adenoviral death protein (ADP).

[0024] In certain embodiments, the foregoing recombinant adenovirus further comprises a nucleotide sequence inserted in an E1b-19K insertion site, an E3 insertion site, or an E4 insertion site. In certain embodiments, the E1b-19K insertion site is located between the start site of Elb-19K and the start site of Elb-55K. In certain embodiments, the E1b-19k insertion site is located between the start site of Elb-19K and the stop codon of Elb-19K. In certain embodiments, the E3 insertion site is located between the stop codon of adenovirus pVIII gene and the start site of adenovirus Fiber gene.

[0025] In certain embodiments, the E1b-19K insertion site comprises a deletion of from about 100 to about 305, about 100 to about 300, about 100 to about 250, about 100 to about 200, about 100 to about 150, about 150 to about 305, about 150 to about 300, about 150 to about 250, or about 150 to about 200 nucleotides adjacent the start site of E1b-19K. In certain embodiments, the E1b-19K insertion site comprises a deletion of about 200 nucleotides, e.g., 202 nucleotides adjacent the start site of E1b-19K. In certain embodiments, the E1b-19K insertion site comprises a deletion corresponding to nucleotides 1714-1917 of the Ad5 genome (SEQ ID NO: 1), or the first therapeutic transgene is inserted between nucleotides corresponding to 1714 and 1917 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments,

the first therapeutic transgene is inserted between CTGACCTC (SEQ ID NO: 3) and TCACCAGG (SEQ ID NO: 2), e.g., the recombinant adenovirus comprises, in a 5' to 3' orientation, CTGACCTC (SEQ ID NO: 3), the first therapeutic transgene, and TCACCAGG (SEQ ID NO: 2).

[0026] In certain embodiments, the E3 insertion site comprises a deletion of from about 500 to about 3185, from about 500 to about 3000, from about 500 to about 2500, from about 500 to about 2000, from about 500 to about 1500, from about 500 to about 1000, from about 1000 to about 3185, from about 1000 to about 3000, from about 1000 to about 2500, from about 1000 to about 2000, from about 1000 to about 1500, from about 1500 to about 3185, from about 1500 to about 3000, from about 1500 to about 2000, from about 2000 to about 3185, from about 2000 to about 3000, from about 2000 to about 2500, from about 2500 to about 3185, from about 2500 to about 3000, or about 3000 to about 3185 nucleotides. In certain embodiments, the E3 insertion site is located between the stop codon of E3-10.5K and the stop codon of E3-14.7K. In certain embodiments, the E3 insertion site comprises a deletion of from about 500 to about 1551, from about 500 to about 1500, from about 500 to about 1000, from about 1000 to about 1551, from about 1000 to about 1500, or from about 1500 to about 1551 nucleotides adjacent the stop codon of E3-10.5K. In certain embodiments, the E3 insertion site comprises a deletion of about 1050 nucleotides adjacent the stop codon of E3-10.5K, e.g., the E3 insertion site comprises a deletion of 1063 nucleotides adjacent the stop codon of E3-10.5K. In certain embodiments, the E3 insertion site comprises a deletion corresponding to the Ad5 dl309 E3 deletion. In certain embodiments, the E3 insertion site comprises a deletion corresponding to nucleotides 29773-30836 of the Ad5 genome (SEQ ID NO: 1), or the second therapeutic transgene is inserted between nucleotides corresponding to 29773 and 30836 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the E3 insertion site comprises a deletion corresponding to nucleotides 29119-30622 of the Ad35 genome (SEQ ID NO: 41).

[0027] In certain embodiments, the recombinant adenovirus comprises an E1a promoter having a deletion of a functional Pea3 binding site. For example, the virus may comprise a deletion of nucleotides corresponding to about -300 to about -250 upstream of the initiation site of Ela or a deletion of nucleotides corresponding to -305 to -255 upstream of the initiation site of Ela. In certain embodiments, the deletion comprises a deletion of nucleotides corresponding to 195-244 of the Ad5 genome (SEQ ID NO: 1), and/or the E1a promoter comprises the sequence GGTGTTTTGG (SEQ ID NO: 4).

[0028] In certain embodiments, the recombinant adenovirus comprises a modified TATA box-based promoter operably linked to a gene, wherein the modified TATA box-based promoter lacks a functional TATA box and permits selective expression of the gene in a hyperproliferative cell and/or a modified CAAT box-based promoter operably linked to a gene, wherein the modified CAAT box-based promoter lacks a functional CAAT box and permits selective expression of the gene in a hyperproliferative cell.

[0029] In certain embodiments, wherein the modified TATA box-based promoter is an early gene promoter. In certain embodiments, the modified TATA box-based promoter is an E1a promoter, E1b promoter, or E4 promoter. In certain embodiments, the modified TATA box-based promoter is an E1a promoter.

[0030] In certain embodiments, the modification included in the modified TATA box-based promoter comprises a deletion of the entire TATA box. In certain embodiments, the recombinant adenovirus comprises a deletion of nucleotides corresponding to -27 to -24, -31 to -24, -44 to +54, or -146 to +54 of the E1a promoter. In certain embodiments, the deletion comprises a deletion of nucleotides corresponding to 472 to 475, 468 to 475, 455 to 552, or 353 to 552 of the Ad5 genome (SEQ ID NO: 1).

[0031] In certain embodiments, the recombinant adenovirus comprises a polynucleotide deletion that results in a virus comprising the sequence CTAGGACTG (SEQ ID NO: 5), AGTGCCCG (SEQ ID NO: 44) and/or TATTCCCG (SEQ ID NO: 45).

[0032] In certain embodiments, the modified CAAT box-based promoter is an early gene promoter. In certain embodiments, the modified CAAT box-based promoter is an E1a promoter, E1b promoter, or E4 promoter. In certain embodiments, the modified CAAT box-based promoter is an E1a promoter.

[0033] In certain embodiments, the modification included in the modified CAAT box-based promoter comprises a deletion of the entire CAAT box. In certain embodiments, the recombinant adenovirus comprises a deletion of nucleotides corresponding to -76 to -68 of the E1a promoter.

[0034] In certain embodiments, the recombinant adenovirus comprises a deletion of nucleotides corresponding to 423 to 431 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the recombinant adenovirus comprises a polynucleotide deletion that results in a virus comprising the sequence TTCCGTGGCG (SEQ ID NO: 46). In certain embodiments, the recombinant adenovirus comprises a deletion of nucleotides corresponding to 477 to 484 of the Ad35 genome (SEQ ID NO: 41).

[0035] In certain embodiments, the inserted nucleotide sequence comprises a first nucleotide sequence comprising a first transgene, and a second nucleotide sequence comprising a second transgene, wherein the first nucleotide sequence and the second nucleotide sequence are separated by a linker. In certain embodiments, the linker encodes a peptide cleavable by a protease or proteases. In certain embodiments, the linker encodes an internal ribosome entry site (IRES) or a self-cleaving 2A peptide. The IRES may, e.g., be selected from the group consisting of the encephalomyocarditis virus IRES, the foot-and-mouth disease virus IRES, and the poliovirus IRES. In certain embodiments, wherein the nucleotide sequence is inserted in the IX-E2 insertion or the L5-E4 insertion site, wherein the recombinant adenovirus further comprise a third nucleotide sequence comprising a third transgene inserted in an E1b-19K insertion site, an E3 insertion site, or an E4 insertion site.

[0036] In certain embodiments, one or more of the nucleotide sequence, the first nucleotide sequence, the second nucleotide sequence, and the third nucleotide sequence comprises one or more transgenes.

[0037] In certain embodiments, one or more of the transgene, the first transgene, and the second transgene encodes a monomeric, dimeric, trimeric, tetrameric, or multimeric protein, or a part thereof. In certain embodiments, one or more of the transgene, the first transgene, and/or the second transgene encodes a RNA that has a therapeutic activity. In certain embodiments, one or more of the transgene, the first transgene, and/or the second transgene encodes a fusion protein comprising at least one binding domain.

[0038] In certain embodiments, one or more of the transgene, the first transgene, and the second transgene encodes an immunomodulatory molecule. In certain embodiments, the immunomodulatory molecule is a costimulatory ligand, a cytokine, or a cytokine receptor. In certain embodiments, the immunomodulatory molecule is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-7, IL-10, IL-10 trap, IL-10R, IL-12A/p35, IL-12B/p40, IL-15, IL-15 receptor fusion protein, IL-23A/p19, IL24, IL-27, IL-33, IL-35, IL-15, an IL-15 receptor fusion protein, TGF-β, a TGF-β trap, an IL-10 trap, VEGF, indoleamin-2,3-dioxygenase (IDO), inducible T-cell co-stimulator ligand (ICOS-L), CD80, CD137L, TNF-α, IFN-α, IFN- β, IFN-γ, or GM-CSF, GITR ligand (GITRL), OX40 ligand (OX40L), CD40 ligand (CD40L), drug-inducible CD40 (iCD40), CD154, CD70, CD86, CD137, CD137L, BORIS/CTCFL, TNFSF9, FGF, ICAM, Podocalyxin, functional fragments thereof, and derivatives thereof.

[0039] In certain embodiments, one or more of the transgenes, the first transgene, and the second transgene encodes an antigen-binding molecule. In certain embodiments, the antigen-binding molecule is an anti-PD-1 antibody, an anti-TGF-β antibody, an anti-PD-L1 antibody, and an anti-CTLA-4 antibody, or functional fragments thereof.

[0040] In certain embodiments, one or more of the transgenes, the first transgene, and the second transgene encodes an antigen or a ligand to the antigen. In certain embodiments, the antigen is selected from the group consisting of CAIX, CEA, CD5, CD7, CD10, CD19, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD80, CD133, CD138, a cytomegalovirus (CMV) infected cell antigen, 4-1BB, EGP-2, EGP-40, EpCAM, erbB2, erbB3, erbB4, FBP, Fetal acetylcholine receptor, KRAS, HPV E6, E7, BING-4, EphA3, calcium activated chloride channel-2, cyclin B1, 9D7, Ep-CAM, PRAME, SSX-2, immature laminin receptor, folate receptor-a, telomerase, tyrosinase, melan-A, NY-ESO-1, GD2, GD3, hTERT, IL13R-a2, x-light chain, KDR, LeY, LI cell adhesion molecule, MAGE-A1, MAGE-A3, MART1, MART2, MUC1, Mesothelin, HER-2/neu, EGFRvIII, NKG2D ligands, NY-ES0-1, gp100, TRP-1/-2, TRP-1/-2, P polypeptide, MC1R, prostate specific antigen, BRAF, androgen-receptor, β-catenin, BRCA1/2, CDK4, CML66, fibronectin, p53, TGF-βRII, T cell receptor, oncofetal antigen, 5T4, PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, functional fragments thereof, and derivatives thereof.

[0041] In certain embodiments, one or more of the transgenes, the first transgene, and the second transgene encodes a toxin. In certain embodiments, the toxin is pseudomonas exotoxin, ricin toxin, or diphtheria toxin.

[0042] In certain embodiments, one or more of the transgenes, the first transgene, and the second transgene encodes an enzyme. In certain embodiments, the enzyme is selected from the group consisting of beta-glucuronidase, beta-galactosidase, beta-glucosidase, carboxypeptidase, beta-lactamase, esterase, metalloproteinase, relaxin, collagenase, streptokinase, arginase, NOS-2, fragments thereof, and derivatives thereof.

[0043] In certain embodiments, one or more of the transgenes, the first transgene, and the second transgene encodes a cell cycle control agent, a growth factor, an anticoagulant, a pro-drug activating gene, a tumor suppressor gene, an apoptotic gene, an anti-platelet agent, a clotting factor, a cystic fibrosis transmembrane conductance regulator (CFTR) protein, fragments thereof, or derivatives thereof.

[0044] In certain embodiments, one or more of the transgenes, the first transgene, and the second transgene encodes angiostatin, endostatin, acetylcholine, DKK1/Wnt, Ox40L, GITRL, secreted flagellin, thymidine kinase, functional fragments thereof, or derivatives thereof.

[0045] In certain embodiments, the recombinant adenovirus is oncolytic. In certain embodiments, the recombinant adenovirus selectively replicates in a hyperproliferative cell. In certain embodiments, the recombinant adenovirus selectively expresses a transgene in a hyperproliferative cell. In certain embodiments, the hyperproliferative cell is a tumor cell.

[0046] In another aspect, the invention provides an isolated nucleotide sequence comprising any of the foregoing recombinant adenovirus sequence, optionally wherein the nucleotide sequence is cDNA. In another aspect, the invention provides an isolated vector comprising the adenovirus nucleotide sequence. In another aspect, the invention provides an isolated cell comprising the adenovirus nucleotide sequence or the vector.

[0047] In another aspect, the invention provides a method of inhibiting proliferation of a tumor cell comprising exposing the tumor cell to an effective amount of any of the foregoing recombinant adenoviruses to inhibit proliferation of the tumor cell.

[0048] In another aspect, the invention provides a method of treating a condition in a subject. In some embodiments, the condition is cancer. The method comprises administering to the subject an effective amount of a recombinant adenoviruses described herein to treat the cancer disease in the subject.

[0049] In another aspect, the invention provides a method of inhibiting tumor growth in a subject in need thereof, wherein the method comprising administering to the subject to an effective amount of any of the foregoing recombinant adenoviruses to inhibit tumor growth. In certain embodiments, the tumor is selected from the group consisting of melanoma, squamous cell carcinoma of the skin, basal cell carcinoma, head and neck tumor, breast tumor, anal cancer, cervical cancer, non-small cell lung cancer, mesothelioma, small cell lung tumor, renal cell carcinoma, prostate tumor, gastroesophageal tumor, colorectal tumor, testicular tumor, bladder tumor, ovarian tumor, hepatocellular carcinoma, cholangiocarcinoma, brain tumor, endometrial tumor, neuroendocrine tumor, merkel cell carcinoma, gastrointestinal stromal tumor, a sarcoma, and pancreatic tumor.

[0050] In another aspect, the invention provides a method of treating a disease or condition in a subject in need thereof, wherein the method comprising administering to the subject an effective amount of any of the foregoing recombinant adenoviruses. In certain embodiments, the disease or condition is selected from the group consisting of an infection, diabetic

retinopathy, psoriasis, rheumatoid arthritis, endometriosis, macular degenerative disorders and benign growth disorders such as prostate enlargement and lipomas, a vascular disorder, a cardiovascular disease, cirrhosis of the liver, a connective tissue disorder, a tumor, a vascular lesion, an ulcerative lesion, an inflammation, thrombosis, and neointima formation.

[0051] In certain embodiments, the subject is a mammal. In certain embodiments, the subject is a human. In certain embodiments, the subject is a pediatric human. In certain embodiments, the subject is an adult human.

[0052] In certain embodiments, the recombinant adenovirus is administered by intramuscular, intravenous, intraarterial, intratumoral, intradermal, inhalation, transdermal, topical, eye drops, intranasal, transmucosal, and/or rectal administration.

[0053] In certain embodiments, the foregoing methods further comprising administering to the subject one or more therapies selected from the group consisting of surgery, radiation, chemotherapy, immunotherapy, hormone therapy, and virotherapy.

[0054] In certain embodiments, the foregoing methods further comprise administering to the subject one or more immune checkpoint modulators. In certain embodiments, the immune checkpoint modulator is an inhibitor, an antagonist, or an agonist of one or more molecules selected from the group consisting of PD-1, PD-L1, PD-L2, 2B4, TIGIT, LAG3, Tim3, BTLA, CD160, GITR, KIR, 4-1BB, and CTLA4.

[0055] In another aspect, the invention provides a pharmaceutical composition comprising any of the foregoing recombinant adenoviruses and at least one pharmaceutically acceptable carrier or diluent.

[0056] In another aspect, the invention provides a formulation for adenoviruses comprising:

a) one or more of any of the foregoing recombinant adenoviruses;
b) at least one buffer;
c) at least one tonicity modifier;
d) at least one sugar or at least one stabilizing agent, or both; and

wherein the formulation has a pH ranging between about 7.0 and about 9.0.

[0057] In certain embodiments, any of the foregoing formulations has an osmolarity of about 200 mOs/L to about 800 mOs/L. In certain embodiments, the recombinant adenovirus in any of the foregoing formulations is at concentration from about 1×10^7 vp/mL to 1×10^{13} vp/mL.

[0058] These and other aspects and advantages of the invention are illustrated by the following figures, detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0059] The invention can be more completely understood with reference to the following drawings.

[0060] FIG. 1 is a graph depicting the genome organization of human Ad5. At the top of the figure the genome is represented as a line with lengths marked in kbp from the conventional left end. Thick arrows represent early and late transcription units (black and grey, respectively). Open boxes represent the major introns. The E4 gene is enlarged as a line scale with lengths in bp. The primary transcript is shown as a black arrow in a 5' to 3' direction and each of the potential encoded proteins is shown as an open box; proteins whose coding regions are split by intron sequences are shown as boxes linked by a line.

[0061] FIG. 2 depicts the mouse GMCSF expression level from A549, ADS-12, and WI-38 cells infected with the virus TAV-(E1B-19K)mGMCSF or TAV-(L5-E4)mGMCSF or kept as non-infected controls. Mouse GMCSF expression was measured in their conditioned media.

[0062] FIG. 3 depicts GMCSF expression level from A549 cells infected with virus TAV IX-WT L5-Empty, TAV IX-WT L5-IL7, TAV IX-WT L5-GMCSF, or TAV IX-GMCSF L5-IL7. GMCSF expression was measured in the conditioned media. Higher expression was seen when GMCSF was expressed from the IX-E2 expression cassette than from the L5-E4 expression cassette.

[0063] FIG. 4 depicts the initial design and revised design of the IX-E2 insertion site.

[0064] FIG. 5 depicts A549 cells infected virus (TAV-Δ19k, TAV-hIL12-furin, TAV-TAV-IXrL5-Empty, WT-IXrL5-hIL12, or TAV-IXrL5-hIL12) in triplicate and stained with crystal violet (staining live cells purple) four days after infection.

[0065] FIG. 6 depicts IL-12 expression level from A549 cells infected with virus (TAV-Δ19k, TAV-hIL12-furin, TAV-TAV-IXrL5-Empty, WT-IXrL5-hIL12, or TAV-IXrL5-hIL12). The A549 cells were infected with the indicated virus in triplicate, and IL12 in the conditioned media was measured with an ELISA four days after infection.

[0066] FIG. 7 depicts IL-17 and GMCSF expression level from A549 cells infected with either TAV-(IXr)mIL7noPA-(L5SV40wt)KozakmGMCSF (labeled SV40wt) or TAV-(IXr)mIL7noPA-(L5EF1A)KozakmGMCSF (labeled hEF1A).

[0067] FIG. 8 depicts IL-17 and GMCSF expression level from A549 cells infected with ethe ADP gene intact [TAV-(IXr)mIL7noPA-(L5EF1A)KozakmGMCSF, labeled as +ADP] or deleted [TAV-(IXr)mIL7noPA-(L5EF1A)KozakmGMCSF-ΔADP, labeled as ΔADP].

Conditioned media was collected at the indicated times after infection and IL-7 and GMCSF were measured in ELISAs.

[0068] FIG. 9 depicts CD80, CD137L, and ICAM1 staining in A549 cells infected with virus [TAV-mCD80(IRES)mCD137L(IRES)mICAM1, labeled as IRES], [TAV-(19k)mCD80-(IX)mCD137L-(L5)mICAM1, labeled as 19K-IX-5], or the control virus [TAV-(19k)Empty-(IX)Empty-(L5)Empty, labeled as Empty].

[0069] FIG. 10 depicts CD80, CD137L, and ICAM1 staining in HT29 cells infected with virus [TAV-mCD80(IRES)mCD137L(IRES)mICAM1, labeled as IRES], [TAV-(19k)mCD80-(IX)mCD137L-(L5)mICAM1, labeled as 19K-IX-5], or the control virus [TAV-(19k)Empty-(IX)Empty-(L5)Empty, labeled as Empty].

[0070] FIG. 11 depicts CD80, CD137L, and ICAM1 staining in ADS12 cells infected with virus [TAV-mCD80(IRES)mCD137L(IRES)mICAM1, labeled as IRES], [TAV-(19k)mCD80-(IX)mCD137L-(L5)mICAM1, labeled as 19K-IX-5], or the control virus [TAV-(19k)Empty-(IX)Empty-(L5)Empty, labeled as Empty].

[0071] FIG. 12 depicts CD80, CD137L, and ICAM1 staining in F244 cells infected with virus [TAV-mCD80(IRES)mCD137L(IRES)mICAM1, labeled as IRES], [TAV-(19k)mCD80-(IX)mCD137L-(L5)mICAM1, labeled as 19K-IX-5], or the control virus [TAV-(19k)Empty-(IX)Empty-(L5)Empty, labeled as Empty].

[0072] FIG. 13 depicts oncolytic activity of the viruses TAV-IX5-Empty (labeled "Empty") and TAV-IX5-mIL12 (labeled "mIL12") in A549 cells after infection at an MOI of 5. Wells were stained with crystal violet on the indicated days after infection.

[0073] FIG. 14 transgene expression of the virus TAV-IX5-mIL12. A549 cells were infected with TAV-IX5-Empty (labeled "Empty") or TAV-IX5-mIL12 (labeled "mIL12") at an MOI of 5, and conditioned media was collected five days after infection and used in an ELISA to measure heterodimeric mouse IL-12. High levels of mouse IL-12 were expressed with the TAV-IX5-mIL12 virus and not the control TAV-IX5-Empty virus. Bars depict the mean IL-12 level of triplicate samples and error bars depict standard deviation.

DETAILED DESCRIPTION

[0074] The invention is based, in part, upon the discovery that recombinant adenoviruses with one or more nucleotide sequences inserted between two viral transcription units in the viral

genome can efficiently replicate and express the nucleotide sequences in targeted cells or tissues.

I. Recombinant Adenovirus

[0075] Adenoviruses are non-enveloped and icosahedral viruses composed of a nucleocapsid and a double-stranded linear DNA genome. Adenoviruses replicate in the nucleus of mammalian cells using the host's replication machinery. The term "adenovirus" refers to any virus in the genus Adenoviridiae including, but not limited to, human, bovine, ovine, equine, canine, porcine, murine, and simian adenovirus subgenera. In particular, human adenoviruses includes the A-F subgenera as well as the individual serotypes thereof, the individual serotypes and A-F subgenera including but not limited to human adenovirus types 1, 2, 3, 4, 4a, 5, 6, 7, 8, 9, 10, 11 (Ad11a and Ad11p), 12, 13, 14, 15, 16, 17, 18, 19, 19a, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 34a, 35, 35p, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, and 91. The term bovine adenoviruses includes, but is not limited to, bovine adenovirus types 1, 2, 3, 4, 7, and 10. The term canine adenoviruses includes, but is not limited to, canine types 1 (strains CLL, Glaxo, RI261, Utrect, Toronto 26-61) and 2. The term equine adenoviruses includes, but is not limited to, equine types 1 and 2. The term porcine adenoviruses includes, but is not limited to, porcine types 3 and 4.

[0076] In some embodiments, provided are recombinant viruses derived from human adenovirus types 5 and 35. The terms "viral vector" and "virus" are used interchangeably herein to refer to any of the obligate intracellular parasites having no protein-synthesizing or energy-generating mechanism.

[0077] The adenovirus replication cycle has two phases: an early phase, during which transcription units E1A, E1B, E2A, E2B, E3, and E4 are expressed. The proteins coded for by genes within these transcription units are mostly involved in regulation of viral transcription, in replication of viral DNA, and in suppression of the host response to infection. The L1-L5 transcription units are transcribed later in the viral reproductive cycle, and code mostly for proteins that make up components of the viral capsid or are involved in assembly of the capsid. The L1-L5 transcription units are expressed primarily from the major late promoter (MLP).

[0078] The general structure of the mature Adenovirion is conserved among different Adenoviral species. The Adenoviral capsid is composed of three major proteins (II, III, and IV) and five minor proteins, VI, VIII, IX, IIIa, and IVa2. "IVa2 gene" used herein refers to the gene

encoding the IVa2 protein, modified versions, and/or fragment thereof. "IX gene" used herein refers to the gene encoding the IX protein, modified versions, and/or fragment thereof.

[0079] A schematic representation of the Ad5 genome and a detail of the E4 gene are shown in FIG. 1. Primary transcripts from E4 are subject to alternative splicing events and are predicted to encode seven different polypeptides: ORF1, ORF2, ORF3, ORF3/4, ORF4, ORF5, ORF6, and ORF6/7. (Leppard et al., Journal of General Virology, 78:2131–8 (1997)) "ORF" is used herein to refer to either the polypeptide or the nucleotide sequence encoding the polypeptide, modified versions, and/or fragment thereof.

[0080] In addition, the fiber protein (also known as protein IV or SPIKE) forms spikes that protrude from each vertex of the icosahedral capsid. "Fiber gene" used herein refers to the gene encoding the fiber protein, also known as L5 gene, modified versions, and/or fragment thereof.

A. Insertion Sites

[0081] In one aspect, the invention provides a recombinant adenovirus comprising a nucleotide sequence inserted in an insertion site, wherein the insertion site is located between the stop codon of a first viral transcription unit and the stop codon of a second viral transcription unit, wherein the stop codon of the first viral transcription unit is nearer to the stop codon of the second viral transcription unit than the start site of the first viral transcription unit is to the stop codon of the second viral transcription unit, wherein the stop codon of the second viral transcription is nearer to the stop codon of the first viral transcription unit than the start site of the second viral transcription unit is to the stop codon of the first viral transcription unit. In some embodiments, the first viral transcription unit and the second viral transcription unit are adjacent to each other in the adenoviral genome, e.g., there is no viral transcription unit between the first viral transcription unit and the second viral transcription unit before the nucleotide sequence is inserted.

[0082] The term "viral transcription unit" used herein refers a linear sequence of nucleotide sequence that extends from a transcription start site to a transcription stop site in the viral genome. The viral transcription unit may be naturally occurring, modified, or fragment thereof. The terms "viral transcription unit" and "virus gene" are used interchangeably herein.

[0083] In certain embodiments, the recombinant adenovirus is a human adenovirus. In some embodiments, the recombinant adenovirus is a human adenovirus type 1, 2, 3, 4, 4a, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 19a, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 34a, 35, 35p, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, or 91. In some

embodiment, the recombinant adenovirus is a type 5 adenovirus (Ad5) or a type 35 adenovirus (Ad35).

[0084] In certain embodiments, the first viral transcription unit is adenovirus IX gene and the second viral transcription unit is adenovirus IVa2 gene. In certain embodiments, the first viral transcription unit is adenovirus fiber gene and the second viral transcription unit is ORF6 or ORF6/7 of adenovirus E4 gene.

[0085] In certain embodiments, the insertion site is the IX-E2 insertion site. In certain embodiments, the IX-E2 insertion site is located between the stop codon of adenovirus IX gene and the stop codon of adenovirus IVa2 gene. In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4093 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4050, nucleotides corresponding to 4051 and 4070, or nucleotides corresponding to 4071 and 4093 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to 3899 and 3970 of the Ad35 genome (SEQ ID NO: 41). In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to 3899 and 3920, nucleotides corresponding to 3920 and 3940, or nucleotides corresponding to 3940 and 3970 of the Ad35 genome (SEQ ID NO: 41).

[0086] In some embodiments, the IX-E2 insertion site has at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% identity to nucleotides corresponding to 4029 and 4093 of the Ad5 genome (SEQ ID NO: 1). In some embodiments, the IX-E2 insertion site has at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% identity to nucleotides corresponding to 3899 and 3970 of the Ad35 genome (SEQ ID NO: 41).

[0087] In certain embodiments, the insertion site is an L5-E4 insertion site. In certain embodiments, the L5-E4 insertion site is located between the stop codon of adenovirus fiber gene and the stop codon of ORF6 or ORF6/7 of the adenovirus E4 gene. In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to 32785 to 32916 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to 32785 and 32800, nucleotides corresponding to 32801 and 32820, nucleotides corresponding to 32821 and 32840, nucleotides corresponding to 32841 and 32860, nucleotides corresponding to 32861 and 32880, nucleotides corresponding to 32881 and 32900, or nucleotides corresponding to 32901 and 32916 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to 31799 and 31821 of the Ad35 genome (SEQ ID NO: 41). In

certain embodiments, the nucleotide sequence is inserted between nucleotides corresponding to 31799 and 32810, or nucleotides corresponding to 32810 and 31821 of the Ad35 genome (SEQ ID NO: 41).

[0088] In some embodiments, the L5-E4 insertion site has at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% identity to nucleotides corresponding to 32785 to 32916 of the Ad5 genome (SEQ ID NO: 1). In some embodiments, the L5-E4 insertion site has at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% identity to nucleotides corresponding to 31799 and 31821 of the Ad35 genome (SEQ ID NO: 41).

[0089] Recombinant adenoviruses with insertions of exogenous nucleotide sequence in the IX-E2 insertion site and/or the L5-E4 insertion site have not been previously described. Such recombinant adenoviruses unexpectedly show very good tumor selective expression in tumor cells compared with in normal cells. In one aspect, the invention provides a method of expressing native proteins. In another aspect, the invention provides a method of expressing native structure, sush as dimeric or multimeric proteins.

[0090] In another aspect, the invention provides a method of expressing two or more therapeutic transgenes in a target cell. The method comprises exposing the cell to an effective amount of the recombinant virus described herein to express the target transgenes.

[0091] In certain embodiments, the nucleotide sequence comprises at least one transgene. In certain embodiments, the nucleotide sequence further comprises a promoter, wherein the transgene is operably linked to the promoter.

[0092] In certain embodiments, the recombinant adenovirus comprises, in a 5' to 3' orientation: (i) a first polyadenylation signal; (ii) a promoter; (iii) a transgene; (iv) a second polyadenylation signal; and (v) a third polyadenylation signal; wherein the transgene is operably linked to the promoter. In some embodiments, the nucleotide sequence, the first nucleotide sequence, and/or the second nucleotide sequence is inserted between the first polyadenylation signal and the third polyadenylation signal. In certain embodiments, wherein the second polyadenylation signal is in the opposite transcriptional direction of the third polyadenylation signal. In certain embodiments, the nucleotide sequence is inserted in the L5-E4 insertion site, and the first polyadenylation signal is the polyadenylation signal of the fiber (L5) gene, the second polyadenylation signal is the polyadenylation signal of the transgene, and the third polyadenylation signal is the polyadenylation signal of the ORF6 or ORF6/7 of the adenovirus E4 gene. In certain embodiments, the nucleotide sequence is inserted in the IX-E2 insertion site, and the first polyadenylation signal is the polyadenylation signal of the IX

gene, the second polyadenylation signal is the polyadenylation signal of the transgene, and the third polyadenylation signal is the polyadenylation signal of the adenovirus IVa2 gene.

[0093] In certain embodiments, the recombinant adenovirus comprises, in a 5' to 3' orientation: (i) a first polyadenylation signal; (ii) a second polyadenylation signal; (iii) a promoter; (iv) a transgene; (v) a third polyadenylation signal; and (vi) a fourth polyadenylation signal, and the transgene is operably linked to the promoter. In some embodiments, the nucleotide sequence, the first nucleotide sequence, and/or the second nucleotide sequence is inserted between the first polyadenylation signal and the fourth polyadenylation signal. In certain embodiments, wherein the second polyadenylation signal is in the opposite transcriptional direction of the first polyadenylation signal. In certain embodiments, wherein the fourth polyadenylation signal is in the opposite transcriptional direction of the third polyadenylation signal. In certain embodiments, the nucleotide sequence is inserted in the L5-E4 insertion site, and the first polyadenylation signal is the polyadenylation signal of the fiber (L5) gene, the third polyadenylation signal is the polyadenylation signal of the transgene, and the fourth polyadenylation signal is the polyadenylation signal of the ORF6 or ORF6/7 of the adenovirus E4 gene. In certain embodiments, the nucleotide sequence is inserted in the IX-E2 insertion site, and the first polyadenylation signal is the polyadenylation signal of the IX gene, the third polyadenylation signal is the polyadenylation signal of the transgene, and the fourth polyadenylation signal is the polyadenylation signal of the adenovirus IVa2 gene.

[0094] The term "promoter" is used herein in its ordinary sense to refer to a nucleotide region comprising a DNA regulatory sequence, wherein the regulatory sequence is derived from a gene which is capable of binding RNA polymerase and initiating transcription of a downstream (3'-direction) coding sequence.

[0095] "Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, control elements operably linked to a coding sequence are capable of affecting the expression of the coding sequence. The control elements need not be contiguous with the coding sequence, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

[0096] In certain embodiments, the promoter is a ubiquitous promoter, a tissue-specific promoter, or tumor-specific promoter.

[0097] In some embodiments, the transgene is operably linked to a ubiquitous promoter, such as βAct promoter, EF1 promoter, EGR1 promoter, eIF4A1 promoter, FerH promoter, FerL promoter, GAPDH promoter, GRP78 promoter, GRP94 promoter, HSP70 promoter, β-Kin promoter, PGK-1 promoter, ROSA promoter, Ubiquitin B promoter, SV40 promoter, or CMV promoter. In one embodiment, high-level constitutive expression will be desired. Examples of useful constitutive promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer) (see, e.g. Boshart et al, Cell, 41:521-530 (1985)), the SV40 promoter, the dihydrofolate reductase promoter, the β-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1α promoter (Invitrogen). Inducible promoters, regulated by exogenously supplied compounds, are also useful and include, the zinc-inducible sheep metallothionine (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, the T7 polymerase promoter system (WO 98/10088); the ecdysone insect promoter (No et al. Proc. Natl. Acad. Sci. USA, 93:3346-3351 (1996)), the tetracycline-repressible system (Gossen et al, Proc. Natl. Acad Sci. USA, 89:5547-5551 (1992)), the tetracycline-inducible system (Gossen et al, Science. 268:1766-1769 (1995), see also Harvey et al, Curr. Opin. Chem. Biol., 2:512-518 (1998)), the RU486-inducible system (Wang et al, Nat. Biotech., 15:239-243 (1997) and Wang et al, Gene Ther., 4:432-441 (1997)) and the rapamycin-inducible system (Magari et al, J. Clin. Invest., 100:2865-2872 (1997)). Other types of inducible promoters which may be useful in this context are those which are regulated by a specific physiological state, e.g., temperature, acute phase, a particular differentiation state of the cell, or in replicating cells only.

[0098] In another embodiment, a native promoter for the transgene will be used. The native promoter may be preferred when it is desired that expression of the transgene should mimic the native expression. The native promoter may be used when expression of the transgene must be regulated temporally or developmentally, or in a tissue-specific manner, or in response to specific transcriptional stimuli. In a further embodiment, other native expression control elements, such as enhancer elements, polyadenylation sites or Kozak consensus sequences may also be used to mimic the native expression.

[0099] Another embodiment of the transgene includes a transgene operably linked to a tissue-specific promoter, such as B29 promoter (B cells), CD14 promoter (Monocytic cells), CD43 promoter (Leukocytes & platelets), CD45 promoter (Haematopoietic cells), CD68 promoter (Macrophages), Desmin promoter (Muscle), Elastase-1 promoter (Pancreatic acinar cells),

Endoglin promoter (Endothelial cells), Endoglin promoter (Endothelial cells), Flt-1 promoter (Endothelial cells) GFAP promoter (Astrocytes), GPIIb promoter (Megakaryocytes), ICAM-2 promoter (Endothelial cells), mouse INF-β promoter (Hematopoietic cells), Mb promoter (Muscle), NphsI promoter (Podocytes), OG-2 promoter (Osteoblasts, Odonblasts), SP-B promoter (Lung), SYN1 promoter (Neurons), WASP promoter (Hematopoietic cells), SV40/bAlb promoter (Liver), or SV40 / hAlb promoter (Liver). Tissue-specific promoters are active in a specific type of cells or tissues. For instance, if expression in skeletal muscle is desired, a promoter active in muscle should be used. These include the promoters from genes encoding skeletal α-actin, myosin light chain 2A, dystrophin, muscle creatine kinase, as well as synthetic muscle promoters with activities higher than naturally-occurring promoters (see Li et al., Nat. Biotech., 17:241-245 (1999)). Examples of promoters that are tissue-specific are known for liver (albumin, Miyatake et al. J. Virol. 71:5124-32 (1997); hepatitis B virus core promoter, Sandig et al., Gene Ther., 3:1002-9 (1996); alpha-fetoprotein (AFP). Arbuthnot et al., Hum. Gene Ther., 7:1503-14 (1996)), bone osteocalcin (Stein et al., Mol. Biol. Rep., 24:185-96 (1997)), bone sialoprotein (Chen et al., J. Bone Miner. Rep., 11:654-64 (1996)), lymphocytes (CD2, Hansal et al., J. Immumnol., 161:1063-8 (1998); immunogllobulin heavy chain; T cell receptor a chain), neuronal such as neuron-specific enolase (NSE) promoter (Andersen et al., Cell. Mol. Neurobiol., 13:503-15 (1993)), neurofilament light-chain gene (Piccioli et al., Proc. Natl. Acad. Sci. USA, 88:5611-5 (1991)), and the neuron-specific vgf gene (Piccioli et al., Neuron. 15:373-84 (1995)), among others.

[00100] Another embodiment of the transgene includes a transgene operably linked to a tumor-specific promoter, such as AFP promoter (Hepatocellular carcinoma), CCKAR promoter (Pancreatic cancer), CEA promoter (Epithelial cancers), c-erbB2 promoter (Breast & pancreas cancer), COX-2 promoter (Tumor), E2F-1 promoter (Tumor), HE4 promoter (Tumor), LP promoter (Tumor), MUC1 promoter (Carcinoma cells), PSA promoter (Prostate and prostate cancers), Survivin promoter (Tumor), TRP1 promoter (Melanocytes & melanoma), Tyr promoter (Melanocytes & melanoma), CXCR4 promoter (Tumor), or AFP/hAFP promoter (Hepatocellular carcinoma). Tumor-specific promoter are active specifically in tumor cells.

[00101] In certain embodiments, the nucleotide sequence further comprises a consensus Kozak sequence. In certain embodiments, the recombinant adenovirus comprises a partial or complete deletion of the nucleotide sequence encoding the adenoviral death protein (ADP).

[00102] In certain embodiments, the invention provides a recombinant adenovirus comprising a first nucleotide sequence inserted in an IX-E2 insertion site and a second nucleotide sequence inserted in an L5-E4 insertion site. These embodiments enable the adenoviruses to express two or more separate exogenous transgenes. This approach has certain advantages over adenoviruses expressing a fusion protein comprising two transgenes with a self-cleavable linker joining them because the cleaved linker may be potentially immunogenic.

[00103] In certain embodiments, the recombinant adenovirus comprises, in a 5' to 3' orientation: (i) a first polyadenylation signal; (ii) a promoter; (iii) a first nucleotide sequence comprising a first transgene; (iv) a linker; (v) a second nucleotide sequence comprising a second transgene; (vi) a second polyadenylation signal; and (vii) a third polyadenylation signal; wherein the transgene is operably linked to the promoter. In certain embodiments, wherein the second polyadenylation signal is in the opposite transcriptional direction of the third polyadenylation signal. In certain embodiments, the recombinant adenovirus comprises, in a 5' to 3' orientation: (i) a first polyadenylation signal; (ii) a second polyadenylation signal; (iii) a promoter; (iv) a first nucleotide sequence comprising a first transgene; (v) a linker; (vi) a second nucleotide sequence comprising a second transgene; (vii) a third polyadenylation signal; and (viii) a fourth polyadenylation signal; wherein the transgene is operably linked to the promoter. In certain embodiments, wherein the second polyadenylation signal is in the opposite transcriptional direction of the first polyadenylation signal. In certain embodiments, wherein the fourth polyadenylation signal is in the opposite transcriptional direction of the third polyadenylation signal.

[00104] In certain embodiments, the IX-E2 insertion site comprises a deletion of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, or 60 nucleotides. In certain embodiments, the L5-E4 insertion site comprises a deletion of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, or 130 nucleotides.

[00105] In certain embodiments, the recombinant adenovirus further comprises a nucleotide sequence inserted in an E1b-19K insertion site, an E3 insertion site, or an E4 insertion site. In certain embodiments, the E1b-19K insertion site is located between the start site of Elb-19K and the start site of Elb-55K. In certain embodiments, the E3 insertion site is located between the stop codon of adenovirus pVIII gene and the start site of adenovirus Fiber gene (L5). In certain embodiments, an E4 insertion site is located between the start codon of ORF1 to the stop codon of ORF6/7 of the adenovirus E4 gene.

[00106] In certain embodiments, the recombinant adenovirus further comprises a first nucleotide sequence inserted in the IX-E2 insertion site and a second nucleotide sequence inserted in the E1b-19K insertion site. In certain embodiments, the recombinant adenovirus further comprises a first nucleotide sequence inserted in the IX-E2 insertion site and a second nucleotide sequence inserted in the E3 insertion site. In certain embodiments, the recombinant adenovirus further comprises a first nucleotide sequence inserted in the IX-E2 insertion site and a second nucleotide sequence inserted in the E4 insertion site.

[00107] In certain embodiments, the recombinant adenovirus further comprises a first nucleotide sequence inserted in the L5-E4 insertion site and a second nucleotide sequence inserted in the E1b-19K insertion site. In certain embodiments, the recombinant adenovirus further comprises a first nucleotide sequence inserted in the L5-E4 insertion site and a second nucleotide sequence inserted in the E3 insertion site. In certain embodiments, the recombinant adenovirus further comprises a first nucleotide sequence inserted in the L5-E4 insertion site and a second nucleotide sequence inserted in the E4 insertion site.

[00108] In certain embodiments, the recombinant adenovirus further comprises a first nucleotide sequence inserted in the IX-E2 insertion site and a second nucleotide sequence inserted in the L5-E4 insertion site, and a third nucleotide sequence inserted in the E1b-19K insertion site, the E3 insertion site, or the E4 insertion site.

[00109] The adenoviral Elb-19k gene functions primarily as an anti-apoptotic gene and is a homolog of the cellular anti-apoptotic gene, BCL-2. Since host cell death prior to maturation of the progeny viral particles would restrict viral replication, Elb-19k is expressed as part of the El cassette to prevent premature cell death thereby allowing the infection to proceed and yield mature virions. Accordingly, in certain embodiments, a recombinant virus is provided that includes an Elb-19K insertion site, e.g., the adenovirus has an exogenous nucleotide sequence inserted into an Elb-19K insertion site. In certain embodiments, the insertion site is located between the start site of Elb-19K and the stop codon of Elb-19K.

[00110] In certain embodiments, the E1b-19K insertion site comprises a deletion of from about 100 to about 305, about 100 to about 300, about 100 to about 250, about 100 to about 200, about 100 to about 150, about 150 to about 305, about 150 to about 300, about 150 to about 250, or about 150 to about 200 nucleotides adjacent to the start site of E1b-19K. In certain embodiments, the E1b-19K insertion site comprises a deletion of about 200 nucleotides, e.g., 202 nucleotides adjacent to the start site of E1b-19K. In certain embodiments, the E1b-19K insertion site comprises a deletion corresponding to nucleotides 1714-1917 of the Ad5

genome (SEQ ID NO: 1), or, an exogenous nucleotide sequence encoding a transgene is inserted between nucleotides corresponding to 1714 and 1917 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, an exogenous nucleotide sequence encoding a transgene is inserted between CTGACCTC (SEQ ID NO: 3) and TCACCAGG (SEQ ID NO: 2), e.g., the recombinant adenovirus comprises, in a 5' to 3' orientation, CTGACCTC (SEQ ID NO: 3), an exogenous nucleotide sequence encoding a transgene, and TCACCAGG (SEQ ID NO: 2). In certain embodiments, the E1b-19K insertion site comprises a deletion corresponding to nucleotides 1611-2153 or 1611-1915 of the Ad35 genome (SEQ ID NO: 41).

[00111] In certain embodiments, the E1b-19K insertion site comprises a deletion of from about 100 to about 305, about 100 to about 300, about 100 to about 250, about 100 to about 200, about 100 to about 150, about 150 to about 305, about 150 to about 300, about 150 to about 250, or about 150 to about 200 nucleotides adjacent the start site of E1b-19K. In certain embodiments, the E1b-19K insertion site comprises a deletion of about 200 nucleotides, e.g., 202 nucleotides adjacent the start site of E1b-19K. In certain embodiments, the E1b-19K insertion site comprises a deletion corresponding to nucleotides 1714-1917 of the Ad5 genome (SEQ ID NO: 1), or the first therapeutic transgene is inserted between nucleotides corresponding to 1714 and 1917 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the first therapeutic transgene is inserted between CTGACCTC (SEQ ID NO: 3) and TCACCAGG (SEQ ID NO: 2), e.g., the recombinant adenovirus comprises, in a 5' to 3' orientation, CTGACCTC (SEQ ID NO: 3), the first therapeutic transgene, and TCACCAGG (SEQ ID NO: 2).

[00112] In certain embodiments, the E3 insertion site comprises a deletion of from about 500 to about 3185, from about 500 to about 3000, from about 500 to about 2500, from about 500 to about 2000, from about 500 to about 1500, from about 500 to about 1000, from about 1000 to about 3185, from about 1000 to about 3000, from about 1000 to about 2500, from about 1000 to about 2000, from about 1000 to about 1500, from about 1500 to about 3185, from about 1500 to about 3000, from about 1500 to about 2000, from about 2000 to about 3185, from about 2000 to about 3000, from about 2000 to about 2500, from about 2500 to about 3185, from about 2500 to about 3000, or about 3000 to about 3185 nucleotides. In certain embodiments, the E3 insertion site is located between the stop codon of E3-10.5K and the stop codon of E3-14.7K. In certain embodiments, the E3 insertion site comprises a deletion of from about 500 to about 1551, from about 500 to about 1500, from about 500 to about 1000, from about 1000 to about 1551, from about 1000 to about 1500, or from about 1500 to about 1551

nucleotides adjacent the stop codon of E3-10.5K. In certain embodiments, the E3 insertion site comprises a deletion of about 1050 nucleotides adjacent the stop codon of E3-10.5K, e.g., the E3 insertion site comprises a deletion of 1063 nucleotides adjacent the stop codon of E3-10.5K. In certain embodiments, the E3 insertion site comprises a deletion corresponding to the Ad5 dl309 E3 deletion. In certain embodiments, the E3 insertion site comprises a deletion corresponding to nucleotides 29773-30836 of the Ad5 genome (SEQ ID NO: 1), or the second therapeutic transgene is inserted between nucleotides corresponding to 29773 and 30836 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the E3 insertion site comprises a deletion corresponding to nucleotides 27199-30622 of the Ad35 genome (SEQ ID NO: 41).

[00113] In certain embodiments, an E4 insertion site comprises any one of the ORF of the E4 gene, i.e., between the start codon of ORF1 to the stop codon of ORF6/7. For example, a nucleotide sequence can be inserted in E4 ORF1, and/or E4 ORF2. In certain embodiments, portions of or the entire E4 region may be deleted. In certain embodiments, in any of the foregoing viruses, the recombinant adenovirus further comprises an E4 deletion. In certain embodiments, the E4 deletion is located between the start site of E4-ORF6/7 (*i.e.,* the nucleotide sequence encoding the start codon of E4-ORF6/7, *e.g.,* corresponding to nucleotides 34075-34077 of SEQ ID NO: 1) and the right inverted terminal repeat (ITR; *e.g.,* corresponding to nucleotides 35836-35938 of SEQ ID NO: 1). In certain embodiments, the E4 deletion is located between the start site of E4-ORF6/7 and the start site of E4-ORF1 (*i.e.,* the nucleotide sequence encoding the start codon of E4-ORF1, *e.g.,* corresponding to nucleotides 35524-35526 of SEQ ID NO: 1). In certain embodiments, the E4 deletion comprises a deletion of a nucleotide sequence between the start site of E4-ORF6/7 and the start site of E4-ORF1. In certain embodiments, the E4 deletion comprises a deletion of from about 500 to about 2500, from about 500 to about 2000, from about 500 to about 1500, from about 500 to about 1000, from about 1000 to about 2500, from about 1000 to about 2000, from about 1000 to about 1500, from about 1500 to about 2500, from about 1500 to about 2000, or from about 2000 to about 2500 nucleotides. In certain embodiments, the E4 deletion comprises a deletion of from about 250 to about 1500, from about 250 to about 1250, from about 250 to about 1000, from about 250 to about 750, from about 250 to about 500, from 500 to about 1500, from about 500 to about 1250, from about 500 to about 1000, from about 500 to about 750, from 750 to about 1500, from about 750 to about 1250, from about 750 to about 1000, from about 1000 to about 1500, or from about 1000 to about 1250 nucleotides adjacent the start site of E4-ORF6/7. In certain embodiments, the E4 deletion comprises a deletion of about 1450 nucleotides adjacent

the start site of E4-ORF6/7, e.g., the E4 deletion comprises a deletion of about 1449 nucleotides adjacent the start site of E4-ORF6/7. In certain embodiments, the E4 deletion comprises a deletion corresponding to nucleotides 34078-35526 or 34083-35541 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the E4 deletion comprises a deletion corresponding to nucleotides 33004-34422 or 31827-34415 of the Ad35 genome (SEQ ID NO: 41).

B. Modified Transcriptional Control Region

[00114] Previously developed oncolytic viruses include the oncolytic serotype 5 adenovirus (Ad5) referred to as TAV-255 in PCT Publication No. WO2010/101921 which is transcriptionally attenuated in normal cells but transcriptionally active in cancer cells. It is believed that the mechanism by which the TAV-255 vector achieves this tumor selectivity is through targeted deletion of three transcriptional factor (TF) binding sites for the transcription factors Pea3 and E2F, proteins that regulate adenovirus expression of E1a, the earliest gene to be transcribed after virus entry into the host cell, through binding to specific DNA sequences. These three Pea3 and E2F deletions attenuate replication in growth-arrested, normal cells but not in malignant ones, indicating that these DNA sequences are only dispensable for transcriptional regulation and growth in cancer cells.

[00115] In certain embodiments, any of the foregoing recombinant adenoviruses comprises a modified E1a regulatory sequence. In certain embodiments, the recombinant adenovirus comprises an E1a promoter having a deletion of a functional Pea3 binding site. For example, the virus may comprise a deletion of nucleotides corresponding to about -300 to about -250 upstream of the initiation site of Ela or a deletion of nucleotides corresponding to -305 to -255 upstream of the initiation site of Ela. In certain embodiments, the deletion comprises a deletion of nucleotides corresponding to 195-244 of the Ad5 genome (SEQ ID NO: 1), and/or the E1a promoter comprises the sequence GGTGTTTTGG (SEQ ID NO: 4).

[00116] In certain embodiments, the recombinant adenovirus comprises a modified TATA box-based promoter operably linked to a gene, wherein the modified TATA box-based promoter lacks a functional TATA box and permits selective expression of the gene in a hyperproliferative cell and/or a modified CAAT box-based promoter operably linked to a gene, wherein the modified CAAT box-based promoter lacks a functional CAAT box and permits selective expression of the gene in a hyperproliferative cell.

[00117] In certain embodiments, wherein the modified TATA box-based promoter is an early gene promoter. In certain embodiments, the modified TATA box-based promoter is an

E1a promoter, E1b promoter, or E4 promoter. In certain embodiments, the modified TATA box-based promoter is an E1a promoter.

[00118] In certain embodiments, the modification included in the modified TATA box-based promoter comprises a deletion of the entire TATA box. In certain embodiments, the recombinant adenovirus comprises a deletion of nucleotides corresponding to -27 to -24, -31 to -24, -44 to +54, or -146 to +54 of the E1a promoter. In certain embodiments, the deletion comprises a deletion of nucleotides corresponding to 472 to 475, 468 to 475, 455 to 552, or 353 to 552 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the deletion comprises a deletion of nucleotides corresponding 477 to 484 of the Ad35 genome (SEQ ID NO: 41).

[00119] In certain embodiments, the recombinant adenovirus comprises a polynucleotide deletion that results in a virus comprising the sequence CTAGGACTG (SEQ ID NO: 5), AGTGCCCG (SEQ ID NO: 44) and/or TATTCCCG (SEQ ID NO: 45).

[00120] In certain embodiments, the modified CAAT box-based promoter is an early gene promoter. In certain embodiments, the modified CAAT box-based promoter is an E1a promoter, E1b promoter, or E4 promoter. In certain embodiments, the modified CAAT box-based promoter is an E1a promoter.

[00121] In certain embodiments, the modification included in the modified CAAT box-based promoter comprises a deletion of the entire CAAT box. In certain embodiments, the recombinant adenovirus comprises a deletion of nucleotides corresponding to -76 to -68 of the E1a promoter.

[00122] In certain embodiments, the recombinant adenovirus comprises a deletion of nucleotides corresponding to 423 to 431 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the recombinant adenovirus comprises a polynucleotide deletion that results in a virus comprising the sequence TTCCGTGGCG (SEQ ID NO: 46). In certain embodiments, the recombinant adenovirus comprises a deletion of nucleotides corresponding to 477 to 484 of the Ad35 genome (SEQ ID NO: 41).

[00123] In certain embodiments, the invention provides a method of expressing two therapeutic transgenes, when expressed, produce a single polypeptide chain, which may be cleaved posttranslationally into two polypeptide chains. In certain embodiments, the recombinant adenovirus further comprises the nucleotide sequence comprises a first nucleotide sequence comprising a first transgene and a second nucleotide sequence comprising a second transgene, wherein the first nucleotide sequence and the second nucleotide sequence are

separated by a linker. In certain embodiments, the linker encodes a peptide cleavable by a protease or proteases. In certain embodiments, the linker encodes an internal ribosome entry site (IRES). The IRES may, e.g., be selected from the group consisting of the encephalomyocarditis virus IRES, the foot-and-mouth disease virus IRES, and the poliovirus IRES. In certain embodiments, the nucleotide sequence is inserted in the IX-E2 insertion or the L5-E4 insertion site, wherein the recombinant adenovirus further comprise a third nucleotide sequence inserted in an E1b-19K insertion site, an E3 insertion site, or an E4 insertion site.

[00124] In certain embodiments, the virus has one or more modifications to a regulatory sequence or promoter. A modification to a regulatory sequence or promoter comprises a deletion, substitution, or addition of one or more nucleotides compared to the wild-type sequence of the regulatory sequence or promoter.

[00125] In one embodiment, the modification of a regulatory sequence or promoter comprises a modification of sequence of a transcription factor binding site to reduce affinity for the transcription factor, for example, by deleting a portion thereof, or by inserting a single point mutation into the binding site. In certain embodiments, the additional modified regulatory sequence enhances expression in neoplastic cells but attenuates expression in normal cells.

[00126] The Ela regulatory sequence contains five binding sites for the transcription factor Pea3, designated Pea3 I, Pea3 II, Pea3 III, Pea3 IV, and Pea3 V, where Pea3 I is the Pea3 binding site most proximal to the Ela start site, and Pea3 V is most distal. The Ela regulatory sequence also contains binding sites for the transcription factor E2F, hereby designated E2F I and E2F II, where E2F I is the E2F binding site most proximal to the Ela start site, and E2F II is more distal. From the Ela start site, the binding sites are arranged: Pea3 I, E2F I, Pea3 II, E2F II, Pea3 III, Pea3 IV, and Pea3 V.

[00127] In one embodiment, at least one of these seven binding sites, or a functional binding site, is deleted. As used herein, a "functional binding site" refers to a binding site that is capable of binding to a respective binding partner, e.g., a transcription factor, e.g., a binding site that has at least 100%, at least 90%, at least 80%, at least 70%, at least 60%, at least 50%, or at least 40%, of the binding activity of a corresponding wild-type binding site sequence. As used herein, a "non-functional binding site" refers to a binding site that, e.g., has less than 30%, less than 20%, less than 10%, or 0% of the binding activity of a corresponding wild-type binding site sequence.

[00128] In certain embodiments, the recombinant adenovirus comprises an E1a promoter having a deletion of a functional Pea3 binding site, e.g., the deletion of an entire Pea3

binding site. As used herein, a "functional Pea3 binding site" refers to a Pea3 binding site that is capable of binding to its respective transcription factor (e.g., Pea3), e.g., a Pea3 binding site that has at least 100%, at least 90%, at least 80%, at least 70%, at least 60%, at least 50%, or at least 40%, of the Pea3 binding activity of a corresponding wild-type Pea3 binding site sequence. As used herein, a "non-functional Pea3 binding site" refers to a Pea3 binding site that, e.g., has less than 30%, less than 20%, less than 10%, or 0% of the Pea3 binding activity of a corresponding wild-type Pea3 binding site sequence. Assays for determining whether a Pea3 binding site binds to Pea3 are known in the art. Exemplary binding assays include electrophoretic mobility shift assays, chromatin immunoprecipitation assays, and DNAse footprinting assays.

[00129] In one embodiment, at least one Pea3 binding site, or a functional Pea3 binding site, is deleted. The deleted Pea3 binding site can be Pea3 I, Pea3 II, Pea3 III, Pea3 IV, and/or Pea3 V. In one embodiment, the deleted Pea3 binding site is Pea3 II, Pea3 III, Pea3 IV, and/or Pea3 V. In another embodiment, the deleted Pea3 binding site is Pea3 IV and/or Pea3 V. In another embodiment, the deleted Pea3 binding site is Pea3 II and/or Pea3 III. In another embodiment, the deleted Pea3 binding site is both Pea3 II and Pea3 III. In another embodiment, the Pea3 I binding site, or a functional Pea3 I binding site, is retained.

[00130] In one embodiment, at least one E2F binding site, or a functional E2F binding site, is deleted. In another embodiment, at least one E2F binding site, or a functional E2F binding site, is retained. In one embodiment, the retained E2F binding site is E2F I and/or E2F II. In another embodiment, the retained E2F binding site is E2F II. In another embodiment, the total deletion consists essentially of one or more of Pea3 II, Pea3 III, Pea3 IV, and/or Pea3 V. In one embodiment, the virus has a deletion of a 50 base pair region located from -305 to -255 upstream of the Ela initiation site, e.g., corresponding to 195-244 of the Ad5 genome (SEQ ID NO: 1), hereafter referred to as the TAV-255 deletion. In certain embodiments, the TAV-255 deletion results in an E1a promoter that comprises the sequence GGTGTTTTGG (SEQ ID NO: 4).

[00131] In one embodiment, the recombinant adenovirus has the same or similar E1a modification as in the oncolytic serotype 5 adenovirus (Ad5) called TAV-255 described in PCT Publication No. WO2010101921 and US Publication No. 20160017294A1, each of which is incorporated by reference herein in its entirety. It is believed that the mechanism by which the TAV-255 vector achieves this tumor selectivity is through targeted deletion of three transcriptional factor (TF) binding sites for the transcription factors Pea3 and E2F, proteins that

regulate adenovirus expression of E1a, the earliest gene to be transcribed after virus entry into the host cell, through binding to specific DNA sequences. These three Pea3 and E2F deletions attenuate replication in growth-arrested, normal cells but not in malignant ones, indicating that these DNA sequences are only dispensable for transcriptional regulation and growth in cancer cells.

[00132] In certain embodiments, the recombinant adenovirus comprises an E1a promoter having one or more deletions of a functional Pea3 binding site. In certain embodiments, the deletion comprises a deletion of nucleotides corresponding to about -300 to about -250 upstream of the initiation site of Ela. In certain embodiments, wherein the deletion comprises a deletion of nucleotides corresponding to -305 to -255 upstream of the initiation site of Ela. In certain embodiments, the deletion comprises a deletion of nucleotides corresponding to 195-244 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the E1a promoter comprises the sequence GGTGTTTTGG (SEQ ID NO: 4).

[00133] In one embodiment, the recombinant adenovirus comprises one or more Pea3 transcription binding site deletions without one or more E2F transcription binding site deletions in the E1A region. In other embodiment, the recombinant adenovirus comprises one or more E2F transcription binding site deletions without one or more Pea3 transcription binding site deletions in the E1A region.

[00134] In certain embodiments, the recombinant oncolytic adenovirus comprises a modified TATA box-based promoter operably linked to a gene, wherein the modified TATA box-based promoter lacks a functional TATA box and permits selective expression of the gene in a hyperproliferative and/or non-growth arrested cell. As used herein, a "functional TATA box" refers to a TATA box that is capable of binding to a TATA box binding protein (TBP), e.g., a TATA box that has at least 100%, at least 90%, at least 80%, at least 70%, at least 60%, at least 50%, or at least 40%, of the TBP binding activity of a corresponding wild-type TATA box sequence. As used herein, a "non-functional TATA box" refers to a TATA box that, e.g., has less than 30%, less than 20%, less than 10%, or 0% of the TBP binding activity of a corresponding wild-type TATA box sequence. Assays for determining whether a TBP binds to a TATA box are known in the art. Exemplary binding assays include electrophoretic mobility shift assays, chromatin immunoprecipitation assays, and DNAse footprinting assays.

[00135] As used herein, a "modified TATA box" refers to a TATA box that has a deletion, substitution, or addition of one or more nucleotides relative to a wild-type TATA box sequence.

[00136] For example, the virus may comprise a deletion of nucleotides corresponding to -29 to -26, -33 to -26, -44 to +52, or -148 to +52 upstream of the initiation site of E1a. In certain embodiments, the deletion comprises a deletion of nucleotides corresponding to 353-552 of the Ad5 genome (SEQ ID NO: 1). In certain embodiments, the TATA box deletion results in an E1a promoter that comprises the sequence CTAGGACTG (SEQ ID NO: 5), AGTGCCCG (SEQ ID NO: 44) and/or TATTCCCG (SEQ ID NO: 45).

[00137] In certain embodiments, the recombinant oncolytic adenovirus comprises a modified CAAT box-based promoter operably linked to a gene, wherein the modified CAAT box-based promoter lacks a functional CAAT box and permits selective expression of the gene in a hyperproliferative cell and/or non-growth arrested. The TATA box-based promoter and the CAAT box-based promoter may be the same promoter (e.g., the Ad5 E1a promoter), or may be different promoters.

[00138] As used herein, "CAAT box" refers to a nucleotide sequence that is capable of binding to a C/EBP or NF-Y protein. A CAAT box typically comprises a consensus sequence of GG(T/C)CAATCT.

[00139] As used herein, a "modified CAAT box" refers to a CAAT box that has a deletion, substitution, or addition of one or more nucleotides relative to a wild-type CAAT box sequence.

[00140] As used herein, a "functional CAAT box" refers to a CAAT box that is capable of binding to a C/EBP or NF-Y protein, *e.g.,* a CAAT box that has at least 100%, at least 90%, at least 80%, at least 70%, at least 60%, at least 50%, or at least 40%, of the a C/EBP or NF-Y binding activity of a corresponding wild-type CAAT box sequence. As used herein, a "non-functional CAAT box" refers to a CAAT box that, *e.g.*, has less than 30%, less than 20%, less than 10%, or 0% of the a C/EBP or NF-Y binding activity of a corresponding wild-type CAAT box sequence. Assays for determining whether a C/EBP or NF-Y protein binds to a CAAT box are known in the art. Exemplary binding assays include electrophoretic mobility shift assays, chromatin immunoprecipitation assays, and DNAse footprinting assays.

[00141] As used herein, "CAAT box-based promoter" refers to any gene promoter that contains a CAAT box.

[00142] As used herein, a "modified CAAT box-based promoter" refers to a CAAT box-based promoter that has been modified by a deletion, substitution, or addition of one or more nucleotides relative to a wild-type CAAT box-based promoter. In certain embodiments, the modification included in the modified CAAT box-based promoter comprises a deletion of one

or more nucleotides of the wild-type CAAT box-based promoter sequence. In certain embodiments, the modification included in the modified CAAT box-based promoter consists of a deletion of one or more nucleotides of the wild-type CAAT box-based promoter sequence. In certain embodiments, the modification included in the modified CAAT box-based promoter comprises a deletion of the entire CAAT box of the wild-type CAAT box-based promoter sequence. In certain embodiments, the modification included in the modified CAAT box-based promoter consists of a deletion of the entire CAAT box of the wild-type CAAT box-based promoter sequence. In certain embodiments, the modification included in the modified CAAT box-based promoter comprises a deletion of the entire CAAT box-based promoter. In certain embodiments, the modification included in the modified CAAT box-based promoter consists of a deletion of the entire CAAT box-based promoter. In certain embodiments, the modification included in the modified CAAT box-based promoter does not comprise an addition of or a substitution with a separate, functional promoter sequence.

[00143] Nucleic acids encoding viral genes can be incorporated into plasmids and introduced into host cells through conventional transfection or transformation techniques. Specific production and purification conditions will vary depending upon the virus and the production system employed. For adenovirus, the traditional method for the generation of viral particles is co-transfection followed by subsequent in vivo recombination of a shuttle plasmid (usually containing a small subset of the adenoviral genome and optionally containing a potential transgene an expression cassette) and an adenoviral helper plasmid (containing most of the entire adenoviral genome). Alternative technologies for the generation of adenovirus include utilization of the bacterial artificial chromosome (BAC) system, in vivo bacterial recombination in a recA+ bacterial strain utilizing two plasmids containing complementary adenoviral sequences, and the yeast artificial chromosome (YAC) system.

[00144] In certain embodiments, a recombinant adenovirus of the invention is an oncolytic virus, e.g., a virus that exhibits tumor-selective replication and/or viral mediated lysis. In certain embodiments, a recombinant adenovirus of the invention exhibits selective expression of a therapeutic transgene in a hyperproliferative cell, e.g., a cancer cell, a tumor cell, relative to a nonhyperproliferative cell. In certain embodiments, the expression of a therapeutic transgene in a non-hyperproliferative cell is about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10%, or about 5% of the expression of the gene in the hyperproliferative cell. In certain embodiments, the virus exhibits

no detectable expression of a therapeutic transgene in a non-hyperproliferative cell. Therapeutic transgene expression may be
determined by any appropriate method known in the art, e.g., Western blot or ELISA. The hyperproliferative cell may be a cancer cell, e.g., a carcinoma, sarcoma, leukemia, lymphoma, prostate cancer, lung cancer, gastrointestinal tract cancer, colorectal cancer, pancreatic cancer, breast cancer, ovarian cancer, cervical cancer, stomach cancer, thyroid cancer, mesothelioma, liver cancer, kidney cancer, skin cancer, head and neck cancer, or brain cancer cell.

C. Transgenes

[00145] The recombinant adenovirus disclosed herein comprise one or more exogenous nucleotide sequences inserted in any of the foregoing insertion sites, e.g., an IX-E2 insertion site, an L5-E4 insertion site, an E1b-19K insertion site, an E3 insertion site, or an E4 insertion site.

[00146] In certain embodiments, the nucleotide sequence comprises at least one transgene. In certain embodiments, the nucleotide sequence further comprises a promoter, wherein the transgene is operably linked to the promoter.

[00147] In certain embodiments, the recombinant adenovirus comprises, in a 5' to 3' orientation: (i) a first polyadenylation signal; (ii) a promoter; (iii) a transgene; (iv) a second polyadenylation signal; and (v) a third polyadenylation signal; wherein the transgene is operably linked to the promoter. In some embodiments, the nucleotide sequence, the first nucleotide sequence, and/or the second nucleotide sequence is inserted between the first polyadenylation signal and the third polyadenylation signal. In certain embodiments, wherein the second polyadenylation signal is in the opposite transcriptional direction of the third polyadenylation signal. In certain embodiments, the nucleotide sequence is inserted in the L5-E4 insertion site, and the first polyadenylation signal is the polyadenylation signal of the L5 transcription unit, the second polyadenylation signal is the polyadenylation signal of the transgene, and the third polyadenylation signal is the polyadenylation signal of the E4 transcription unit. In certain embodiments, the nucleotide sequence is inserted in the IX-E2 insertion site, and the first polyadenylation signal is the polyadenylation signal of the IX transcription unit, the second polyadenylation signal is the polyadenylation signal of the transgene, and the third polyadenylation signal is the polyadenylation signal of the adenovirus IVa2 gene.

[00148] In certain embodiments, the recombinant adenovirus comprises, in a 5' to 3' orientation: (i) a first polyadenylation signal; (ii) a second polyadenylation signal; (iii) a

promoter; (iv) a transgene; (v) a third polyadenylation signal; and (vi) a fourth polyadenylation signal, and the transgene is operably linked to the promoter. In some embodiments, the nucleotide sequence, the first nucleotide sequence, and/or the second nucleotide sequence is inserted between the first polyadenylation signal and the fourth polyadenylation signal. In certain embodiments, wherein the second polyadenylation signal is in the opposite transcriptional direction of the first polyadenylation signal. In certain embodiments, wherein the fourth polyadenylation signal is in the opposite transcriptional direction of the third polyadenylation signal. In certain embodiments, the nucleotide sequence is inserted in the L5-E4 insertion site, and the first polyadenylation signal is the polyadenylation signal of the L5 transcription unit, the third polyadenylation signal is the polyadenylation signal of the transgene, and the fourth polyadenylation signal is the polyadenylation signal of the E4 transcription unit. In certain embodiments, the nucleotide sequence is inserted in the IX-E2 insertion site, and the first polyadenylation signal is the polyadenylation signal of the IX transcription unit, the third polyadenylation signal is the polyadenylation signal of the transgene, and the fourth polyadenylation signal is the polyadenylation signal of the adenovirus IVa2 gene.

[00149] In certain embodiments, the recombinant adenovirus further comprises the nucleotide sequence comprises a first nucleotide sequence comprising a first transgene and a second nucleotide sequence comprising a second transgene, wherein the first nucleotide sequence and the second nucleotide sequence are separated by a linker.

[00150] In certain embodiments, the nucleotide sequence comprises, in a 5' to 3' orientation: (i) a first polyadenylation signal; (ii) a promoter; (iii) a first nucleotide sequence comprising a first transgene; (iv) a linker; (v) a second nucleotide sequence comprising a second transgene; (vi) a second polyadenylation signal; and (vii) a third polyadenylation signal; wherein the transgene is operably linked to the promoter. In certain embodiments, wherein the second polyadenylation signal is in the opposite transcriptional direction of the third polyadenylation signal. In certain embodiments, the nucleotide sequence comprises, in a 5' to 3' orientation: (i) a first polyadenylation signal; (ii) a second polyadenylation signal; (iii) a promoter; (iv) a first nucleotide sequence comprising a first transgene; (v) a linker; (vi) a second nucleotide sequence comprising a second transgene; (vii) a third polyadenylation signal; and (viii) a fourth polyadenylation signal; wherein the transgene is operably linked to the promoter. In certain embodiments, wherein the second polyadenylation signal is in the opposite transcriptional direction of the first polyadenylation signal. In certain embodiments,

wherein the fourth polyadenylation signal is in the opposite transcriptional direction of the third polyadenylation signal.

[00151] In certain embodiments, the linker encodes a peptide cleavable by a protease or proteases. In certain embodiments, the linker encodes internal ribosome entry site (IRES) or a self-cleaving 2A peptide. The IRES may, e.g., be selected from the group consisting of the encephalomyocarditis virus IRES, the foot-and-mouth disease virus IRES, and the poliovirus IRES. In certain embodiments, wherein the nucleotide sequence is inserted in the IX-E2 insertion or the L5-E4 insertion site, wherein the recombinant adenovirus further comprise a third nucleotide sequence inserted in an E1b-19K insertion site, an E3 insertion site, or an E4 insertion site.

[00152] In certain embodiments, one or more of the nucleotide sequence, the first nucleotide sequence, the second nucleotide sequence, and the third nucleotide sequence comprises one or more transgenes.

[00153] In certain embodiments, one or more of the nucleotide sequence, the first nucleotide sequence, the second nucleotide sequence, and the third nucleotide sequence comprises:

a) a transcriptional initiation region;

b) a nucleotide sequence comprising a transgene, wherein the transgene is under transcriptional control of the transcriptional initiation region; and

c) a transcriptional termination region.

[00154] In some embodiments, the transcriptional initiation region comprises a promoter.

[00155] In certain embodiments, one or more of the transgenes, the first transgene, and the second transgene encodes a monomeric, dimeric, trimeric, tetrameric, or multimeric protein, or a part thereof. In certain embodiments, one or more of the transgene, the first transgene, and the second transgene encodes a RNA that has a therapeutic activity. In certain embodiments, one or more of the transgene, the first transgene, and the second transgene encodes a fusion protein comprising at least one binding domain.

[00156] In certain embodiments, one or more of the transgene, the first transgene, and the second transgene encodes an immunomodulatory molecule. In certain embodiments, the immunomodulatory molecule is a costimulatory ligand, a cytokine, or a cytokine receptor. In certain embodiments, the immunomodulatory molecule is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-7, IL-10, IL-10 trap, IL-10R, IL-

12A/p35, IL-12B/p40, IL-15, IL-23A/p19, IL-24, IL-27, IL-33, IL-35, IL-15, an IL-15 receptor fusion protein, TGF-β, a TGF-β trap, an IL-10 trap, VEGF, VEGF trap, indoleamin-2,3-dioxygenase (IDO), inducible T-cell co-stimulator ligand (ICOS-L), CD80, CD137L, TNF-α, IFN-α, IFN- β, IFN-γ, GM-CSF, GITR ligand (GITRL), OX40 ligand (OX40L), CD40 ligand (CD40L)/CD154, CD70, CD86, CD137, CD137L, BORIS/CTCFL, bone morphogenetic protein (BMP), TNFSF9, FGF, ICAM, Podocalyxin, functional fragments thereof, and derivatives thereof.

[00157] In certain embodiments, the transgene encodes a fusion protein that comprise, in an N- to C-terminal orientation: a soluble portion of an extracellular domain of a cytokine receptor; an amino acid linker; an immunoglobulin (Ig) hinge region; and an immunoglobulin (Ig) Fc domain. In some embodiments, the cytokine receptor is TGFβ type II (TβRII) receptor.

[00158] In certain embodiments, a nucleotide sequence encoding CD80 or a functional fragment thereof is inserted in the IX-E2 insertion site, and a nucleotide sequence encoding CD137L or a functional fragment thereof is inserted in the L5-E4 insertion site. In certain embodiments, a nucleotide sequence encoding CD137L or a functional fragment thereof is inserted in the IX-E2 insertion site, and a nucleotide sequence encoding CD80 or a functional fragment thereof is inserted in the L5-E4 insertion site.

[00159] In certain embodiments, the recombinant adenovirus comprises a nucleotide sequence encoding IL-12A/p35 or a functional fragment thereof, a nucleotide sequence encoding IL-12B/p40 or a functional fragment thereof, and a nucleotide sequence encoding IFN-α or a functional fragment thereof. These nucleotide sequences may be inserted in the IX-E2 insertion site, the L5-E4 insertion site, the E1b-19K insertion site, the E3 insertion site, and/or the E4 insertion site.

[00160] In certain embodiments, one or more of the transgenes, the first transgene, and/or the second transgene encodes an antigen-binding molecule. In certain embodiments, the antigen-binding molecule is an anti-PD-1 antibody, an anti-TGF-β antibody, an anti-PD-L1 antibody, and an anti-CTLA-4 antibody, or functional fragments thereof. Exemplary anti-PD-1 antibodies include nivolumab (Bristol-Myers Squibb Co.), pembrolizumab (KEYTRUDA®, Merck & Co.) and Atezolizumab (formerly MPDL3280A), MEDI4736, Avelumab, and PDR001.

[00161] In certain embodiments, one or more of the transgenes, the first transgene, and the second transgene encodes an antigen or a ligand to the antigen. In certain embodiments, the antigen is selected from the group consisting of CAIX, CEA, CD5, CD7, CD10, CD19, CD20,

CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD80, CD133, CD135 (Flt3), Flt3I, CD138, a cytomegalovirus (CMV) infected cell antigen, 4-1BB, EGP-2, EGP-40, EpCAM, erbB2, erbB3, erbB4, FBP, Fetal acetylcholine receptor, KRAS, HPV E6, E7, BING-4, EphA3, calcium activated chloride channel-2, cyclin B1, 9D7, SAP-1, PRAME, SSX-2, immature laminin receptor, folate receptor-a, telomerase, tyrosinase, melan-A, NY-ESO-1, GD2, GD3, hTERT, IL13R-a2, x-light chain, KDR, LeY, LI cell adhesion molecule, MAGE-A1, MAGE-A3, MART1, MART2, MUC1, Mesothelin, HER-2/neu, EGFRvIII, NKG2D ligands, NY-ES0-1, gp100, TRP-1/-2, TRP-1/-2, P polypeptide, MC1R, prostate specific antigen, BRAF, androgen-receptor, β-catenin, BRCA1/2, CDK4, CML66, fibronectin, p53, TGF-βRII, T cell receptor, oncofetal antigen, 5T4, PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, functional fragments thereof, and derivatives thereof.

[00162] In certain embodiments, one or more of the transgene, the first transgene, and the second transgene encodes a toxin. In certain embodiments, the toxin is pseudomonas exotoxin, ricin toxin, or diphtheria toxin.

[00163] In certain embodiments, one or more of the transgene, the first transgene, and the second transgene encodes an enzyme. In certain embodiments, the enzyme is selected from the group consisting of beta-glucuronidase, beta-galactosidase, beta-glucosidase, carboxypeptidase, beta-lactamase, esterase, metalloproteinase, relaxin, collagenase, streptokinase, arginase, NOS-2, fragments thereof, and derivatives thereof.

[00164] In certain embodiments, one or more of the transgene, the first transgene, and the second transgene encodes a cell cycle control agent, a growth factor, an anticoagulant, a pro-drug activating gene, a tumor suppressor gene, an apoptotic gene, an anti-platelet agent, a clotting factor, a cystic fibrosis transmembrane conductance regulator (CFTR) protein, fragments thereof, or derivatives thereof.

[00165] In certain embodiments, one or more of the transgene, the first transgene, and the second transgene encodes angiostatin, endostatin, acetylcholine, DKK1/Wnt, Ox40L, GITRL, secreted flagellin, thymidine kinase, functional fragments thereof, or derivatives thereof.

II. Methods of Treatment

[00166] In another aspect, the invention provides a method of inhibiting proliferation of a tumor cell comprising exposing the tumor cell to an effective amount of any of the foregoing recombinant adenoviruses to inhibit proliferation of the tumor cell.

[00167] In another aspect, the invention provides a method of inhibiting tumor growth in a subject in need thereof, wherein the method comprising administering to the subject to an effective amount of any of the foregoing recombinant adenoviruses to inhibit tumor growth. In some embodiments, the tumor is a HER2/neu positive tumor, and wherein the recombinant adenovirus comprises an E1a promoter having no more than one deletion of a functional Pea3 binding site. In some embodiments, the HER2/neu positive tumor is from breast cancer, gastric cancer, ovarian cancer, bladder cancer, salivary gland cancer, endometrial cancer, pancreatic cancer, or non-small-cell lung cancer (NSCLC).

[00168] In certain embodiments, the tumor is selected from the group consisting of melanoma, squamous cell carcinoma of the skin, basal cell carcinoma, head and neck tumor, breast tumor, anal cancer, cervical cancer, non-small cell lung cancer, mesothelioma, small cell lung tumor, renal cell carcinoma, prostate tumor, gastroesophageal tumor, colorectal tumor, testicular tumor, bladder tumor, ovarian tumor, hepatocellular carcinoma, cholangiocarcinoma, brain tumor, endometrial tumor, neuroendocrine tumor, merkel cell carcinoma, gastrointestinal stromal tumor, a sarcoma, and pancreatic tumor.

[00169] The recombinant adenoviruses disclosed herein can be can be used to treat various medical indications, for example, cancers. As used herein, "treat", "treating" and "treatment" mean the treatment of a disease in a subject, e.g., in a human. This includes: (a) inhibiting the disease, i.e., arresting its development; and (b) relieving the disease, i.e., causing regression of the disease state. As used herein, the terms "subject" and "patient" refer to an organism to be treated by the methods and compositions described herein. Such organisms preferably include, but are not limited to, mammals (e.g., murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably includes humans.

[00170] In one aspect, the invention provides a method of treating a hyperproliferative disease, in a subject. The method comprises administering to the subject an effective amount of a recombinant virus described herein to treat the hyperproliferative disease in the subject. In certain embodiments, the hyperproliferative disease is selected from the group consisting of cancer, atherosclerosis, rheumatoid arthritis, psoriasis, lupus, idiopathic pulmonary fibrosis, scleroderma and cirrhosis. In certain embodiments, the hyperproliferative disease is cancer.

[00171] In some embodiments, the invention provides a method of treating cancer in a subject. The method comprises administering to the subject an effective amount of a recombinant adenoviruses described herein to treat the cancer disease in the subject.

[00172] Examples of cancers include solid tumors, soft tissue tumors, hematopoietic tumors and metastatic lesions. Examples of hematopoietic tumors include, leukemia, acute leukemia, acute lymphoblastic leukemia (ALL), B-cell, T-cell or FAB ALL, acute myeloid leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), e.g., transformed CLL, diffuse large B-cell lymphomas (DLBCL), follicular lymphoma, hairy cell leukemia, myelodysplastic syndrome (MDS), a lymphoma, Hodgkin's disease, a malignant lymphoma, non-Hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, or Richter's Syndrome (Richter's Transformation). Examples of solid tumors include malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting head and neck (including pharynx), thyroid, lung (small cell or non-small cell lung carcinoma (NSCLC)), breast, lymphoid, gastrointestinal (e.g., oral, esophageal, stomach, liver, pancreas, small intestine, colon and rectum, anal canal), genitals and genitourinary tract (e.g., renal, urothelial, bladder, ovarian, uterine, cervical, endometrial, prostate, testicular), CNS (e.g., neural or glial cells, e.g., neuroblastoma or glioma), or skin (e.g., melanoma).

[00173] In certain embodiments, the cancer is selected from melanoma, squamous cell carcinoma of the skin, basal cell carcinoma, head and neck cancer, breast cancer, anal cancer, cervical cancer, non-small cell lung cancer, mesothelioma, small cell lung cancer, renal cell carcinoma, prostate cancer, gastroesophageal cancer, colorectal cancer, testicular cancer, bladder cancer, ovarian cancer, hepatocellular carcinoma, cholangiocarcinoma, brain cancer, endometrial cancer, neuroendocrine cancer, and pancreatic cancer.

[00174] In certain embodiments, the cancer is selected from nasopharyngeal cancer, basal cell carcinoma, synovial cancer, hepatocellular cancer, renal cancer, cancer of connective tissues, melanoma, lung cancer, bowel cancer, colon cancer, rectal cancer, colorectal cancer, brain cancer, throat cancer, oral cancer, liver cancer, bone cancer, pancreatic cancer, choriocarcinoma, gastrinoma, neuroendocrine, pheochromocytoma, prolactinoma, T-cell leukemia/lymphoma, neuroma, von Hippel-Lindau disease, Zollinger-Ellison syndrome, adrenal cancer, anal cancer, bile duct cancer, bladder cancer, ureter cancer, brain cancer, oligodendroglioma, neuroblastoma, meningioma, spinal cord tumor, bone cancer, osteochondroma, chondrosarcoma, Ewing's sarcoma, cancer of unknown primary site, carcinoid, carcinoid of gastrointestinal tract, fibrosarcoma, breast cancer, Paget's disease, cervical cancer, colorectal cancer, rectal cancer, esophagus cancer, gall bladder cancer, head cancer, eye cancer, neck cancer, kidney cancer, Wilms' tumor, liver cancer, Kaposi's sarcoma, prostate cancer, lung cancer, testicular cancer, Hodgkin's disease, non-Hodgkin's lymphoma,

oral cancer, skin cancer, mesothelioma, multiple myeloma, ovarian cancer, endocrine pancreatic cancer, glucagonoma, pancreatic cancer, parathyroid cancer, penis cancer, pituitary cancer, soft tissue sarcoma, retinoblastoma, small intestine cancer, stomach cancer, thymus cancer, thyroid cancer, trophoblastic cancer, hydatidiform mole, uterine cancer, endometrial cancer, vagina cancer, vulva cancer, acoustic neuroma, mycosis fungoides, insulinoma, carcinoid syndrome, somatostatinoma, gum cancer, heart cancer, lip cancer, meninges cancer, mouth cancer, nerve cancer, palate cancer, parotid gland cancer, peritoneum cancer, pharynx cancer, pleural cancer, salivary gland cancer, tongue cancer and tonsil cancer.

[00175] In some aspects, the invention provides a method of inhibiting proliferation of a tumor cell comprising exposing the tumor cell to an effective amount of any of the foregoing recombinant adenoviruses.

[00176] In another aspect, the invention provides a method of inhibiting tumor growth in a subject in need thereof, wherein the method comprising administering to the subject to an effective amount of any of the foregoing recombinant adenoviruses. In certain embodiments, the tumor is selected from the group consisting of melanoma, squamous cell carcinoma of the skin, basal cell carcinoma, head and neck tumor, breast tumor, anal cancer, cervical cancer, non-small cell lung cancer, mesothelioma, small cell lung tumor, renal cell carcinoma, prostate tumor, gastroesophageal tumor, colorectal tumor, testicular tumor, bladder tumor, ovarian tumor, hepatocellular carcinoma, cholangiocarcinoma, brain tumor, endometrial tumor, neuroendocrine tumor, merkel cell carcinoma, gastrointestinal stromal tumor, a sarcoma, and pancreatic tumor.

[00177] In another aspect, the invention provides a method of treating a disease or condition in a subject in need thereof, wherein the method comprising administering to the subject to an effective amount of any of the foregoing recombinant adenoviruses. In certain embodiments, the disease or condition is selected from the group consisting of an infection, diabetic retinopathy, psoriasis, rheumatoid arthritis, endometriosis, macular degenerative disorders and benign growth disorders such as prostate enlargement and lipomas, a vascular disorder, a cardiovascular disease, an infection, cirrhosis of the liver, a connective tissue disorder, a tumor, a vascular lesion, an ulcerative lesion, an inflammation, thrombosis, and neointima formation.

[00178] In certain embodiments, the subject is a mammal. In certain embodiments, the subject is a human. In certain embodiments, the subject is a pediatric human. In certain embodiments, the subject is an adult human.

[00179] In certain embodiments, the recombinant adenovirus is administered by intramuscular, intravenous, intraarterial, or intratumoral injection. In certain embodiments, the recombinant adenovirus is administered by intradermal, inhalation, transdermal, topical, eye drops, intranasal, transmucosal, and rectal administration.

[00180] In certain embodiments, the foregoing recombinant adenoviruses are administered to the subject in combination with one or more therapies selected from the group consisting of surgery, radiation, chemotherapy, immunotherapy, hormone therapy, and virotherapy.

[00181] In certain embodiments, the recombinant adenoviruses of the invention are administered in combination with a tyrosine kinase inhibitor, e.g., erlotinib.

[00182] In certain embodiments, the recombinant adenoviruses of the invention are administered in combination with one or more immune checkpoint modulators. In certain embodiments, the immune checkpoint modulator is an inhibitor, an antagonist, or an agonist of one or more molecules selected from the group consisting of PD-1, PD-L1, PD-L2, 2B4, TIGIT, LAG3, Tim3, BTLA, CD160, GITR, KIR, 4-1BB, and CTLA4. In some embodiments the immune checkpoint modulators are antibodies to PD-1, PD-L1, PD-L2, 2B4, TIGIT, LAG3, Tim3, BTLA, CD160, GITR, KIR, 4-1BB, and/or CTLA4. Exemplary anti-PD-1 antibodies include nivolumab (Bristol-Myers Squibb Co.), pembrolizumab (KEYTRUDA®, Merck & Co.) and Atezolizumab (formerly MPDL3280A), MEDI4736, Avelumab, and PDR001.

[00183] Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished by any suitable method, *e.g.*, filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

[00184] The term "effective amount" as used herein refers to the amount of an active component (*e.g.*, the amount of a recombinant virus of the present invention) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route.

[00185] In certain embodiments, a therapeutically effective amount of active component is in the range of 0.1 mg/kg to 100 mg/kg, *e.g.*, 1 mg/kg to 100 mg/kg, 1 mg/kg to 10 mg/kg. In certain embodiments, a therapeutically effective amount of a recombinant virus is in the range of 10^2 to 10^{15} plaque forming units (pfus), *e.g.*, 10^2 to 10^{10}, 10^2 to 10^5, 10^5 to 10^{15}, 10^5 to

10^{10}, or 10^{10} to 10^{15} plaque forming units. The amount administered will depend on variables such as the type and extent of disease or indication to be treated, the overall health of the patient, the *in vivo* potency of the antibody, the pharmaceutical formulation, and the route of administration. The initial dosage can be increased beyond the upper level in order to rapidly achieve the desired blood-level or tissue-level. Alternatively, the initial dosage can be smaller than the optimum, and the daily dosage may be progressively increased during the course of treatment. Human dosage can be optimized, e.g., in a conventional Phase I dose escalation study designed to run from 0.5 mg/kg to 20 mg/kg. Dosing frequency can vary, depending on factors such as route of administration, dosage amount, serum half-life of the virus, and the disease being treated. Exemplary dosing frequencies are once per day, once per week and once every two weeks. One route of administration is parenteral, e.g., intravenous infusion. Formulation of virus -based drugs is within ordinary skill in the art. In certain embodiments, a recombinant virus is lyophilized, and then reconstituted in buffered saline, at the time of administration.

[00186] The term administered "in combination," as used herein, is understood to mean that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, such that the effects of the treatments on the patient overlap at a point in time. In certain embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery." In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In certain embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

III. Pharmaceutical Composition/Formulation

[00187] The present disclosure also provides a pharmaceutical composition comprising any of the foregoing recombinant adenoviruses and at least one pharmaceutically acceptable carrier or diluent. As used herein, "pharmaceutically acceptable carrier" means buffers, carriers, and excipients suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. The carrier(s) should be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient. Pharmaceutically acceptable carriers include buffers, solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration.

[00188] Pharmaceutical compositions and formulations containing the recombinant adenoviruses disclosed herein can be formulated to be compatible with its intended route of administration. Examples of routes of administration are intramuscular, intravenous, intraarterial, or intratumoral intradermal, inhalation, transdermal, topical, transmucosal, and rectal administration.

[00189] In one aspect, the present disclosure provides an adenovirus formulation for the stabilization and storage of recombinant adenoviruses. In some embodiments, the invention provides a formulation for adenoviruses comprising:

e) one or more of any of the foregoing recombinant adenoviruses;

f) at least one buffer;

g) at least one tonicity modifier;

h) at least one sugar or at least one stabilizing agent, or both; and

wherein the formulation has a pH ranging between about 7.0 and about 9.0.

[00190] In certain embodiments, the stabilizing agent is glycerol. In certain embodiments, the stabilizing agent is at about 2% to about 5% (v/v).

[00191] In certain embodiments, the buffer is Tris (includes Tris-HCl and/or mono-Tris), TES, HEPES, brucine tetrahydrate, EPPS, tricine, or histidine. In certain embodiments, the buffer is at concentration of about 1mM to about 30 mM.

[00192] In some embodiments, the tonicity modifier is $MgCl_2$, $MnCl_2$, $CaCl_2$, $ZnCl_2$, NaCl, or KCl. In one embodiment, the tonicity modifier is NaCl. In one embodiment, the tonicity modifier is at concentration of about 0.1 mM to about 5 mM. In one embodiment, the tonicity modifier is at concentration of about 10 mM to about 250 mM. In one embodiment,

the the tonicity modifier is at concentration of about 25 mM to about 100 mM. In one embodiment, the tonicity modifier is at concentration of about 25 mM.

[00193] In certain embodiments, the formulation comprises a first tonicity modifier and a second tonicity modifier, wherein the first tonicity modifier is a monovalent cation, and wherein the second tonicity modifier is a divalent cation. In certain embodiments, the monovalent cation is NaCl or KCl. In certain embodiments, the divalent cation is $MgCl_2$, $MnCl_2$, $CaCl_2$, or $ZnCl_2$. In certain embodiments, the tonicity modifier or the divalent cation is at a concentration of about 0.1 mM to about 5 mM.

[00194] In some embodiments, the sugar is sucrose or trehalose. In one embodiment, the sugar is sucrose. In one embodiment, the sugar is at weight to volume percentage from about 2% to about 8%. In one embodiment, the sugar is at weight to volume percentage from about 3% to about 5%. In one embodiment, the sugar is at weight to volume percentage of about 5%.

[00195] In certain embodiments, any of the foregoing formulations further comprise at least one non-ionic surfactant. In certain embodiments, the non-ionic surfactant is polysorbate-80 or polysorbate-40. In one embodiment, the non-ionic surfactant is at a concentration of about 0.001% to about 1%. In one embodiment, the non-ionic surfactant is at a concentration of about 0.02 %.

[00196] In certain embodiments, any of the foregoing formulations further comprise at least one inhibitor of free radical oxidation. In certain embodiments, the inhibitor of free radical oxidation is EDTA. In one embodiment, the inhibitor of free radical oxidation is at a concentration of about 0.01 mM to about 5 mM. In one embodiment, the inhibitor of free radical oxidation is at a concentration of about 0.05 mM to about 2 mM. In one embodiment, the inhibitor of free radical oxidation is at a concentration of about 0.1 mM.

[00197] In certain embodiments, any of the foregoing formulations further comprise at least one cryoprotectant. In certain embodiments, the cryoprotectant is EtOH. In some embodiments, the cryoprotectant is a concentration of about 0.01% to 5%. In some embodiments, the cryoprotectant is a concentration of about 0.1% to 2%. In one embodiment, the cryoprotectant is at a concentration of about 0.5%.

[00198] In some embodiments, the formulation has an osmolarity of about 200 mOs/L to about 800 mOs/L. In some embodiments, the formulation has an osmolarity of about 300 mOs/L to about 600 mOs/L. In some embodiments, the formulation has an osmolarity of about 400 mOs/L to about 500 mOs/L.

[00199] In certain embodiments, the recombinant oncolytic adenovirus in any of the foregoing formulations is at concentration from about 1 x 10^7 vp/mL to 1 x 10^{13} vp/mL.

[00200] In certain embodiments, the formulation comprises about 20 mM Tris, about 25 mM NaCl, about 2.5% glycerol, and wherein the formulation has a pH of about 8.0. In certain embodiments, the formulation comprises about 20 mM Tris, about 25 mM NaCl, about 3-5% sucrose, and wherein the formulation has a pH of about 8.0. In certain embodiments, the formulation comprises about 10 mM Tris, about 75 mM NaCl, about 5% sucrose, about 0.02% polysorbate-80, about 1 mM MgCl2, about 0.1 mM EDTA, about 0.5% EtOH, and wherein the formulation has a pH of about 8.0.

[00201] In certain embodiments, any of the foregoing formulations further comprise at least one immunoadjuvant. In certain embodiments, the immunoadjuvant is selected from 1) Alum, 2) Saponins, 3) non-ionic polymer surfactants, 4) monophosphoryl lipid A, 5) muramyl dipeptides, and 6) cytokines.

[00202] In certain embodiments, any of the foregoing formulations further comprise at least one dye. In certain embodiments, any of the foregoing formulations further comprise at least one reversible protease inhibitor. In certain embodiments, the reversible protease inhibitor is an inhibitor of an L3/p23 cysteine protease. In certain embodiments, any of the foregoing formulations further comprise an antioxidant. In certain embodiments, the antioxidant is vitamin A, vitamin C, vitamin E, vitamin B6, vitamin B12, folic acid, or folate.

[00203] It should be understood that the expression "at least one of" includes individually each of the recited objects after the expression and the various combinations of two or more of the recited objects unless otherwise understood from the context and use. The expression "and/or" in connection with three or more recited objects should be understood to have the same meaning unless otherwise understood from the context.

[00204] The use of the term "include," "includes," "including," "have," "has," "having," "contain," "contains," or "containing," including grammatical equivalents thereof, should be understood generally as open-ended and non-limiting, for example, not excluding additional unrecited elements or steps, unless otherwise specifically stated or understood from the context.

[00205] Where the use of the term "about" is before a quantitative value, the present invention also includes the specific quantitative value itself, unless specifically stated otherwise. As used herein, the term "about" refers to a ±10% variation from the nominal value unless otherwise indicated or inferred.

[00206] It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present invention remain operable. Moreover, two or more steps or actions may be conducted simultaneously.

[00207] The use of any and all examples, or exemplary language herein, for example, "such as" or "including," is intended merely to illustrate better the present invention and does not pose a limitation on the scope of the invention unless claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the present invention.

EXAMPLES

[00208] The following working examples are illustrative and are not intended to be limiting and it will be readily understood by one of skill in the art that other embodiments may be utilized.

[00209] **Example 1**

[00210] The nucleotide sequence of an exemplary IX-E2 insertion site (nucleotide 4029 to 4093 numbering according to NCBI Reference Sequence AC_000008.1 (SEQ ID NO: 1)) is as follows. The stop codon of adenovirus IX gene ("TAA" on left; SEQ ID NO: 8) and the stop codon of adenovirus IVa2 gene ("TTA" on the right; SEQ ID NO: 9) are underlined.

TAAAACATAAATAAAAAACCAGACTCTGTTTGGATTTGGATCAAGCAAGTGTCTTGCTGTCT
TTA (SEQ ID NO: 6)

[00211] **Example 2**

[00212] The nucleotide sequence of an exemplary L5-E4 insertion site (nucleotide 32785 to 32916 numbering according to NCBI Reference Sequence AC_000008.1 (SEQ ID NO: 1)) is as follows. The stop codon of adenovirus fiber gene ("TAA" on left; SEQ ID NO: 8) and the stop codon of ORF6/7 of adenovirus E4 gene ("TCA" on the right; SEQ ID NO: 10) are underlined.

TAAGAATCGTTTGTGTTATGTTTCAACGTGTTTATTTTTCAATTGCAGAAAATTTCAAGTC
ATTTTTCATTCAGTAGTATAGCCCCACCACCACATAGCTTATACAGATCACCGTACCTTAAT
CAAACTCA (SEQ ID NO: 7)

[00213] **Example 3**

[00214] To generate viruses with transgenes cloned into an expression cassette in the L5-E4 site, a plasmid with adenoviral nucleotide sequence (which contained deletions of the

RIDα, RIDβ, and 14.7k genes in the E3 region and the ORF1-ORF4 genes in the E4 region) was modified by inserting into the L5-E4 site an expression cassette with the SV40 promoter and terminator with an intervening SwaI restriction site ("ATTTAAAT" SEQ ID NO: 11). The nucleotide sequence of this modification, from the polyadenylation signal of the L5 transcription unit ("AATAAA" SEQ ID NO: 12) to the polyadenylation signal of the E4 transcription unit ("TTTATT" SEQ ID NO: 13) is:

SEQ ID NO: 14 [L5 initial Empty]

AATAAAGAATCGTTTGTGTTATGTTTCAACCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAA
GTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCA
GGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAG
TCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGC
CCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTG
CCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAG
CTTTGCAAAGATTTAAATAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAG
CATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAAC
TCATCAATGTATCTTATCATGTCTGGTGTTTATT

[00215] In SEQ ID NO: 14, the polyadenylation signals of the L5 and E4 transcription units, and the nucleotides of the SwaI restriction site, are underlined.

[00216] A transgene encoding the mouse GMCSF was then cloned into the SwaI site, generating the sequence:

SEQ ID NO: 15 [L5 initial mGMCSF]

AATAAAGAATCGTTTGTGTTATGTTTCAACCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAA
GTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCA
GGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAG
TCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGC
CCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTG
CCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAG
CTTTGCAAAGATTT**ATGTGGCTGCAGAACCTGCTGTTCCTGGGCATCGTGGTGTACAGCCTG**
AGCGCCCCCACCAGATCCCCCATCACCGTGACCAGACCCTGGAAGCACGTGGAAGCCATCAA
AGAGGCCCTGAACCTGCTGGACGACATGCCCGTGACCCTGAACGAAGAGGTGGAAGTGGTGT
CCAACGAGTTCAGCTTCAAGAAACTGACCTGCGTGCAGACCAGACTGAAGATCTTCGAGCAG
GGCCTGAGAGGCAACTTCACCAAGCTGAAGGGCGCTCTGAACATGACCGCCAGCTACTACCA
GACCTACTGCCCTCCCACACCCGAGACAGACTGCGAGACACAGGTCACAACCTACGCCGACT
TCATCGACAGCCTGAAAACCTTCCTGACCGACATCCCCTTCGAGTGCAAGAAACCCGGCCAG
AAGTGAAAATAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAA
ATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAAT
GTATCTTATCATGTCTGGTGTTTATT

[00217] In SEQ ID NO: 15, the polyadenylation signals of the L5 and E4 transcription units, and the residual nucleotides of the SwaI restriction site are underlined, and the transgene encoding the mouse GMCSF are bolded.

[00218] The virus TAV-(L5-E4)mGMCSF was generated to carry the following modifications (compared to the dl309 strain of adenovirus type 5): the TAV-255 deletion in the E1A promoter to confer selective replication in cancerous cells, a deletion of the 5' end of the viral E1B-19K gene which does not extend into the viral E1B-55K gene, a deletion of the E3 RIDα, RIDβ, and 14.7k genes, the sequence of SEQ ID NO: 15, and a deletion of the E4 ORF1-ORF4 genes.

[00219] To test for mGMCSF expression: A549 cells (human cancer cell line), ADS-12 cells (mouse cancer cell line), and WI38 cells (human normal cell line) were infected with TAV-(L5-E4)mGMCSF at an MOI (multiplicity of infection) of 5. As a control, additional cells were cultured without infection or were infected with the virus TAV-(E1B-19K)mGMCSF which carries the following modifications compared to the dl309 strain of adenovirus type 5: the TAV-255 deletion in the E1A promoter, and the mGMCSF gene replacing the 5' end of the E1B-19K gene without disrupting the E1B-55K gene. Four days after infection, the conditioned media was used in an ELISA to measure mouse GMCSF expression. Results are shown in FIG. 2: both viruses gave high levels of expression in A549 cells, moderate expression in ADS-12 cells, and low levels of expression in WI38 cells.

[00220] **Example 4**

[00221] To investigate an expression cassette insertion at the IX-E2 site, initially, we inserted an expression cassette with the cytomegalovirus immediate early promoter (CMV promoter) and the transcription terminator of bovine growth hormone (BGH terminator) including a NotI restriction site ("GCGGCCGC" SEQ ID NO: 16) between the promoter and terminator to facilitate insertion of a transgene. Its nucleotide sequence from the polyadenylation signal of IX ("AATAAA" SEQ ID NO: 12) to the polyadenylation signal of the E2 transcription unit ("TTTATT" SEQ ID NO: 13) is:

SEQ ID NO: 17 [IX initial Empty]

AATAAAAAACCAGACTCTGTTTGGATTTGGATCAAGCAAGTGTCTTGCTGTCTTACGGTAAA
TGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTC
CCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACT
GCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGA
CGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGC
AGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAAT
GGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGG
GAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCAT
TGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTA
ACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCG
CGGCCGCCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTT

GACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATT
GTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGAT
TGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGTTTATT

[00222] The polyadenylation signals of the IX and E2 transcripts, and the NotI restriction site, are underlined.

[00223] The virus TAV IX-WT L5-Empty carried the TAV-255 deletion in the E1A promoter, wild-type viral sequence in the IX-E2 site, and the sequence SEQ ID NO: 14 (the empty expression cassette in the L5-E4 site). The virus TAV IX-WT L5-IL7 carried the TAV-255 deletion in the E1A promoter, wild-type viral sequence in the IX-E2 site, and the sequence SEQ ID NO: 18 (see below with capital letters indicating the mouse IL-7 gene and lower case letters representing flanking nucleotides from the L5-E4 expression cassette including underlined residual nucleotides from the SwaI restriction site) cloned into the L5-E4 cassette of SEQ ID NO: 14.

SEQ ID NO: 18 [L5 mIL7]

gctttgcaaagatttATGTTCCATGTTTCTTTTAGATATATCTTTGGAATTCCTCCACTGAT
CCTTGTTCTGCTGCCTGTCACATCATCTGAGTGCCACATTAAAGACAAAGAAGGTAAAGCAT
ATGAGAGTGTACTGATGATCAGCATCGATGAATTGGACAAAATGACAGGAACTGATAGTAAT
TGCCCGAATAATGAACCAAACTTTTTTAGAAAACATGTATGTGATGATACAAAGGAAGCTGC
TTTTCTAAATCGTGCTGCTCGCAAGTTGAAGCAATTTCTTAAAATGAATATCAGTGAAGAAT
TCAATGTCCACTTACTAACAGTATCACAAGGCACACAAACACTGGTGAACTGCACAAGTAAG
GAAGAAAAAAACGTAAAGGAACAGAAAAAGAATGATGCATGTTTCCTAAAGAGACTACTGAG
AGAAATAAAAACTTGTTGGAATAAAATTTTGAAGGGCAGTATATAAaaataacttgtttatt
gcag

[00224] The virus TAV IX-WT L5-GMCSF carried the TAV-255 deletion in the E1A promoter, wild-type viral sequence in the IX-E2 site, and the sequence SEQ ID NO: 19 (with capital letters indicating the mouse GMCSF gene and lower case letters representing flanking nucleotides from the L5-E4 expression cassette including underlined residual nucleotides from the SwaI restriction site) cloned into the L5-E4 cassette of SEQ ID NO: 14. This virus carries wild-type mouse GMCSF and not the codon-optimized form of mouse GMCSF used in the virus TAV-(L5-E4)mGMCSF shown in SEQ ID NO: 15.

SEQ ID NO: 19 [L5 wt mGMCSF]

gctttgcaaagatttATGTGGCTGCAGAATTTACTTTTCCTGGGCATTGTGGTCTACAGCCT
CTCAGCACCCACCCGCTCACCCATCACTGTCACCCGGCCTTGGAAGCATGTAGAGGCCATCA
AAGAAGCCCTGAACCTCCTGGATGACATGCCTGTCACGTTGAATGAAGAGGTAGAAGTCGTC
TCTAACGAGTTCTCCTTCAAGAAGCTAACATGTGTGCAGACCCGCCTGAAGATATTCGAGCA
GGGTCTACGGGGCAATTTCACCAAACTCAAGGGCGCCTTGAACATGACAGCCAGCTACTACC
AGACATACTGCCCCCCAACTCCGGAAACGGACTGTGAAACACAAGTTACCACCTATGCGGAT

TTCATAGACAGCCTTAAAACCTTTCTGACTGATATCCCCTTTGAATGCAAAAAACCAGGCCA
AAAATGAaaataacttgtttattgcag

[00225] The virus TAV IX-GMCSF L5-IL7 carried the TAV-255 deletion in the E1A promoter, the sequence SEQ ID NO: 20 (with capital letters indicating the mouse GMCSF gene and lower case letters representing the flanking nucleotides of the IX-E2 expression cassette including underlined residual nucleotides from the NotI restriction site) cloned into the IX-E2 cassette of SEQ ID NO: 17, and mouse IL-7 cloned into the L5-E4 cassette as depicted in SEQ ID NO: 18 and SEQ ID NO: 14. The sequence of mouse GMCSF was identical in the viruses TAV IX-WT L5-GMCSF and TAV IX-GMCSF L5-IL7 but was inserted in the IX-E2 expression cassette of one virus and the L5-E4 expression cassette in the other virus.

SEQ ID NO: 20 [IX wt mGMCSF]

atagggagacccgcggccATGTGGCTGCAGAATTTACTTTTCCTGGGCATTGTGGTCTACAG
CCTCTCAGCACCCACCCGCTCACCCATCACTGTCACCCGGCCTTGGAAGCATGTAGAGGCCA
TCAAAGAAGCCCTGAACCTCCTGGATGACATGCCTGTCACGTTGAATGAAGAGGTAGAAGTC
GTCTCTAACGAGTTCTCCTTCAAGAAGCTAACATGTGTGCAGACCCGCCTGAAGATATTCGA
GCAGGGTCTACGGGGCAATTTCACCAAACTCAAGGGCGCCTTGAACATGACAGCCAGCTACT
ACCAGACATACTGCCCCCCAACTCCGGAAACGGACTGTGAAACACAAGTTACCACCTATGCG
GATTTCATAGACAGCCTTAAAACCTTTCTGACTGATATCCCCTTTGAATGCAAAAAACCAGG
CCAAAAATGAggccgctgtgccttctagt

[00226] To test for transgene expression from these viruses, A549 cells were infected with the viruses at an MOI of 5 and four days later the conditioned media was collected and used in ELISAs for IL-7 and GMCSF. The ELISA for GMCSF showed substantially higher expression from the cassette in the IX-E2 site driven by the CMV promoter than from the cassette in the L5-E4 site driven by the SV40 promoter, as depicted in FIG. 3.

[00227] **Example 5**

[00228] In viruses of the initial IX-E2 design (FIG. 4), we suspected that viral growth could be further optimized by blocking any potential for the CMV promoter driving transcription in the opposite direction from what was intended (Seila et al., *Science*. (2008) 322(5909):1849-51). We therefore revised the insert in this site so both the 5' and 3' ends of the insert contain polyadenylation signals oriented to polyadenylate transcripts going both into the insert from the normal viral genes and out of the insert toward the normal viral genes. See the revised IX-E2 design in FIG. 4.

[00229] The nucleotide sequence of the revised insert, from the polyadenylation signal of the IX transcript to the polyadenylation signal of the E2 transcript, is:

SEQ ID NO: 21 [IX revised Empty]

AATAAAATACACCTTTTTTCGATTGTACGTATTTTTATTTACGGTAAATGGCCCGCCTGGCT
GACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCA
ATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
ACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
CCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTA
TTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCG
GTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGC
ACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGC
GGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCAC
TGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCGCGGCCGCTGTGCCT
TCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGC
CACTCCCACTGTCCTTTCCTAATAAAAAACCAGACTCTGTTTGGATTTGGATCAAGCAAGTG
TCTTGCTGTCTTTATT

[00230] Each forward polyadenylation signal ("AATAAA" SEQ ID NO: 12) and reverse polyadenylation signal ("TTTATT" SEQ ID NO: 13), and the NotI site ("GCGGCCGC" SEQ ID NO: 16), are underlined. Viruses carrying the expression cassette with the revised IX-E2 design grew more efficiently than the viruses with the initial IX-E2 design.

[00231] **Example 6**

[00232] The virus TAV-IXrL5-Empty carried the TAV-255 deletion in the viral E1A promoter, SEQ ID NO: 21 (the empty expression cassette of the revised IX-E2 design) and SEQ ID NO: 14 (the empty expression cassette in the L5-E4 site). The virus TAV-IXrL5-hIL12 carries the TAV-255 deletion in the viral E1A promoter, a gene encoding the human IL12A chain in the revised IX-E2 expression cassette (depicted in SEQ ID NO: 22), and a gene encoding the human IL12B chain in the L5-E4 expression cassette (depicted in SEQ ID NO: 23). In SEQ ID NO: 22, each forward polyadenylation signal ("AATAAA" SEQ ID NO: 12) and reverse polyadenylation signal ("TTTATT" SEQ ID NO: 13), and the residual nucleotides from the NotI site are underlined, and the gene encoding the human IL12A chain are bolded. In SEQ ID NO: 23, the polyadenylation signals of the L5 and E4 transcription units, and the residual nucleotides of the SwaI restriction site are underlined, and the gene encoding the human IL12B chain are bolded.

SEQ ID NO: 22 [IX revised hIL12A]

AATAAAATACACCTTTTTTCGATTGTACGTATTTTTATTTACGGTAAATGGCCCGCCTGGCT
GACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCA
ATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
ACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
CCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTA
TTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCG

GTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGC
ACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGC
GGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCAC
TGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCGCGGCC**ATGTGGCCC**
CCTGGGTCAGCCTCCCAGCCACCGCCCTCACCTGCCGCGGCCACAGGTCTGCATCCAGCGGC
TCGCCCTGTGTCCCTGCAGTGCCGGCTCAGCATGTGTCCAGCGCGCAGCCTCCTCCTTGTGG
CTACCCTGGTCCTCCTGGACCACCTCAGTTTGGCCAGAAACCTCCCCGTGGCCACTCCAGAC
CCAGGAATGTTCCCATGCCTTCACCACTCCCAAAACCTGCTGAGGGCCGTCAGCAACATGCT
CCAGAAGGCCAGACAAACTCTAGAATTTTACCCTTGCACTTCTGAAGAGATTGATCATGAAG
ATATCACAAAAGATAAAACCAGCACAGTGGAGGCCTGTTTACCATTGGAATTAACCAAGAAT
GAGAGTTGCCTAAATTCCAGAGAGACCTCTTTCATAACTAATGGGAGTTGCCTGGCCTCCAG
AAAGACCTCTTTTATGATGGCCCTGTGCCTTAGTAGTATTTATGAAGACTTGAAGATGTACC
AGGTGGAGTTCAAGACCATGAATGCAAAGCTTCTGATGGATCCTAAGAGGCAGATCTTTCTA
GATCAAAACATGCTGGCAGTTATTGATGAGCTGATGCAGGCCCTGAATTTCAACAGTGAGAC
TGTGCCACAAAAATCCTCCCTTGAAGAACCGGATTTTTATAAAACTAAAATCAAGCTCTGCA
TACTTCTTCATGCTTTCAGAATTCGGGCAGTGACTATTGATAGAGTGATGAGCTATCTGAAT
GCTTCCTAAGGCCGCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGC
CTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAAAACCAGACTCTGT
TTGGATTTGGATCAAGCAAGTGTCTTGCTGTCTTTATT

SEQ ID NO: 23 [L5 initial hIL12B]

AATAAAGAATCGTTTGTGTTATGTTTCAACCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAA
GTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCA
GGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAG
TCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGC
CCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTG
CCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAG
CTTTGCAAAGATTT**ATGTGTCACCAGCAGTTGGTCATCTCTTGGTTTTCCCTGGTTTTTCTG**
GCATCTCCCCTCGTGGCCATATGGGAACTGAAGAAAGATGTTTATGTCGTAGAATTGGATTG
GTATCCGGATGCCCCTGGAGAAATGGTGGTCCTCACCTGTGACACCCCTGAAGAAGATGGTA
TCACCTGGACCTTGGACCAGAGCAGTGAGGTCTTAGGCTCTGGCAAAACCCTGACCATCCAA
GTCAAAGAGTTTGGAGATGCTGGCCAGTACACCTGTCACAAAGGAGGCGAGGTTCTAAGCCA
TTCGCTCCTGCTGCTTCACAAAAAGGAAGATGGAATTTGGTCCACTGATATTTTAAAGGACC
AGAAAGAACCCAAAAATAAGACCTTTCTAAGATGCGAGGCCAAGAATTATTCTGGACGTTTC
ACCTGCTGGTGGCTGACGACAATCAGTACTGATTTGACATTCAGTGTCAAAAGCAGCAGAGG
CTCTTCTGACCCCCAAGGGGTGACGTGCGGAGCTGCTACACTCTCTGCAGAGAGAGTCAGAG
GGGACAACAAGGAGTATGAGTACTCAGTGGAGTGCCAGGAGGACAGTGCCTGCCCAGCTGCT
GAGGAGAGTCTGCCCATTGAGGTCATGGTGGATGCCGTTCACAAGCTCAAGTATGAAAACTA
CACCAGCAGCTTCTTCATCAGGGACATCATCAAACCTGACCCACCCAAGAACTTGCAGCTGA
AGCCATTAAAGAATTCTCGGCAGGTGGAGGTCAGCTGGGAGTACCCTGACACCTGGAGTACT
CCACATTCCTACTTCTCCCTGACATTCTGCGTTCAGGTCCAGGGCAAGAGCAAGAGAGAAAA
GAAAGATAGAGTCTTCACGGACAAGACCTCAGCCACGGTCATCTGCCGCAAAAATGCCAGCA
TTAGCGTGCGGGCCCAGGACCGCTACTATAGCTCATCTTGGAGCGAATGGGCATCTGTGCCC
TGCAGTTAGAAATAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCA
CAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATC
AATGTATCTTATCATGTCTGGTGTTTATT

[00233] The virus WT-IXrL5-hIL12 was created with an identical genomic structure as TAV-IXrL5-hIL12 except that it carries a wild-type E1A promoter instead of carrying the

TAV-255 deletion in the E1A promoter. Each of these viruses also has a deletion of the E3 RIDα, RIDβ, and 14.7k genes and a deletion of the E4 ORF1-ORF4 genes.

[00234] To compare this design approach with another strategy to incorporate IL12 into an oncolytic adenovirus, we tested an adenovirus carrying a gene encoding the human IL12A and IL12B chains linked by a furin cleavage site (amino acids RAKR; SEQ ID NO: 24) carried in the E1B-19K site. When the fusion protein was synthesized by the cell, the furin site was cleaved between the final R of the RAKR sequence and the next amino acid (the first amino acid of mature IL12A) by the enzyme furin in the Golgi. We previously found that using a furin cleavage site as a linker led to high level expression of the heterodimeric IL12 protein. The nucleic acid sequence of that fusion gene (capitalized), the flanking SalI and XhoI restriction sites used for cloning (underlined), and the adenoviral nucleotides indicating the site where it was inserted in the adenoviral genome (lower case) is:

SEQ ID NO: 25 [hIL12 furin]

atctgacctcgtcgacATGTGTCACCAGCAGTTGGTCATCTCTTGGTTTTCCCTGGTTTTTC
TGGCATCTCCCCTCGTGGCCATATGGGAACTGAAGAAAGATGTTTATGTCGTAGAATTGGAT
TGGTATCCGGATGCCCCTGGAGAAATGGTGGTCCTCACCTGTGACACCCCTGAAGAAGATGG
TATCACCTGGACCTTGGACCAGAGCAGTGAGGTCTTAGGCTCTGGCAAAACCCTGACCATCC
AAGTCAAAGAGTTTGGAGATGCTGGCCAGTACACCTGTCACAAAGGAGGCGAGGTTCTAAGC
CATTCGCTCCTGCTGCTTCACAAAAAGGAAGATGGAATTTGGTCCACTGATATTTTAAAGGA
CCAGAAAGAACCCAAAAATAAGACCTTTCTAAGATGCGAGGCCAAGAATTATTCTGGACGTT
TCACCTGCTGGTGGCTGACGACAATCAGTACTGATTTGACATTCAGTGTCAAAAGCAGCAGA
GGCTCTTCTGACCCCCAAGGGGTGACGTGCGGAGCTGCTACACTCTCTGCAGAGAGAGTCAG
AGGGGACAACAAGGAGTATGAGTACTCAGTGGAGTGCCAGGAGGACAGTGCCTGCCCAGCTG
CTGAGGAGAGTCTGCCCATTGAGGTCATGGTGGATGCCGTTCACAAGCTCAAGTATGAAAAC
TACACCAGCAGCTTCTTCATCAGGGACATCATCAAACCTGACCCACCCAAGAACTTGCAGCT
GAAGCCATTAAAGAATTCTCGGCAGGTGGAGGTCAGCTGGGAGTACCCTGACACCTGGAGTA
CTCCACATTCCTACTTCTCCCTGACATTCTGCGTTCAGGTCCAGGGCAAGAGCAAGAGAGAA
AAGAAAGATAGAGTCTTCACGGACAAGACCTCAGCCACGGTCATCTGCCGCAAAAATGCCAG
CATTAGCGTGCGGGCCCAGGACCGCTACTATAGCTCATCTTGGAGCGAATGGGCATCTGTGC
CCTGCAGTCGTGCTAAGCGAAGAAACCTCCCCGTGGCCACTCCAGACCCAGGAATGTTCCCA
TGCCTTCACCACTCCCAAAACCTGCTGAGGGCCGTCAGCAACATGCTCCAGAAGGCCAGACA
AACTCTAGAATTTTACCCTTGCACTTCTGAAGAGATTGATCATGAAGATATCACAAAAGATA
AAACCAGCACAGTGGAGGCCTGTTTACCATTGGAATTAACCAAGAATGAGAGTTGCCTAAAT
TCCAGAGAGACCTCTTTCATAACTAATGGGAGTTGCCTGGCCTCCAGAAAGACCTCTTTTAT
GATGGCCCTGTGCCTTAGTAGTATTTATGAAGACTTGAAGATGTACCAGGTGGAGTTCAAGA
CCATGAATGCAAAGCTTCTGATGGATCCTAAGAGGCAGATCTTTCTAGATCAAAACATGCTG
GCAGTTATTGATGAGCTGATGCAGGCCCTGAATTTCAACAGTGAGACTGTGCCACAAAAATC
CTCCCTTGAAGAACCGGATTTTTATAAAACTAAAATCAAGCTCTGCATACTTCTTCATGCTT
TCAGAATTCGGGCAGTGACTATTGATAGAGTGATGAGCTATCTGAATGCTTCCTAATAActc
gagtcaccaggcg

[00235] The virus TAV-hIL12-furin carries the TAV-255 deletion in the E1A promoter and SEQ ID NO: 25 in the E1B-19K region. The control virus TAV-Δ19k carries the TAV-255 deletion in the E1A promoter and a deletion of the E1B-19K region.

[00236] To test for oncolytic activity and IL12 expression, A549 cells were infected with TAV-Δ19k, TAV-hIL12-furin, TAV-TAV-IXrL5-Empty, and TAV-IXrL5-hIL12 at an MOI of 5 in triplicate. Four days after infection, the conditioned media was collected and IL12 was measured in an ELISA that detects only the assembled IL12A-IL12B heterodimer, and the remaining cells were stained with crystal violet. As shown in FIG. 5, TAV-hIL12-furin was slightly less lytic than the corresponding control virus TAV-Δ19k which is in agreement with our general experience when transgenes are inserted in the E1B-19K region, while TAV-IXrL5-hIL12 was as lytic as TAV-IXrL5-Empty and TAV-Δ19k. This demonstrated that insertion of expression cassettes in the IX-E2 and L5-E4 regions has minimal impact on viral fitness and may be superior to insertion of transgenes in the E1B-19K region in this regard.

[00237] As shown in FIG. 6, TAV-hIL12-furin expressed higher levels of IL12 than the viruses using IX-E2 and L5-E4 cassettes. This suggested that at least one of the two expression cassettes may be suboptimal and prompted further investigation into improving the design. However, the IL12 protein generated from TAV-hIL12-furin contains a non-native RAKR sequence (the remaining furin recognition site) at the C-terminus of the IL12B chain which might lead to undesirable *in vivo* effects such as immunogenicity against the transgene or against native IL12, whereas the IL12 generated by TAV-IXrL5-hIL12 has the advantage that it is completely native.

[00238] **Example 7**

[00239] Based on the relatively low expression from the L5-E4 cassette compared to the IX-E2 cassette observed, we hypothesized that the L5-E4 cassette was the cause of the low expression of the IL-12 heterodimer. We revised the L5-E4 region to include bidirectional polydenylation signals at both ends, similar to the approach that was used for the IX-E2 region.

[00240] The nucleotide sequence SEQ ID NO: 26 was cloned into the L5-E4 region, showing the polyadenylation signals of the L5 and E4 transcripts at the 5' and 3' ends and all polyadenylation signals and the SwaI restriction site underlined.

SEQ ID NO: 26 [L5 revised SV40]

AATAAAAGGTTTATTCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCC
AGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCC
CAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTC
CCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCA

TGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGAGCTATTCC
AGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTTGCAAAGATTTA
AATAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGAATCGTTTGTGTTATGTTTCA
ACGTGTTTATT

[00241] The virus TAV-(IXr)Empty-(L5r)Empty was made with the TAV-255 deletion in the E1A promoter, the revised IX-E2 cassette without a transgene in the IX-E2 region shown in SEQ ID NO: 21, and the revised L5-E4 cassette without a transgene in the L5-E4 region shown in SEQ ID NO: 26.

[00242] The virus TAV-(IXr)mIL7-(L5r)mGMCSF contained the TAV-255 deletion in the E1A promoter, the revised IX-E2 cassette shown in SEQ ID NO: 21 with the mouse GMCSF gene cloned into the NotI site as shown in SEQ ID NO: 20, and the revised L5-E4 cassette shown in SEQ ID NO: 26 with the mouse IL-7 gene cloned into the SwaI site as shown in SEQ ID NO: 18.

[00243] The virus TAV-(IXr)mGMCSF-(L5r)mIL7 contained the TAV-255 deletion in the E1A promoter, the revised IX-E2 cassette shown in SEQ ID NO: 21 with the mouse IL-7 gene cloned into the NotI site as shown in SEQ ID NO: 27, and the revised L5-E4 cassette shown in SEQ ID NO: 26 with the mouse GMCSF gene cloned into the SwaI site as shown in SEQ ID NO: 19. Thus, the viruses TAV-(IXr)mGMCSF-(L5r)mIL7 and TAV-(IXr)mIL7-(L5r)mGMCSF differed only in which gene of IL-7 and GMCSF was inserted into which site: the revised IX-E2 or the revised L5-E4 site.

SEQ ID NO: 27 [IX mIL7]

atagggagacccgcggccATGTTCCATGTTTCTTTTAGATATATCTTTGGAATTCCTCCACT
GATCCTTGTTCTGCTGCCTGTCACATCATCTGAGTGCCACATTAAAGACAAAGAAGGTAAAG
CATATGAGAGTGTACTGATGATCAGCATCGATGAATTGGACAAAATGACAGGAACTGATAGT
AATTGCCCGAATAATGAACCAAACTTTTTTAGAAAACATGTATGTGATGATACAAAGGAAGC
TGCTTTTCTAAATCGTGCTGCTCGCAAGTTGAAGCAATTTCTTAAAATGAATATCAGTGAAG
AATTCAATGTCCACTTACTAACAGTATCACAAGGCACACAAACACTGGTGAACTGCACAAGT
AAGGAAGAAAAAAACGTAAAGGAACAGAAAAAGAATGATGCATGTTTCCTAAAGAGACTACT
GAGAGAAATAAAAACTTGTTGGAATAAAATTTTGAAGGGCAGTATATAAggccgctgtgcct
tctagt

[00244] To test transgene expression from these viruses, A549 cells were infected with each virus at an MOI of 5 in triplicate. Conditioned media was collected four days later and GMCSF and IL-7 were measured in ELISAs. For both IL-7 and GMCSF, expression was higher from the virus carrying the gene in the revised IX-E2 site than the revised L5-E4 site. This confirmed the previous finding that the expression cassette at IX-E2 using the CMV

promoter expressed at higher levels than the cassette at L5-E4 using the SV40 promoter and this was not affected by revising the L5-E4 site to include bidirectional polyadenylation signals.

[00245] Example 8

[00246] We next investigated further improving the promoter in the L5-E4 site. The SV40 promoter that was initially used had a point mutation of G (in the wild-type SV40 sequence) to T (in the L5-E4 insert) at the major transcription start site, so we generated an L5-E4 insert with that nucleotide changed back to the wild-type G as shown in SEQ ID NO: 28 with the previously mutated nucleotide, the polyadenylation signals, and the SwaI restriction site underlined. We also investigated using a different promoter, and generated an L5-E4 insert that used the human EF1A promoter instead of the SV40 promoter. SEQ ID NO: 29 shows the L5-E4 insert using the human EF1A promoter, shown from the polyadenylation signal from the L5 transcript to the polyadenylation signal of the E4 transcript, with the polyadenylation signals and the SwaI restriction site underlined.

SEQ ID NO: 28 [L5 revised SV40 wt Empty]

AATAAAAGGTTTATTCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCC
AGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCC
CAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTC
CCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCA
TGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCC
AGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTTGCAAAGATTTA
AATAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGAATCGTTTGTGTTATGTTTCA
ACGTGTTTATT

SEQ ID NO: 29 [L5 revised EF1A Empty]

AATAAAAGGTTTATTAGGCGGCCTCCCCGTCACCACCCCCCCCAACCCGCCCCGACCGGAGC
TGAGAGTAATTCATACAAAAGGACTCGCCCCTGCCTTGGGGAATCCCAGGGACCGTCGTTAA
ACTCCCACTAACGTAGAACCCAGAGATCGCTGCGTTCCCGCCCCCTCACCCGCCCGCTCTCG
TCATCACTGAGGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCA
CATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGA
AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGG
TGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTG
CCGCCAGAACACAATTTAAATAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGAAT
CGTTTGTGTTATGTTTCAACGTGTTTATT

[00247] To test these revised expression cassettes in L5-E4, we generated viruses carrying the mouse IL-7 gene in the IX-E2 site and the mouse GMCSF gene in the new L5-E4 sites. The mouse IL-7 gene used in the IX-E2 site was modified by introducing a silent mutations in two regions near the 3' end of the gene with sequence AATAAA that might by processed as a polyadenylation signal to lead to reduced expression, substituting synonymous

sequences that would not change the encoded protein sequence but would eliminate the internal AATAAA sequences; SEQ ID NO: 30 shows that nucleotide sequence with the IL-7 coding nucleotides capitalized and the mutations underlined, and the flanking nucleotides of the IX-E2 cassette in lower case with the residual nucleotides from the NotI site underlined. To attempt to improve expression of the mouse GMCSF gene in the L5-E4 sites, a consensus Kozak sequence (nucleotides GCCACC) was included between the residual nucleotides of the SwaI site and the start codon of the GMCSF gene; SEQ ID NO: 31 shows the nucleotide sequence with the mouse GMCSF gene and the Kozak sequence capitalized with the Kozak sequence underlined, and with the flanking nucleotides of the L5-E4 cassette in lower case with the residual nucleotides from the SwaI site underlined.

SEQ ID NO: 30 [mIL7 no poly-A]

atagggagacccgcggccATGTTCCATGTTTCTTTTAGATATATCTTTGGAATTCCTCCACT
GATCCTTGTTCTGCTGCCTGTCACATCATCTGAGTGCCACATTAAAGACAAAGAAGGTAAAG
CATATGAGAGTGTACTGATGATCAGCATCGATGAATTGGACAAAATGACAGGAACTGATAGT
AATTGCCCGAATAATGAACCAAACTTTTTTAGAAAACATGTATGTGATGATACAAAGGAAGC
TGCTTTTCTAAATCGTGCTGCTCGCAAGTTGAAGCAATTTCTTAAAATGAATATCAGTGAAG
AATTCAATGTCCACTTACTAACAGTATCACAAGGCACACAAACACTGGTGAACTGCACAAGT
AAGGAAGAAAAAAACGTAAAGGAACAGAAAAAGAATGATGCATGTTTCCTAAAGAGACTACT
GAGAGAAATCAAAACTTGTTGGAACAAAATTTTGAAGGGCAGTATATAAggccgctgtgcct
tctagt

SEQ ID NO: 31 [mGMCSF Kozak]

atttGCCACCATGTGGCTGCAGAATTTACTTTTCCTGGGCATTGTGGTCTACAGCCTCTCAG
CACCCACCCGCTCACCCATCACTGTCACCCGGCCTTGGAAGCATGTAGAGGCCATCAAAGAA
GCCCTGAACCTCCTGGATGACATGCCTGTCACGTTGAATGAAGAGGTAGAAGTCGTCTCTAA
CGAGTTCTCCTTCAAGAAGCTAACATGTGTGCAGACCCGCCTGAAGATATTCGAGCAGGGTC
TACGGGGCAATTTCACCAAACTCAAGGGCGCCTTGAACATGACAGCCAGCTACTACCAGACA
TACTGCCCCCCAACTCCGGAAACGGACTGTGAAACACAAGTTACCACCTATGCGGATTTCAT
AGACAGCCTTAAAACCTTTCTGACTGATATCCCCTTTGAATGCAAAAAACCAGGCCAAAAAT
GAaaataacttgtttattgcag

[00248] The virus TAV-(IXr)mIL7noPA-(L5SV40wt)KozakmGMCSF contained the TAV-255 deletion in the E1A promoter, the revised IX-E2 insert shown in SEQ ID NO: 21 with the mouse IL-7 gene with synonymous mutations at the potential internal polyadenylation sites cloned into the NotI site as shown in SEQ ID NO: 30, and the L5-E4 insert with the wild-type SV40 promoter shown in SEQ ID NO: 28 with the mouse GMCSF gene including a consensus Kozak sequence cloned into the SwaI site as shown in SEQ ID NO: 31. The virus TAV-(IXr)mIL7noPA-(L5EF1A)KozakmGMCSF contains the TAV-255 deletion in the E1A promoter, the revised IX-E2 insert shown in SEQ ID NO: 21 with the mouse IL-7 gene with synonymous mutations at the potential internal polyadenylation sites cloned into the NotI site

as shown in SEQ ID NO: 30, and the L5-E4 insert with the human EF1A promoter shown in SEQ ID NO: 29 with the mouse GMCSF gene including a consensus Kozak sequence cloned into the SwaI site as shown in SEQ ID NO: 31.

[00249] To test these viruses for transgene expression, A549 cells were infected with the two viruses in triplicate at an MOI of 5. Four days later, conditioned media was collected and used in ELISAs to measure IL-7 and GMCSF expression. Results are shown in FIG. 7. The human EF1A promoter had moderately higher expression and was chosen for further development. The virus TAV-(IXr)mIL7noPA-(L5EF1A)KozakmGMCSF was prepared as a pharmaceutical agent for mouse experiments by amplifying the virus in serum-free suspension culture of SF-BMAdR 281 cells (derived from A549 cells), lysing the cells, purifying the virus with chromatography, and dialyzing into a buffer with 25 mM NaCl, 20 mM Tris, 2.5% glycerol at pH 8. The virus thus prepared had a viral particle concentration of 5.8×10^{11} vp/ml and an infectious titer of 3.0×10^{10} IU/ml.

[00250] **Example 9**

[00251] We further investigated whether deletion of the adenoviral death protein (ADP) could improve expression of the transgenes. ADP is expressed late during viral replication and lyses the host cell to release progeny virions, so its removal might allow cells to live and express the transgenes longer before they are killed. The nucleotide sequence of the ADP gene in the context of the E3 RIDα, RIDβ, and 14.7K deletion used in the TAV-(IXr)mIL7noPA-(L5EF1A)KozakmGMCSF virus is shown in SEQ ID NO: 32 with the nucleotides encoding ADP capitalized, the site of the E3 RIDα, RIDβ, and 14.7K deletion as a hyphen, and the flanking adenoviral nucleotides in lowercase. To create the ΔADP deletion, the underlined nucleotides within SEQ ID NO: 32 were deleted.

SEQ ID NO: 32 [ADP]

Gaaaatgccttaatttactaagttacaaagctaatgtcaccactaactgctttactcgctgc
ttgcaaaacaaattcaaaaagttagcattataattagaataggatttaaacccccggtcat
ttcctgctcaataccattcccctgaacaattgactctatgtgggatatgctccagcgctaca
accttgaagtcaggcttcctggatgtcagcatctgactttggccagcacctgtcccgcggat
ttgttccagtccaactacagcgacccaccctaacagagATGACCAACACAACCAACGCGGCC
GCCGCTACCGGACTTACATCTACCACAAATACACCCCAAGTTTCTGCCTTTGTCAATAACTG
GGATAACTTGGGCATGTGGTGGTTCTCCATAGCGCTTATGTTTGTATGCCTTATTATTATGT
GGCTCATCTGCTGCCTAAAGCGCAAACGCGCCCGACCACCCATCTATAGTCCCATCATTGTG
CTACACCCAAACAATGATGGAATCCATAGATTGGACGGACTGAAACACATGTTCTTTTCTCT
TACAGTATGAtaataaaaaaaaataataaagca

[00252] The virus TAV-(IXr)mIL7noPA-(L5EF1A)KozakmGMCSF-ΔADP was created with an identical genome to TAV-(IXr)mIL7noPA-(L5EF1A)KozakmGMCSF except that it also contains a deletion of the nucleotides of the ADP gene as underlined of SEQ ID NO: 32.

[00253] To test whether ADP deletion leads to longer term and higher transgene expression, A549 cells were infected with TAV-(IXr)mIL7noPA-(L5EF1A)KozakmGMCSF and TAV-(IXr)mIL7noPA-(L5EF1A)KozakmGMCSF-ΔADP at an MOI of 5, and every three days after infection the conditioned media was collected to measure IL-7 and GMCSF in an ELISA. Results are shown in FIG. 8. There was no convincing difference in transgene expression levels between the two viruses, although the cells were observed during the course of the experiment and cell death was delayed from about 3 days after infection with TAV-(IXr)mIL7noPA-(L5EF1A)KozakmGMCSF to about 9 days after infection with TAV-(IXr)mIL7noPA-(L5EF1A)KozakmGMCSF-ΔADP. So although expression of the soluble cytokines as in this experiment was not enhanced, for some applications such as expression of costimulatory molecules on the infected cell's surface, it might be advantageous to delete ADP so the cell survives longer and the costimulatory molecule on its surface can activate target cells over a longer period of time.

[00254] **Example 10**

[00255] We then used the IX-E2 and L5-E4 sites as part of the design of a virus expressing three transgenes: the costimulatory molecules CD80 and CD137L and the adhesion molecule ICAM1. We previously created a virus carrying all three of these transgenes in place of the 5' end of the viral E1B-19K gene with an IRES between each of the three transgenes. The nucleotide sequence of that virus in the E1B-19K region is shown in SEQ ID NO: 33, with 5' flanking adenoviral nucleotide sequence and a SalI restriction site used for cloning (lower case), mouse CD80 (capitalized), the IRES from encephalomyocarditis virus (lower case), mouse CD137L (capitalized), the IRES from foot and mouth disease virus (lower case), mouse ICAM1 (capitalized), and 3' flanking adenoviral nucleotide sequence including an XhoI restriction site used for cloning. That virus, TAV-mCD80(IRES)mCD137L(IRES)mICAM1, contains the TAV-255 deletion in the E1A promoter, SEQ ID NO: 33 in the E1B-19K site, and deletions of the E3 region ADP, RIDα, RIDβ, and 14.7K genes and the E4 region ORF1-4 genes.

SEQ ID NO: 33 [Costim IRES]

atctgacctcgtcgacATGGCTTGCAATTGTCAGTTGATGCAGGATACACCACTCCTCAAGT
TTCCATGTCCAAGGCTCATTCTTCTCTTTGTGCTGCTGATTCGTCTTTCACAAGTGTCTTCA
GATGTTGATGAACAACTGTCCAAGTCAGTGAAAGATAAGGTATTGCTGCCTTGCCGTTACAA
CTCTCCTCATGAAGATGAGTCTGAAGACCGAATCTACTGGCAAAAACATGACAAAGTGGTGC
TGTCTGTCATTGCTGGGAAACTAAAAGTGTGGCCCGAGTATAAGAACCGGACTTTATATGAC
AACACTACCTACTCTCTTATCATCCTGGGCCTGGTCCTTTCAGACCGGGGCACATACAGCTG
TGTCGTTCAAAAGAAGGAAAGAGGAACGTATGAAGTTAAACACTTGGCTTTAGTAAAGTTGT
CCATCAAAGCTGACTTCTCTACCCCCAACATAACTGAGTCTGGAAACCCATCTGCAGACACT
AAAAGGATTACCTGCTTTGCTTCCGGGGGTTTCCCAAAGCCTCGCTTCTCTTGGTTGGAAAA
TGGAAGAGAATTACCTGGCATCAATACGACAATTTCCCAGGATCCTGAATCTGAATTGTACA
CCATTAGTAGCCAACTAGATTTCAATACGACTCGCAACCACACCATTAAGTGTCTCATTAAA
TATGGAGATGCTCACGTGTCAGAGGACTTCACCTGGGAAAAACCCCCAGAAGACCCTCCTGA
TAGCAAGAACACACTTGTGCTCTTTGGGGCAGGATTCGGCGCAGTAATAACAGTCGTCGTCA
TCGTTGTCATCATCAAATGCTTCTGTAAGCACAGAAGCTGTTTCAGAAGAAATGAGGCAAGC
AGAGAAACAAACAACAGCCTTACCTTCGGGCCTGAAGAAGCATTAGCTGAACAGACCGTCTT
CCTTTAGtaacgttactggccgaagccgcttggaataaggccggtgtgcgtttgtctatatg
ttattttccaccatattgccgtcttttggcaatgtgagggcccggaaacctggccctgtctt
cttgacgagcattcctaggggtctttcccctctgccaaaggaatgcaaggtctgttgaatg
tcgtgaaggaagcagttcctctggaagcttcttgaagacaaacaacgtctgtagcgacccttt
tgcaggcagcggaacccccccacctggcgacaggtgcctctgcggccaaaagccacgtgtata
agatacacctgcaaaggcggcacaaccccagtgccacgttgtgagttggatagttgtggaaa
gagtcaaatggctctcctcaagcgtattcaacaaggggctgaaggatgcccagaaggtaccc
cattgtatgggatctgatctggggcctcggtgcacatgctttacatgtgtttagtcgaggtt
aaaaaacgtctaggccccccgaaccacggggacgtggttttcctttgaaaaacacgatgata
atATGGACCAGCACACACTTGATGTGGAGGATACCGCGGATGCCAGACATCCAGCAGGTACT
TCGTGCCCCTCGGATGCGGCGCTCCTCAGAGATACCGGGCTCCTCGCGGACGCTGCGCTCCT
CTCAGATACTGTGCGCCCCACAAATGCCGCGCTCCCCACGGATGCTGCCTACCCTGCGGTTA
ATGTTCGGGATCGCGAGGCCGCGTGGCCGCCTGCACTGAACTTCTGTTCCCGCCACCCAAAG
CTCTATGGCCTAGTCGCTTTGGTTTTGCTGCTTCTGATCGCCGCCTGTGTTCCTATCTTCAC
CCGCACCGAGCCTCGGCCAGCGCTCACAATCACCACCTCGCCCAACCTGGGTACCCGAGAGA
ATAATGCAGACCAGGTCACCCCTGTTTCCCACATTGGCTGCCCCAACACTACACAACAGGGC
TCTCCTGTGTTCGCCAAGCTACTGGCTAAAAACCAAGCATCGTTGTGCAATACAACTCTGAA
CTGGCACAGCCAAGATGGAGCTGGGAGCTCATACCTATCTCAAGGTCTGAGGTACGAAGAAG
ACAAAAAGGAGTTGGTGGTAGACAGTCCCGGGCTCTACTACGTATTTTTGGAACTGAAGCTC
AGTCCAACATTCACAAACACAGGCCACAAGGTGCAGGGCTGGGTCTCTCTTGTTTTGCAAGC
AAAGCCTCAGGTAGATGACTTTGACAACTTGGCCCTGACAGTGGAACTGTTCCCTTGCTCCA
TGGAGAACAAGTTAGTGGACCGTTCCTGGAGTCAACTGTTGCTCCTGAAGGCTGGCCACCGC
CTCAGTGTGGGTCTGAGGGCTTATCTGCATGGAGCCCAGGATGCATACAGAGACTGGGAGCT
GTCTTATCCCAACACCACCAGCTTTGGACTCTTTCTTGTGAAACCCGACAACCCATGGGAAT
GAggtttccacaactgataaaactcgtgcaacttgaaactccgcctggtctttccaggtcta
gaggggttacactttgtactgtgctcgactccacgcccggtccactggcgggtgttagtagc
agcactgttgtttcgtagcggagcatggtggccgtgggaactcctccttggtgacaagggcc
cacggggccgaaagccacgtccagacggacccaccatgtgtgcaaccccagcacggcaactt
ttactgcgaacaccaccttaaggtgacactggtactggtactcggtcactggtgacaggcta
aggatgcccttcaggtaccccgaggtaacacgggacactcgggatctgagaaggggattggg
acttctttaaaagtgcccagtttaaaaagcttctacgcctgaataggcgaccggaggccggc
gcctttccattacccactactaaatccATGGCTTCAACCCGTGCCAAGCCCACGCTACCTCT
GCTCCTGGCCCTGGTCACCGTTGTGATCCCTGGGCCTGGTGATGCTCAGGTATCCATCCATC
CCAGAGAAGCCTTCCTGCCCCAGGGTGGGTCCGTGCAGGTGAACTGTTCTTCCTCATGCAAG
GAGGACCTCAGCCTGGGCTTGGAGACTCAGTGGCTGAAAGATGAGCTCGAGAGTGGACCCAA

CTGGAAGCTGTTTGAGCTGAGCGAGATCGGGGAGGACAGCAGTCCGCTGTGCTTTGAGAACT
GTGGCACCGTGCAGTCGTCCGCTTCCGCTACCATCACCGTGTATTCGTTTCCGGAGAGTGTG
GAGCTGAGACCTCTGCCAGCCTGGCAGCAAGTAGGCAAGGACCTCACCCTGCGCTGCCACGT
GGATGGTGGAGCACCGCGGACCCAGCTCTCAGCAGTGCTGCTCCGTGGGGAGGAGATACTGA
GCCGCCAGCCAGTGGGTGGGCACCCCAAGGACCCCAAGGAGATCACATTCACGGTGCTGGCT
AGCAGAGGGGACCACGGAGCCAATTTCTCATGCCGCACAGAACTGGATCTCAGGCCGCAAGG
GCTGGCATTGTTCTCTAATGTCTCCGAGGCCAGGAGCCTCCGGACTTTCGATCTTCCAGCTA
CCATCCCAAAGCTCGACACCCCTGACCTCCTGGAGGTGGGCACCCAGCAGAAGTTGTTTTGC
TCCCTGGAAGGCCTGTTTCCTGCCTCTGAAGCTCGGATATACCTGGAGCTGGGAGGCCAGAT
GCCGACCCAGGAGAGCACAAACAGCAGTGACTCTGTGTCAGCCACTGCCTTGGTAGAGGTGA
CTGAGGAGTTCGACAGAACCCTGCCGCTGCGCTGCGTTTTGGAGCTAGCGGACCAGATCCTG
GAGACGCAGAGGACCTTAACAGTCTACAACTTTTCAGCTCCGGTCCTGACCCTGAGCCAGCT
GGAGGTCTCGGAAGGGAGCCAAGTAACTGTGAAGTGTGAAGCCCACAGTGGGTCGAAGGTGG
TTCTTCTGAGCGGCGTCGAGCCTAGGCCACCCACCCCGCAGGTCCAATTCACACTGAATGCC
AGCTCGGAGGATCACAAACGAAGCTTCTTTTGCTCTGCCGCTCTGGAGGTGGCGGGAAAGTT
CCTGTTTAAAAACCAGACCCTGGAACTGCACGTGCTGTATGGTCCTCGGCTGGACGAGACGG
ACTGCTTGGGGAACTGGACCTGGCAAGAGGGGTCTCAGCAGACTCTGAAATGCCAGGCCTGG
GGGAACCCATCTCCTAAGATGACCTGCAGACGGAAGGCAGATGGTGCCCTGCTGCCCATCGG
GGTGGTGAAGTCTGTCAAACAGGAGATGAATGGTACATACGTGTGCCATGCCTTTAGCTCCC
ATGGGAATGTCACCAGGAATGTGTACCTGACAGTACTGTACCACTCTCAAAATAACTGGACT
ATAATCATTCTGGTGCCAGTACTGCTGGTCATTGTGGGCCTCGTGATGGCAGCCTCTTATGT
TTATAACCGCCAGAGAAAGATCAGGATATACAAGTTACAGAAGGCTCAGGAGGAGGCCATAA
AACTCAAGGGACAAGCCCCACCTCCCTGActcgagtcaccaggcg

[00256] We found that expression of genes after an IRES has generally been poor compared to genes where translation is not initiated by an IRES, so we investigated using the IX-E2 and L5-E4 sites as an alternative strategy. We generated the virus TAV-(19k)mCD80-(IX)mCD137L-(L5)mICAM1 carrying mouse CD80 in the E1B-19K site as shown in SEQ ID NO: 34 the CD80 gene capitalized and the flanking adenoviral sequence and restriction sites lower case (this used a Bsu36I restriction site instead of the SalI and XhoI restriction sites used in the other viruses), the mouse CD137L gene in the IX-E2 site of SEQ ID NO: 21 with the CD137L gene inserted in the NotI site as shown in SEQ ID NO: 35 with the CD137L gene capitalized and the flanking expression cassette sequence and residual NotI restriction site in lowercase, and the mouse ICAM1 gene in the L5-E4 site of SEQ ID NO: 29 with the ICAM1 gene inserted in the SwaI site as shown in SEQ ID NO: 36 with the ICAM1 gene capitalized and the flanking expression cassette sequence and residual SwaI site in lowercase. This virus also contains the TAV-255 deletion in the E1A promoter, deletion of the E3 region ADP, RIDα, RIDβ, and 14.7K genes, and deletion of the E4 region ORF1-4 genes.

SEQ ID NO: 34 [19k mCD80]

atctgacctcATGGCTTGCAATTGTCAGTTGATGCAGGATACACCACTCCTCAAGTTTCCAT
GTCCAAGGCTCATTCTTCTCTTTGTGCTGCTGATTCGTCTTTCACAAGTGTCTTCAGATGTT
GATGAACAACTGTCCAAGTCAGTGAAAGATAAGGTATTGCTGCCTTGCCGTTACAACTCTCC

TCATGAAGATGAGTCTGAAGACCGAATCTACTGGCAAAAACATGACAAAGTGGTGCTGTCTG
TCATTGCTGGGAAACTAAAAGTGTGGCCCGAGTATAAGAACCGGACTTTATATGACAACACT
ACCTACTCTCTTATCATCCTGGGCCTGGTCCTTTCAGACCGGGGCACATACAGCTGTGTCGT
TCAAAAGAAGGAAAGAGGAACGTATGAAGTTAAACACTTGGCTTTAGTAAAGTTGTCCATCA
AAGCTGACTTCTCTACCCCCAACATAACTGAGTCTGGAAACCCATCTGCAGACACTAAAAGG
ATTACCTGCTTTGCTTCCGGGGGTTTCCCAAAGCCTCGCTTCTCTTGGTTGGAAAATGGAAG
AGAATTACCTGGCATCAATACGACAATTTCCCAGGATCCTGAATCTGAATTGTACACCATTA
GTAGCCAACTAGATTTCAATACGACTCGCAACCACACCATTAAGTGTCTCATTAAATATGGA
GATGCTCACGTGTCAGAGGACTTCACCTGGGAAAAACCCCCAGAAGACCCTCCTGATAGCAA
GAACACACTTGTGCTCTTTGGGGCAGGATTCGGCGCAGTAATAACAGTCGTCGTCATCGTTG
TCATCATCAAATGCTTCTGTAAGCACAGAAGCTGTTTCAGAAGAAATGAGGCAAGCAGAGAA
ACAAACAACAGCCTTACCTTCGGGCCTGAAGAAGCATTAGCTGAACAGACCGTCTTCCTTTA
Gtcaggtgaatctgggtcacc

SEQ ID NO: 35 [IX mCD137L]

atagggagacccgcggccATGGACCAGCACACACTTGATGTGGAGGATACCGCGGATGCCAG
ACATCCAGCAGGTACTTCGTGCCCCTCGGATGCGGCGCTCCTCAGAGATACCGGGCTCCTCG
CGGACGCTGCGCTCCTCTCAGATACTGTGCGCCCCACAAATGCCGCGCTCCCCACGGATGCT
GCCTACCCTGCGGTTAATGTTCGGGATCGCGAGGCCGCGTGGCCGCCTGCACTGAACTTCTG
TTCCCGCCACCCAAAGCTCTATGGCCTAGTCGCTTTGGTTTTGCTGCTTCTGATCGCCGCCT
GTGTTCCTATCTTCACCCGCACCGAGCCTCGGCCAGCGCTCACAATCACCACCTCGCCCAAC
CTGGGTACCCGAGAGAATAATGCAGACCAGGTCACCCCTGTTTCCCACATTGGCTGCCCCAA
CACTACACAACAGGGCTCTCCTGTGTTCGCCAAGCTACTGGCTAAAAACCAAGCATCGTTGT
GCAATACAACTCTGAACTGGCACAGCCAAGATGGAGCTGGGAGCTCATACCTATCTCAAGGT
CTGAGGTACGAAGAAGACAAAAAGGAGTTGGTGGTAGACAGTCCCGGGCTCTACTACGTATT
TTTGGAACTGAAGCTCAGTCCAACATTCACAAACACAGGCCACAAGGTGCAGGGCTGGGTCT
CTCTTGTTTTGCAAGCAAAGCCTCAGGTAGATGACTTTGACAACTTGGCCCTGACAGTGGAA
CTGTTCCCTTGCTCCATGGAGAACAAGTTAGTGGACCGTTCCTGGAGTCAACTGTTGCTCCT
GAAGGCTGGCCACCGCCTCAGTGTGGGTCTGAGGGCTTATCTGCATGGAGCCCAGGATGCAT
ACAGAGACTGGGAGCTGTCTTATCCCAACACCACCAGCTTTGGACTCTTTCTTGTGAAACCC
GACAACCCATGGGAATGAggccgctgtgccttctagt

SEQ ID NO: 36 [L5 mICAM1]

cgccagaacacatttATGGCTTCAACCCGTGCCAAGCCCACGCTACCTCTGCTCCTGGCCCT
GGTCACCGTTGTGATCCCTGGGCCTGGTGATGCTCAGGTATCCATCCATCCCAGAGAAGCCT
TCCTGCCCCAGGGTGGGTCCGTGCAGGTGAACTGTTCTTCCTCATGCAAGGAGGACCTCAGC
CTGGGCTTGGAGACTCAGTGGCTGAAAGATGAGCTCGAGAGTGGACCCAACTGGAAGCTGTT
TGAGCTGAGCGAGATCGGGGAGGACAGCAGTCCGCTGTGCTTTGAGAACTGTGGCACCGTGC
AGTCGTCCGCTTCCGCTACCATCACCGTGTATTCGTTTCCGGAGAGTGTGGAGCTGAGACCT
CTGCCAGCCTGGCAGCAAGTAGGCAAGGACCTCACCCTGCGCTGCCACGTGGATGGTGGAGC
ACCGCGGACCCAGCTCTCAGCAGTGCTGCTCCGTGGGGAGGAGATACTGAGCCGCCAGCCAG
TGGGTGGGCACCCCAAGGACCCCAAGGAGATCACATTCACGGTGCTGGCTAGCAGAGGGGAC
CACGGAGCCAATTTCTCATGCCGCACAGAACTGGATCTCAGGCCGCAAGGGCTGGCATTGTT
CTCTAATGTCTCCGAGGCCAGGAGCCTCCGGACTTTCGATCTTCCAGCTACCATCCCAAAGC
TCGACACCCCTGACCTCCTGGAGGTGGGCACCCAGCAGAAGTTGTTTTGCTCCCTGGAAGGC
CTGTTTCCTGCCTCTGAAGCTCGGATATACCTGGAGCTGGGAGGCCAGATGCCGACCCAGGA
GAGCACAAACAGCAGTGACTCTGTGTCAGCCACTGCCTTGGTAGAGGTGACTGAGGAGTTCG
ACAGAACCCTGCCGCTGCGCTGCGTTTTGGAGCTAGCGGACCAGATCCTGGAGACGCAGAGG
ACCTTAACAGTCTACAACTTTTCAGCTCCGGTCCTGACCCTGAGCCAGCTGGAGGTCTCGGA

AGGGAGCCAAGTAACTGTGAAGTGTGAAGCCCACAGTGGGTCGAAGGTGGTTCTTCTGAGCG
GCGTCGAGCCTAGGCCACCCACCCCGCAGGTCCAATTCACACTGAATGCCAGCTCGGAGGAT
CACAAACGAAGCTTCTTTTGCTCTGCCGCTCTGGAGGTGGCGGGAAAGTTCCTGTTTAAAAA
CCAGACCCTGGAACTGCACGTGCTGTATGGTCCTCGGCTGGACGAGACGGACTGCTTGGGGA
ACTGGACCTGGCAAGAGGGGTCTCAGCAGACTCTGAAATGCCAGGCCTGGGGGAACCCATCT
CCTAAGATGACCTGCAGACGGAAGGCAGATGGTGCCCTGCTGCCCATCGGGGTGGTGAAGTC
TGTCAAACAGGAGATGAATGGTACATACGTGTGCCATGCCTTTAGCTCCCATGGGAATGTCA
CCAGGAATGTGTACCTGACAGTACTGTACCACTCTCAAAATAACTGGACTATAATCATTCTG
GTGCCAGTACTGCTGGTCATTGTGGGCCTCGTGATGGCAGCCTCTTATGTTTATAACCGCCA
GAGAAAGATCAGGATATACAAGTTACAGAAGGCTCAGGAGGAGGCCATAAAACTCAAGGGAC
AAGCCCCACCTCCCTGAaaataacttgtttattgcag

[00257] To test for expression from these two viruses: A549 cells, HT29 cells, ADS12 cells, and F244 cells were infected at an MOI of 3 with TAV-mCD80(IRES)mCD137L(IRES)mICAM1, TAV-(19k)mCD80-(IX)mCD137L-(L5)mICAM1, or the control virus TAV-(19k)Empty-(IX)Empty-(L5)Empty which has the same structure as TAV-(19k)mCD80-(IX)mCD137L-(L5)mICAM1 but without the transgenes. Two days later, the cells were stained for CD80, CD137L, and ICAM1 and results are shown in FIG. 9-FIG. 12. While there was poor expression of the CD137L and ICAM1 genes when expressed after the IRESes in TAV-mCD80(IRES)mCD137L(IRES)mICAM1, there was robust expression of those genes from the IX-E2 and L5-E4 sites with TAV-(19k)mCD80-(IX)mCD137L-(L5)mICAM1.

[00258] **Example 11**

[00259] While the experiments described above used an adenovirus based on human adenovirus type 5, other adenoviruses have a very similar structure and have clearly identifiable sites homologous to the IX-E2 and L5-E4 sites described above. For example, human adenovirus type 35 has the sequence in the IX-E2 site shown in SEQ ID NO: 37 and has the sequence in the L5-E4 site shown in SEQ ID NO: 38 where the polyadenylation signals are underlined in each sequence.

SEQ ID NO: 37 [Ad35 wt IX-E2]
AATAAAAAAAATTCCAGAATCAATGAATAAATAAACGAGCTTGTTGTTGATTTAAAATCAAG
TGTTTTTATT

SEQ ID NO: 38 [Ad35 wt L5-E4]
AATAAAGTTTAAGTGTTTTTATT

[00260] To determine whether expression cassettes could be inserted into these sites, the IX-E2 site was modified with the same sequence used in the adenovirus type 5 revised IX-E2 site as shown in SEQ ID NO: 39 (the expression cassette was inserted in the opposite

orientation as with adenovirus type 5, so the flanking viral sequence in lowercase is the reverse complement of conventional annotation which is shown in SEQ ID NO: 37, and the L5-E4 site was modified with the same sequence used in the adenovirus type 5 site with the EF1A promoter as shown in SEQ ID NO: 40. An adenovirus type 35 carrying both of those expression cassettes in site IX-E2 and L5-E4 as well as deletions in the E3 RIDα, RIDβ, and 14.7K genes and the E4 ORF1-4 genes was rescued, demonstrating that these sites can be used for insertion of expression cassettes in other serotypes of adenovirus. The strategy of inserting an expression cassette between two adjacent transcription units with polyadenylation sites facing each other is not in principle restricted to adenoviruses and could potentially be applied to other viruses as well.

SEQ ID NO: 39 [Ad35 IX-E2 cassette]

tcgagatcggtggtccagggcataccgtgcgcgaaaaatgaaataaaATACACCTTTTTTCG
ATTGTACGTATTTTTATTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC
CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGT
CAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCC
AAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACA
TGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATG
GTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCC
AAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTC
CAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAG
GTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAAT
TAATACGACTCACTATAGGGAGACCCGCGGCCGCTGTGCCTTCTAGTTGCCAGCCATCTGTT
GTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTA
ATAAAaacacttgattttaaatcaacaacaagctcgtttatttat

SEQ ID NO: 40 [Ad35 L5-E4 cassette]

tttcttttcttacattacagaagacgacaactaaaataaaAGGTTTATTAGGCGGCCTCCCC
GTCACCACCCCCCCCAACCCGCCCCGACCGGAGCTGAGAGTAATTCATACAAAAGGACTCGC
CCCTGCCTTGGGGAATCCCAGGGACCGTCGTTAAACTCCCACTAACGTAGAACCCAGAGATC
GCTGCGTTCCCGCCCCCTCACCCGCCCGCTCTCGTCATCACTGAGGTGGAGAAGAGCATGCG
TGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGG
GGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTG
ATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTA
GTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAATTTAAATAACTTGT
TTATTGCAGCTTATAATGGTTACAaataaagtttaagtgtttttatttaaaatcacaaaatt
cg

[00261] Example 12

[00262] We used the revised IX-E2 and L5-E4 sites to generate a virus carrying the mouse IL12A and IL12B genes for use as a model in preclinical experiments. The gene for mouse IL12A was cloned into the NotI restriction site of the revised IX-E2 site with an

expression cassette shown in SEQ ID NO: 21 to generate the sequence of SEQ ID NO: 42, with the residual nucleotides of the NotI restriction site underlined. The gene for mouse IL12B was cloned into the SwaI restriction site of the L5-E4 site with an expression cassette using the EF1A promoter shown in SEQ ID NO: 29 to generate the sequence of SEQ ID NO: 43, with the residual nucleotides of the SwaI restriction site underlined.

SEQ ID NO: 42

CTATAGGGAGACCCGCGGCCATGTGTCAATCACGCTACCTCCTCTTTTTGGCCACCCTTGCC
CTCCTAAACCACCTCAGTTTGGCCAGGGTCATTCCAGTCTCTGGACCTGCCAGGTGTCTTAG
CCAGTCCCGAAACCTGCTGAAGACCACAGATGACATGGTGAAGACGGCCAGAGAAAAACTGA
AACATTATTCCTGCACTGCTGAAGACATCGATCATGAAGACATCACACGGGACCAAACCAGC
ACATTGAAGACCTGTTTACCACTGGAACTACACAAGAACGAGAGTTGCCTGGCTACTAGAGA
GACTTCTTCCACAACAAGAGGGAGCTGCCTGCCCCCACAGAAGACGTCTTTGATGATGACCC
TGTGCCTTGGTAGCATCTATGAGGACTTGAAGATGTACCAGACAGAGTTCCAGGCCATCAAC
GCAGCACTTCAGAATCACAACCATCAGCAGATCATTCTAGACAAGGGCATGCTGGTGGCCAT
CGATGAGCTGATGCAGTCTCTGAATCATAATGGCGAGACTCTGCGCCAGAAACCTCCTGTGG
GAGAAGCAGACCCTTACAGAGTGAAAATGAAGCTCTGCATCCTGCTTCACGCCTTCAGCACC
CGCGTCGTGACCATCAACAGGGTGATGGGCTATCTGAGCTCCGCCTGAGGCCGCTGTGCCTT
CTAGTT

SEQ ID NO: 43

TTGCCGCCAGAACACAATTTATGTGTCCTCAGAAGCTAACCATCTCCTGGTTTGCCATCGTT
TTGCTGGTGTCTCCACTCATGGCCATGTGGGAGCTGGAGAAAGACGTTTATGTTGTAGAGGT
GGACTGGACTCCCGATGCCCCTGGAGAAACAGTGAACCTCACCTGTGACACGCCTGAAGAAG
ATGACATCACCTGGACCTCAGACCAGAGACATGGAGTCATAGGCTCTGGAAAGACCCTGACC
ATCACTGTCAAAGAGTTTCTAGATGCTGGCCAGTACACCTGCCACAAAGGAGGCGAGACTCT
GAGCCACTCACATCTGCTGCTCCACAAGAAGGAAAATGGAATTTGGTCCACTGAAATTTTAA
AAAATTTCAAAAACAAGACTTTCCTGAAGTGTGAAGCACCAAATTACTCCGGACGGTTCACG
TGCTCATGGCTGGTGCAAAGAAACATGGACTTGAAGTTCAACATCAAGAGCAGTAGCAGTTC
CCCTGACTCTCGGGCAGTGACATGTGGAATGGCGTCTCTGTCTGCAGAGAAGGTCACACTGG
ACCAAAGGGACTATGAGAAGTATTCAGTGTCCTGCCAGGAGGATGTCACCTGCCCAACTGCC
GAGGAGACCCTGCCCATTGAACTGGCGTTGGAAGCACGGCAGCAGAATAAATATGAGAACTA
CAGCACCAGCTTCTTCATCAGGGACATCATCAAACCAGACCCGCCCAAGAACTTGCAGATGA
AGCCTTTGAAGAACTCACAGGTGGAGGTCAGCTGGGAGTACCCTGACTCCTGGAGCACTCCC
CATTCCTACTTCTCCCTCAAGTTCTTTGTTCGAATCCAGCGCAAGAAAGAAAAGATGAAGGA
GACAGAGGAGGGGTGTAACCAGAAAGGTGCGTTCCTCGTAGAGAAGACATCTACCGAAGTCC
AATGCAAAGGCGGGAATGTCTGCGTGCAAGCTCAGGATCGCTATTACAATTCCTCATGCAGC
AAGTGGGCATGTGTTCCCTGCAGGGTCCGATCCTAGAAATAACTTGTTTATTGCAG

[00263] The virus TAV-IX5-Empty was generated carrying the TAV-255 deletion in the E1A promoter, the IX-E2 expression cassette without a transgene shown in SEQ ID NO: 21, and the L5-E4 expression cassette without a transgene shown in SEQ ID NO: 29. The virus TAV-IX5-mIL12 was generated carrying the TAV-255 deletion in the E1A promoter, the IX-E2 expression cassette including the mouse IL12A gene of SEQ ID NO: 42, and the L5-E4 expression cassette including the mouse IL12B gene of SEQ ID NO: 43.

[00264] To test these viruses for oncolysis, A549 cells were infected with the TAV-IX5-Empty or TAV-IX5-mIL12 viruses at an MOI of 5 or kept as non-infected controls, and wells were stained with crystal violet every two days after infection. As shown in FIG. 13, both the empty control virus and the virus carrying mouse IL-12 were lytic within 4-6 days.

[00265] To test for transgene expression, A549 cells were infected with the TAV-IX5-Empty or TAV-IX5-mIL12 viruses at an MOI of 5 in triplicate and conditioned media was collected five days after infection to measure mouse IL-12 with an ELISA detecting only the heterodimer with both the mouse IL12A and mouse IL12B chains. As shown in FIG. 14, TAV-IX5-mIL12 induced expression of high levels of the IL-12 heterodimer.

[00266] **Human adenovirus 5, complete genome**

NCBI Reference Sequence: AC_000008.1 (SEQ ID NO: 1)

>AC_000008.1 Human adenovirus 5, complete genome

CATCATCAATAATATACCTTATTTTGGATTGAAGCCAATATGATAATGAGGGGGTGGAGTTTGTGACGTG
GCGCGGGGCGTGGGAACGGGGCGGGTGACGTAGTAGTGTGGCGGAAGTGTGATGTTGCAAGTGTGGCGGA
ACACATGTAAGCGACGGATGTGGCAAAAGTGACGTTTTTGGTGTGCGCCGGTGTACACAGGAAGTGACAA
TTTTCGCGCGGTTTTAGGCGGATGTTGTAGTAAATTTGGGCGTAACCGAGTAAGATTTGGCCATTTTCGC
GGGAAAACTGAATAAGAGGAAGTGAAATCTGAATAATTTTGTGTTACTCATAGCGCGTAATATTTGTCTA
GGGCCGCGGGGACTTTGACCGTTTACGTGGAGACTCGCCCAGGTGTTTTTCTCAGGTGTTTTCCGCGTTC
CGGGTCAAAGTTGGCGTTTTATTATTATAGTCAGCTGACGTGTAGTGTATTTATACCCGGTGAGTTCCTC
AAGAGGCCACTCTTGAGTGCCAGCGAGTAGAGTTTTCTCCTCCGAGCCGCTCCGACACCGGGACTGAAAA
TGAGACATATTATCTGCCACGGAGGTGTTATTACCGAAGAAATGGCCGCCAGTCTTTTGGACCAGCTGAT
CGAAGAGGTACTGGCTGATAATCTTCCACCTCCTAGCCATTTTGAACCACCTACCCTTCACGAACTGTAT
GATTTAGACGTGACGGCCCCCGAAGATCCCAACGAGGAGGCGGTTTCGCAGATTTTTCCCGACTCTGTAA
TGTTGGCGGTGCAGGAAGGGATTGACTTACTCACTTTTCCGCCGGCGCCCGGTTCTCCGGAGCCGCCTCA
CCTTTCCCGGCAGCCCGAGCAGCCGGAGCAGAGAGCCTTGGGTCCGGTTTCTATGCCAAACCTTGTACCG
GAGGTGATCGATCTTACCTGCCACGAGGCTGGCTTTCCACCCAGTGACGACGAGGATGAAGAGGGTGAGG
AGTTTGTGTTAGATTATGTGGAGCACCCCGGGCACGGTTGCAGGTCTTGTCATTATCACCGGAGGAATAC
GGGGGACCCAGATATTATGTGTTCGCTTTGCTATATGAGGACCTGTGGCATGTTTGTCTACAGTAAGTGA
AAATTATGGGCAGTGGGTGATAGAGTGGTGGGTTTGGTGTGGTAATTTTTTTTTTAATTTTTACAGTTTT
GTGGTTTAAAGAATTTTGTATTGTGATTTTTTTAAAAGGTCCTGTGTCTGAACCTGAGCCTGAGCCCGAG
CCAGAACCGGAGCCTGCAAGACCTACCCGCCGTCCTAAAATGGCGCCTGCTATCCTGAGACGCCCGACAT
CACCTGTGTCTAGAGAATGCAATAGTAGTACGGATAGCTGTGACTCCGGTCCTTCTAACACACCTCCTGA
GATACACCCGGTGGTCCCGCTGTGCCCCATTAAACCAGTTGCCGTGAGAGTTGGTGGGCGTCGCCAGGCT
GTGGAATGTATCGAGGACTTGCTTAACGAGCCTGGGCAACCTTTGGACTTGAGCTGTAAACGCCCCAGGC
CATAAGGTGTAAACCTGTGATTGCGTGTGTGGTTAACGCCTTTGTTTGCTGAATGAGTTGATGTAAGTTT
AATAAAGGGTGAGATAATGTTTAACTTGCATGGCGTGTTAAATGGGGCGGGGCTTAAAGGGTATATAATG
CGCCGTGGGCTAATCTTGGTTACATCTGACCTCATGGAGGCTTGGGAGTGTTTGGAAGATTTTTCTGCTG
TGCGTAACTTGCTGGAACAGAGCTCTAACAGTACCTCTTGGTTTTGGAGGTTTCTGTGGGGCTCATCCCA
GGCAAAGTTAGTCTGCAGAATTAAGGAGGATTACAAGTGGGAATTTGAAGAGCTTTTGAAATCCTGTGGT

GAGCTGTTTGATTCTTTGAATCTGGGTCACCAGGCGCTTTTCCAAGAGAAGGTCATCAAGACTTTGGATT
TTTCCACACCGGGGCGCGCTGCGGCTGCTGTTGCTTTTTTGAGTTTTATAAAGGATAAATGGAGCGAAGA
AACCCATCTGAGCGGGGGGTACCTGCTGGATTTTCTGGCCATGCATCTGTGGAGAGCGGTTGTGAGACAC
AAGAATCGCCTGCTACTGTTGTCTTCCGTCCGCCCGGCGATAATACCGACGGAGGAGCAGCAGCAGCAGC
AGGAGGAAGCCAGGCGGCGGCGGCAGGAGCAGAGCCCATGGAACCCGAGAGCCGGCCTGGACCCTCGGGA
ATGAATGTTGTACAGGTGGCTGAACTGTATCCAGAACTGAGACGCATTTTGACAATTACAGAGGATGGGC
AGGGGCTAAAGGGGGTAAAGAGGGAGCGGGGGGCTTGTGAGGCTACAGAGGAGGCTAGGAATCTAGCTTT
TAGCTTAATGACCAGACACCGTCCTGAGTGTATTACTTTTCAACAGATCAAGGATAATTGCGCTAATGAG
CTTGATCTGCTGGCGCAGAAGTATTCCATAGAGCAGCTGACCACTTACTGGCTGCAGCCAGGGGATGATT
TTGAGGAGGCTATTAGGGTATATGCAAAGGTGGCACTTAGGCCAGATTGCAAGTACAAGATCAGCAAACT
TGTAAATATCAGGAATTGTTGCTACATTTCTGGGAACGGGGCCGAGGTGGAGATAGATACGGAGGATAGG
GTGGCCTTTAGATGTAGCATGATAAATATGTGGCCGGGGGTGCTTGGCATGGACGGGGTGGTTATTATGA
ATGTAAGGTTTACTGGCCCCAATTTTAGCGGTACGGTTTTCCTGGCCAATACCAACCTTATCCTACACGG
TGTAAGCTTCTATGGGTTTAACAATACCTGTGTGGAAGCCTGGACCGATGTAAGGGTTCGGGGCTGTGCC
TTTTACTGCTGCTGGAAGGGGGTGGTGTGTCGCCCCAAAAGCAGGGCTTCAATTAAGAAATGCCTCTTTG
AAAGGTGTACCTTGGGTATCCTGTCTGAGGGTAACTCCAGGGTGCGCCACAATGTGGCCTCCGACTGTGG
TTGCTTCATGCTAGTGAAAAGCGTGGCTGTGATTAAGCATAACATGGTATGTGGCAACTGCGAGGACAGG
GCCTCTCAGATGCTGACCTGCTCGGACGGCAACTGTCACCTGCTGAAGACCATTCACGTAGCCAGCCACT
CTCGCAAGGCCTGGCCAGTGTTTGAGCATAACATACTGACCCGCTGTTCCTTGCATTTGGGTAACAGGAG
GGGGGTGTTCCTACCTTACCAATGCAATTTGAGTCACACTAAGATATTGCTTGAGCCCGAGAGCATGTCC
AAGGTGAACCTGAACGGGGTGTTTGACATGACCATGAAGATCTGGAAGGTGCTGAGGTACGATGAGACCC
GCACCAGGTGCAGACCCTGCGAGTGTGGCGGTAAACATATTAGGAACCAGCCTGTGATGCTGGATGTGAC
CGAGGAGCTGAGGCCCGATCACTTGGTGCTGGCCTGCACCCGCGCTGAGTTTGGCTCTAGCGATGAAGAT
ACAGATTGAGGTACTGAAATGTGTGGGCGTGGCTTAAGGGTGGGAAAGAATATATAAGGTGGGGGTCTTA
TGTAGTTTTGTATCTGTTTTGCAGCAGCCGCCGCCGCCATGAGCACCAACTCGTTTGATGGAAGCATTGT
GAGCTCATATTTGACAACGCGCATGCCCCCATGGGCCGGGGTGCGTCAGAATGTGATGGGCTCCAGCATT
GATGGTCGCCCCGTCCTGCCCGCAAACTCTACTACCTTGACCTACGAGACCGTGTCTGGAACGCCGTTGG
AGACTGCAGCCTCCGCCGCCGCTTCAGCCGCTGCAGCCACCGCCCGCGGGATTGTGACTGACTTTGCTTT
CCTGAGCCCGCTTGCAAGCAGTGCAGCTTCCCGTTCATCCGCCCGCGATGACAAGTTGACGGCTCTTTTG
GCACAATTGGATTCTTTGACCCGGGAACTTAATGTCGTTTCTCAGCAGCTGTTGGATCTGCGCCAGCAGG
TTTCTGCCCTGAAGGCTTCCTCCCCTCCCAATGCGGTTTAAAACATAAATAAAAAACCAGACTCTGTTTG
GATTTGGATCAAGCAAGTGTCTTGCTGTCTTTATTTAGGGGTTTTGCGCGCGCGGTAGGCCCGGGACCAG
CGGTCTCGGTCGTTGAGGGTCCTGTGTATTTTTTCCAGGACGTGGTAAAGGTGACTCTGGATGTTCAGAT
ACATGGGCATAAGCCCGTCTCTGGGGTGGAGGTAGCACCACTGCAGAGCTTCATGCTGCGGGGTGGTGTT
GTAGATGATCCAGTCGTAGCAGGAGCGCTGGGCGTGGTGCCTAAAAATGTCTTTCAGTAGCAAGCTGATT
GCCAGGGGCAGGCCCTTGGTGTAAGTGTTTACAAAGCGGTTAAGCTGGGATGGGTGCATACGTGGGGATA
TGAGATGCATCTTGGACTGTATTTTTAGGTTGGCTATGTTCCCAGCCATATCCCTCCGGGGATTCATGTT
GTGCAGAACCACCAGCACAGTGTATCCGGTGCACTTGGGAAATTTGTCATGTAGCTTAGAAGGAAATGCG
TGGAAGAACTTGGAGACGCCCTTGTGACCTCCAAGATTTTCCATGCATTCGTCCATAATGATGGCAATGG
GCCCACGGGCGGCGGCCTGGGCGAAGATATTTCTGGGATCACTAACGTCATAGTTGTGTTCCAGGATGAG
ATCGTCATAGGCCATTTTTACAAAGCGCGGGCGGAGGGTGCCAGACTGCGGTATAATGGTTCCATCCGGC
CCAGGGGCGTAGTTACCCTCACAGATTTGCATTTCCCACGCTTTGAGTTCAGATGGGGGGATCATGTCTA
CCTGCGGGGCGATGAAGAAAACGGTTTCCGGGGTAGGGGAGATCAGCTGGGAAGAAAGCAGGTTCCTGAG
CAGCTGCGACTTACCGCAGCCGGTGGGCCCGTAAATCACACCTATTACCGGGTGCAACTGGTAGTTAAGA

GAGCTGCAGCTGCCGTCATCCCTGAGCAGGGGGGCCACTTCGTTAAGCATGTCCCTGACTCGCATGTTTT
CCCTGACCAAATCCGCCAGAAGGCGCTCGCCGCCCAGCGATAGCAGTTCTTGCAAGGAAGCAAAGTTTTT
CAACGGTTTGAGACCGTCCGCCGTAGGCATGCTTTTGAGCGTTTGACCAAGCAGTTCCAGGCGGTCCCAC
AGCTCGGTCACCTGCTCTACGGCATCTCGATCCAGCATATCTCCTCGTTTCGCGGGTTGGGGCGGCTTTC
GCTGTACGGCAGTAGTCGGTGCTCGTCCAGACGGGCCAGGGTCATGTCTTTCCACGGGCGCAGGGTCCTC
GTCAGCGTAGTCTGGGTCACGGTGAAGGGGTGCGCTCCGGGCTGCGCGCTGGCCAGGGTGCGCTTGAGGC
TGGTCCTGCTGGTGCTGAAGCGCTGCCGGTCTTCGCCCTGCGCGTCGGCCAGGTAGCATTTGACCATGGT
GTCATAGTCCAGCCCCTCCGCGGCGTGGCCCTTGGCGCGCAGCTTGCCCTTGGAGGAGGCGCCGCACGAG
GGGCAGTGCAGACTTTTGAGGGCGTAGAGCTTGGGCGCGAGAAATACCGATTCCGGGGAGTAGGCATCCG
CGCCGCAGGCCCCGCAGACGGTCTCGCATTCCACGAGCCAGGTGAGCTCTGGCCGTTCGGGGTCAAAAAC
CAGGTTTCCCCCATGCTTTTTGATGCGTTTCTTACCTCTGGTTTCCATGAGCCGGTGTCCACGCTCGGTG
ACGAAAAGGCTGTCCGTGTCCCCGTATACAGACTTGAGAGGCCTGTCCTCGAGCGGTGTTCCGCGGTCCT
CCTCGTATAGAAACTCGGACCACTCTGAGACAAAGGCTCGCGTCCAGGCCAGCACGAAGGAGGCTAAGTG
GGAGGGGTAGCGGTCGTTGTCCACTAGGGGGTCCACTCGCTCCAGGGTGTGAAGACACATGTCGCCCTCT
TCGGCATCAAGGAAGGTGATTGGTTTGTAGGTGTAGGCCACGTGACCGGGTGTTCCTGAAGGGGGGCTAT
AAAAGGGGGTGGGGGCGCGTTCGTCCTCACTCTCTTCCGCATCGCTGTCTGCGAGGGCCAGCTGTTGGGG
TGAGTACTCCCTCTGAAAAGCGGGCATGACTTCTGCGCTAAGATTGTCAGTTTCCAAAAACGAGGAGGAT
TTGATATTCACCTGGCCCGCGGTGATGCCTTTGAGGGTGGCCGCATCCATCTGGTCAGAAAAGACAATCT
TTTTGTTGTCAAGCTTGGTGGCAAACGACCCGTAGAGGGCGTTGGACAGCAACTTGGCGATGGAGCGCAG
GGTTTGGTTTTTGTCGCGATCGGCGCGCTCCTTGGCCGCGATGTTTAGCTGCACGTATTCGCGCGCAACG
CACCGCCATTCGGGAAAGACGGTGGTGCGCTCGTCGGGCACCAGGTGCACGCGCCAACCGCGGTTGTGCA
GGGTGACAAGGTCAACGCTGGTGGCTACCTCTCCGCGTAGGCGCTCGTTGGTCCAGCAGAGGCGGCCGCC
CTTGCGCGAGCAGAATGGCGGTAGGGGGTCTAGCTGCGTCTCGTCCGGGGGGTCTGCGTCCACGGTAAAG
ACCCCGGGCAGCAGGCGCGCGTCGAAGTAGTCTATCTTGCATCCTTGCAAGTCTAGCGCCTGCTGCCATG
CGCGGGCGGCAAGCGCGCGCTCGTATGGGTTGAGTGGGGGACCCCATGGCATGGGGTGGGTGAGCGCGGA
GGCGTACATGCCGCAAATGTCGTAAACGTAGAGGGGCTCTCTGAGTATTCCAAGATATGTAGGGTAGCAT
CTTCCACCGCGGATGCTGGCGCGCACGTAATCGTATAGTTCGTGCGAGGGAGCGAGGAGGTCGGGACCGA
GGTTGCTACGGGCGGGCTGCTCTGCTCGGAAGACTATCTGCCTGAAGATGGCATGTGAGTTGGATGATAT
GGTTGGACGCTGGAAGACGTTGAAGCTGGCGTCTGTGAGACCTACCGCGTCACGCACGAAGGAGGCGTAG
GAGTCGCGCAGCTTGTTGACCAGCTCGGCGGTGACCTGCACGTCTAGGGCGCAGTAGTCCAGGGTTTCCT
TGATGATGTCATACTTATCCTGTCCCTTTTTTTTCCACAGCTCGCGGTTGAGGACAAACTCTTCGCGGTC
TTTCCAGTACTCTTGGATCGGAAACCCGTCGGCCTCCGAACGGTAAGAGCCTAGCATGTAGAACTGGTTG
ACGGCCTGGTAGGCGCAGCATCCCTTTTCTACGGGTAGCGCGTATGCCTGCGCGGCCTTCCGGAGCGAGG
TGTGGGTGAGCGCAAAGGTGTCCCTGACCATGACTTTGAGGTACTGGTATTTGAAGTCAGTGTCGTCGCA
TCCGCCCTGCTCCCAGAGCAAAAAGTCCGTGCGCTTTTTGGAACGCGGATTTGGCAGGGCGAAGGTGACA
TCGTTGAAGAGTATCTTTCCCGCGCGAGGCATAAAGTTGCGTGTGATGCGGAAGGGTCCCGGCACCTCGG
AACGGTTGTTAATTACCTGGGCGGCGAGCACGATCTCGTCAAAGCCGTTGATGTTGTGGCCCACAATGTA
AAGTTCCAAGAAGCGCGGGATGCCCTTGATGGAAGGCAATTTTTTAAGTTCCTCGTAGGTGAGCTCTTCA
GGGGAGCTGAGCCCGTGCTCTGAAAGGGCCCAGTCTGCAAGATGAGGGTTGGAAGCGACGAATGAGCTCC
ACAGGTCACGGGCCATTAGCATTTGCAGGTGGTCGCGAAAGGTCCTAAACTGGCGACCTATGGCCATTTT
TTCTGGGGTGATGCAGTAGAAGGTAAGCGGGTCTTGTTCCCAGCGGTCCCATCCAAGGTTCGCGGCTAGG
TCTCGCGCGGCAGTCACTAGAGGCTCATCTCCGCCGAACTTCATGACCAGCATGAAGGGCACGAGCTGCT
TCCCAAAGGCCCCCATCCAAGTATAGGTCTCTACATCGTAGGTGACAAAGAGACGCTCGGTGCGAGGATG
CGAGCCGATCGGGAAGAACTGGATCTCCCGCCACCAATTGGAGGAGTGGCTATTGATGTGGTGAAAGTAG

AAGTCCCTGCGACGGGCCGAACACTCGTGCTGGCTTTTGTAAAAACGTGCGCAGTACTGGCAGCGGTGCA
CGGGCTGTACATCCTGCACGAGGTTGACCTGACGACCGCGCACAAGGAAGCAGAGTGGGAATTTGAGCCC
CTCGCCTGGCGGGTTTGGCTGGTGGTCTTCTACTTCGGCTGCTTGTCCTTGACCGTCTGGCTGCTCGAGG
GGAGTTACGGTGGATCGGACCACCACGCCGCGCGAGCCCAAAGTCCAGATGTCCGCGCGCGGCGGTCGGA
GCTTGATGACAACATCGCGCAGATGGGAGCTGTCCATGGTCTGGAGCTCCCGCGGCGTCAGGTCAGGCGG
GAGCTCCTGCAGGTTTACCTCGCATAGACGGGTCAGGGCGCGGGCTAGATCCAGGTGATACCTAATTTCC
AGGGGCTGGTTGGTGGCGGCGTCGATGGCTTGCAAGAGGCCGCATCCCCGCGGCGCGACTACGGTACCGC
GCGGCGGGCGGTGGGCCGCGGGGGTGTCCTTGGATGATGCATCTAAAAGCGGTGACGCGGGCGAGCCCCC
GGAGGTAGGGGGGGCTCCGGACCCGCCGGGAGAGGGGGCAGGGGCACGTCGGCGCCGCGCGCGGGCAGGA
GCTGGTGCTGCGCGCGTAGGTTGCTGGCGAACGCGACGACGCGGCGGTTGATCTCCTGAATCTGGCGCCT
CTGCGTGAAGACGACGGGCCCGGTGAGCTTGAGCCTGAAAGAGAGTTCGACAGAATCAATTTCGGTGTCG
TTGACGGCGGCCTGGCGCAAAATCTCCTGCACGTCTCCTGAGTTGTCTTGATAGGCGATCTCGGCCATGA
ACTGCTCGATCTCTTCCTCCTGGAGATCTCCGCGTCCGGCTCGCTCCACGGTGGCGGCGAGGTCGTTGGA
AATGCGGGCCATGAGCTGCGAGAAGGCGTTGAGGCCTCCCTCGTTCCAGACGCGGCTGTAGACCACGCCC
CCTTCGGCATCGCGGGCGCGCATGACCACCTGCGCGAGATTGAGCTCCACGTGCCGGGCGAAGACGGCGT
AGTTTCGCAGGCGCTGAAAGAGGTAGTTGAGGGTGGTGGCGGTGTGTTCTGCCACGAAGAAGTACATAAC
CCAGCGTCGCAACGTGGATTCGTTGATATCCCCCAAGGCCTCAAGGCGCTCCATGGCCTCGTAGAAGTCC
ACGGCGAAGTTGAAAAACTGGGAGTTGCGCGCCGACACGGTTAACTCCTCCTCCAGAAGACGGATGAGCT
CGGCGACAGTGTCGCGCACCTCGCGCTCAAAGGCTACAGGGGCCTCTTCTTCTTCTTCAATCTCCTCTTC
CATAAGGGCCTCCCCTTCTTCTTCTTCTGGCGGCGGTGGGGGAGGGGGGACACGGCGGCGACGACGGCGC
ACCGGGAGGCGGTCGACAAAGCGCTCGATCATCTCCCCGCGGCGACGGCGCATGGTCTCGGTGACGGCGC
GGCCGTTCTCGCGGGGGCGCAGTTGGAAGACGCCGCCCGTCATGTCCCGGTTATGGGTTGGCGGGGGGCT
GCCATGCGGCAGGGATACGGCGCTAACGATGCATCTCAACAATTGTTGTGTAGGTACTCCGCCGCCGAGG
GACCTGAGCGAGTCCGCATCGACCGGATCGGAAAACCTCTCGAGAAAGGCGTCTAACCAGTCACAGTCGC
AAGGTAGGCTGAGCACCGTGGCGGGCGGCAGCGGGCGGCGGTCGGGGTTGTTTCTGGCGGAGGTGCTGCT
GATGATGTAATTAAAGTAGGCGGTCTTGAGACGGCGGATGGTCGACAGAAGCACCATGTCCTTGGGTCCG
GCCTGCTGAATGCGCAGGCGGTCGGCCATGCCCCAGGCTTCGTTTTGACATCGGCGCAGGTCTTTGTAGT
AGTCTTGCATGAGCCTTTCTACCGGCACTTCTTCTTCTCCTTCCTCTTGTCCTGCATCTCTTGCATCTAT
CGCTGCGGCGGCGGCGGAGTTTGGCCGTAGGTGGCGCCCTCTTCCTCCCATGCGTGTGACCCCGAAGCCC
CTCATCGGCTGAAGCAGGGCTAGGTCGGCGACAACGCGCTCGGCTAATATGGCCTGCTGCACCTGCGTGA
GGGTAGACTGGAAGTCATCCATGTCCACAAAGCGGTGGTATGCGCCCGTGTTGATGGTGTAAGTGCAGTT
GGCCATAACGGACCAGTTAACGGTCTGGTGACCCGGCTGCGAGAGCTCGGTGTACCTGAGACGCGAGTAA
GCCCTCGAGTCAAATACGTAGTCGTTGCAAGTCCGCACCAGGTACTGGTATCCCACCAAAAAGTGCGGCG
GCGGCTGGCGGTAGAGGGGCCAGCGTAGGGTGGCCGGGGCTCCGGGGGCGAGATCTTCCAACATAAGGCG
ATGATATCCGTAGATGTACCTGGACATCCAGGTGATGCCGGCGGCGGTGGTGGAGGCGCGCGGAAAGTCG
CGGACGCGGTTCCAGATGTTGCGCAGCGGCAAAAAGTGCTCCATGGTCGGGACGCTCTGGCCGGTCAGGC
GCGCGCAATCGTTGACGCTCTAGACCGTGCAAAAGGAGAGCCTGTAAGCGGGCACTCTTCCGTGGTCTGG
TGGATAAATTCGCAAGGGTATCATGGCGGACGACCGGGGTTCGAGCCCCGTATCCGGCCGTCCGCCGTGA
TCCATGCGGTTACCGCCCGCGTGTCGAACCCAGGTGTGCGACGTCAGACAACGGGGGAGTGCTCCTTTTG
GCTTCCTTCCAGGCGCGGCGGCTGCTGCGCTAGCTTTTTTGGCCACTGGCCGCGCGCAGCGTAAGCGGTT
AGGCTGGAAAGCGAAAGCATTAAGTGGCTCGCTCCCTGTAGCCGGAGGGTTATTTTCCAAGGGTTGAGTC
GCGGGACCCCCGGTTCGAGTCTCGGACCGGCCGGACTGCGGCGAACGGGGGTTTGCCTCCCCGTCATGCA
AGACCCCGCTTGCAAATTCCTCCGGAAACAGGGACGAGCCCCTTTTTTGCTTTTCCCAGATGCATCCGGT
GCTGCGGCAGATGCGCCCCCCTCCTCAGCAGCGGCAAGAGCAAGAGCAGCGGCAGACATGCAGGGCACCC

TCCCCTCCTCCTACCGCGTCAGGAGGGGCGACATCCGCGGTTGACGCGGCAGCAGATGGTGATTACGAAC
CCCCGCGGCGCCGGGCCCGGCACTACCTGGACTTGGAGGAGGGCGAGGGCCTGGCGCGGCTAGGAGCGCC
CTCTCCTGAGCGGTACCCAAGGGTGCAGCTGAAGCGTGATACGCGTGAGGCGTACGTGCCGCGGCAGAAC
CTGTTTCGCGACCGCGAGGGAGAGGAGCCCGAGGAGATGCGGGATCGAAAGTTCCACGCAGGGCGCGAGC
TGCGGCATGGCCTGAATCGCGAGCGGTTGCTGCGCGAGGAGGACTTTGAGCCCGACGCGCGAACCGGGAT
TAGTCCCGCGCGCGCACACGTGGCGGCCGCCGACCTGGTAACCGCATACGAGCAGACGGTGAACCAGGAG
ATTAACTTTCAAAAAAGCTTTAACAACCACGTGCGTACGCTTGTGGCGCGCGAGGAGGTGGCTATAGGAC
TGATGCATCTGTGGGACTTTGTAAGCGCGCTGGAGCAAAACCCAAATAGCAAGCCGCTCATGGCGCAGCT
GTTCCTTATAGTGCAGCACAGCAGGGACAACGAGGCATTCAGGGATGCGCTGCTAAACATAGTAGAGCCC
GAGGGCCGCTGGCTGCTCGATTTGATAAACATCCTGCAGAGCATAGTGGTGCAGGAGCGCAGCTTGAGCC
TGGCTGACAAGGTGGCCGCCATCAACTATTCCATGCTTAGCCTGGGCAAGTTTTACGCCCGCAAGATATA
CCATACCCCTTACGTTCCCATAGACAAGGAGGTAAAGATCGAGGGGTTCTACATGCGCATGGCGCTGAAG
GTGCTTACCTTGAGCGACGACCTGGGCGTTTATCGCAACGAGCGCATCCACAAGGCCGTGAGCGTGAGCC
GGCGGCGCGAGCTCAGCGACCGCGAGCTGATGCACAGCCTGCAAAGGGCCCTGGCTGGCACGGGCAGCGG
CGATAGAGAGGCCGAGTCCTACTTTGACGCGGGCGCTGACCTGCGCTGGGCCCCAAGCCGACGCGCCCTG
GAGGCAGCTGGGGCCGGACCTGGGCTGGCGGTGGCACCCGCGCGCGCTGGCAACGTCGGCGGCGTGGAGG
AATATGACGAGGACGATGAGTACGAGCCAGAGGACGGCGAGTACTAAGCGGTGATGTTTCTGATCAGATG
ATGCAAGACGCAACGGACCCGGCGGTGCGGGCGGCGCTGCAGAGCCAGCCGTCCGGCCTTAACTCCACGG
ACGACTGGCGCCAGGTCATGGACCGCATCATGTCGCTGACTGCGCGCAATCCTGACGCGTTCCGGCAGCA
GCCGCAGGCCAACCGGCTCTCCGCAATTCTGGAAGCGGTGGTCCCGGCGCGCGCAAACCCCACGCACGAG
AAGGTGCTGGCGATCGTAAACGCGCTGGCCGAAAACAGGGCCATCCGGCCCGACGAGGCCGGCCTGGTCT
ACGACGCGCTGCTTCAGCGCGTGGCTCGTTACAACAGCGGCAACGTGCAGACCAACCTGGACCGGCTGGT
GGGGGATGTGCGCGAGGCCGTGGCGCAGCGTGAGCGCGCGCAGCAGCAGGGCAACCTGGGCTCCATGGTT
GCACTAAACGCCTTCCTGAGTACACAGCCCGCCAACGTGCCGCGGGGACAGGAGGACTACACCAACTTTG
TGAGCGCACTGCGGCTAATGGTGACTGAGACACCGCAAAGTGAGGTGTACCAGTCTGGGCCAGACTATTT
TTTCCAGACCAGTAGACAAGGCCTGCAGACCGTAAACCTGAGCCAGGCTTTCAAAAACTTGCAGGGGCTG
TGGGGGGTGCGGGCTCCCACAGGCGACCGCGCGACCGTGTCTAGCTTGCTGACGCCCAACTCGCGCCTGT
TGCTGCTGCTAATAGCGCCCTTCACGGACAGTGGCAGCGTGTCCCGGGACACATACCTAGGTCACTTGCT
GACACTGTACCGCGAGGCCATAGGTCAGGCGCATGTGGACGAGCATACTTTCCAGGAGATTACAAGTGTC
AGCCGCGCGCTGGGGCAGGAGGACACGGGCAGCCTGGAGGCAACCCTAAACTACCTGCTGACCAACCGGC
GGCAGAAGATCCCCTCGTTGCACAGTTTAAACAGCGAGGAGGAGCGCATTTTGCGCTACGTGCAGCAGAG
CGTGAGCCTTAACCTGATGCGCGACGGGGTAACGCCCAGCGTGGCGCTGGACATGACCGCGCGCGAACATG
GAACCGGGCATGTATGCCTCAAACCGGCCGTTTATCAACCGCCTAATGGACTACTTGCATCGCGCGGCCG
CCGTGAACCCCGAGTATTTCACCAATGCCATCTTGAACCCGCACTGGCTACCGCCCCCTGGTTTCTACAC
CGGGGGATTCGAGGTGCCCGAGGGTAACGATGGATTCCTCTGGGACGACATAGACGACAGCGTGTTTTCC
CCGCAACCGCAGACCCTGCTAGAGTTGCAACAGCGCGAGCAGGCAGAGGCGGCGCTGCGAAAGGAAAGCT
TCCGCAGGCCAAGCAGCTTGTCCGATCTAGGCGCTGCGGCCCCGCGGTCAGATGCTAGTAGCCCATTTCC
AAGCTTGATAGGGTCTCTTACCAGCACTCGCACCACCCGCCCGCGCCTGCTGGGCGAGGAGGAGTACCTA
AACAACTCGCTGCTGCAGCCGCAGCGCGAAAAAAACCTGCCTCCGGCATTTCCCAACAACGGGATAGAGA
GCCTAGTGGACAAGATGAGTAGATGGAAGACGTACGCGCAGGAGCACAGGGACGTGCCAGGCCCGCGCCC
GCCCACCCGTCGTCAAAGGCACGACCGTCAGCGGGGTCTGGTGTGGGAGGACGATGACTCGGCAGACGAC
AGCAGCGTCCTGGATTTGGGAGGGAGTGGCAACCCGTTTGCGCACCTTCGCCCCAGGCTGGGGAGAATGT
TTTAAAAAAAAAAAAAGCATGATGCAAAATAAAAAACTCACCAAGGCCATGGCACCGAGCGTTGGTTTTCT
TGTATTCCCCTTAGTATGCGGCGCGCGGCGATGTATGAGGAAGGTCCTCCTCCCTCCTACGAGAGTGTGG

TGAGCGCGGCGCCAGTGGCGGCGGCGCTGGGTTCTCCCTTCGATGCTCCCCTGGACCCGCCGTTTGTGCC
TCCGCGGTACCTGCGGCCTACCGGGGGGGAGAAACAGCATCCGTTACTCTGAGTTGGCACCCCTATTCGAC
ACCACCCGTGTGTACCTGGTGGACAACAAGTCAACGGATGTGGCATCCCTGAACTACCAGAACGACCACA
GCAACTTTCTGACCACGGTCATTCAAAACAATGACTACAGCCCGGGGGAGGCAAGCACACAGACCATCAA
TCTTGACGACCGGTCGCACTGGGGCGGCGACCTGAAAACCATCCTGCATACCAACATGCCAAATGTGAAC
GAGTTCATGTTTACCAATAAGTTTAAGGCGCGGGTGATGGTGTCGCGCTTGCCTACTAAGGACAATCAGG
TGGAGCTGAAATACGAGTGGGTGGAGTTCACGCTGCCCGAGGGCAACTACTCCGAGACCATGACCATAGA
CCTTATGAACAACGCGATCGTGGAGCACTACTTGAAAGTGGGCAGACAGAACGGGGTTCTGGAAAGCGAC
ATCGGGGTAAAGTTTGACACCCGCAACTTCAGACTGGGGTTTGACCCCGTCACTGGTCTTGTCATGCCTG
GGGTATATACAAACGAAGCCTTCCATCCAGACATCATTTTGCTGCCAGGATGCGGGGTGGACTTCACCCA
CAGCCGCCTGAGCAACTTGTTGGGCATCCGCAAGCGGCAACCCTTCCAGGAGGGCTTTAGGATCACCTAC
GATGATCTGGAGGGTGGTAACATTCCCGCACTGTTGGATGTGGACGCCTACCAGGCGAGCTTGAAAGATG
ACACCGAACAGGGCGGGGGTGGCGCAGGCGGCAGCAACAGCAGTGGCAGCGGCGCGGAAGAGAACTCCAA
CGCGGCAGCCGCGGCAATGCAGCCGGTGGAGGACATGAACGATCATGCCATTCGCGGCGACACCTTTGCC
ACACGGGCTGAGGAGAAGCGCGCTGAGGCCGAAGCAGCGGCCGAAGCTGCCGCCCCCGCTGCGCAACCCG
AGGTCGAGAAGCCTCAGAAGAAACCGGTGATCAAACCCCTGACAGAGGACAGCAAGAAACGCAGTTACAA
CCTAATAAGCAATGACAGCACCTTCACCCAGTACCGCAGCTGGTACCTTGCATACAACTACGGCGACCCT
CAGACCGGAATCCGCTCATGGACCCTGCTTTGCACTCCTGACGTAACCTGCGGCTCGGAGCAGGTCTACT
GGTCGTTGCCAGACATGATGCAAGACCCCGTGACCTTCCGCTCCACGCGCCAGATCAGCAACTTTCCGGT
GGTGGGCGCCGAGCTGTTGCCCGTGCACTCCAAGAGCTTCTACAACGACCAGGCCGTCTACTCCCAACTC
ATCCGCCAGTTTACCTCTCTGACCCACGTGTTCAATCGCTTTCCCGAGAACCAGATTTTGGCGCGCCCGC
CAGCCCCCACCATCACCACCGTCAGTGAAAACGTTCCTGCTCTCACAGATCACGGGACGCTACCGCTGCG
CAACAGCATCGGAGGAGTCCAGCGAGTGACCATTACTGACGCCAGACGCCGCACCTGCCCCTACGTTTAC
AAGGCCCTGGGCATAGTCTCGCCGCGCGTCCTATCGAGCCGCACTTTTTGAGCAAGCATGTCCATCCTTA
TATCGCCCAGCAATAACACAGGCTGGGGCCTGCGCTTCCCAAGCAAGATGTTTGGCGGGGCCAAGAAGCG
CTCCGACCAACACCCAGTGCGCGTGCGCGGGCACTACCGCGCGCCCTGGGGCGCGCACAAACGCGGCCGC
ACTGGGCGCACCACCGTCGATGACGCCATCGACGCGGTGGTGGAGGAGGCGCGCAACTACACGCCCACGC
CGCCACCAGTGTCCACAGTGGACGCGGCCATTCAGACCGTGGTGCGCGGAGCCCGGCGCTATGCTAAAAT
GAAGAGACGGCGGAGGCGCGTAGCACGTCGCCACCGCCGCCGACCCGGCACTGCCGCCCAACGCGCGGCG
GCGGCCCTGCTTAACCGCGCACGTCGCACCGGCCGACGGGCGGCCATGCGGGCCGCTCGAAGGCTGGCCG
CGGGTATTGTCACTGTGCCCCCCAGGTCCAGGCGACGAGCGGCCGCCGCAGCAGCCGCGGCCATTAGTGC
TATGACTCAGGGTCGCAGGGGCAACGTGTATTGGGTGCGCGACTCGGTTAGCGGCCTGCGCGTGCCCGTG
CGCACCCGCCCCCCGCGCAACTAGATTGCAAGAAAAAACTACTTAGACTCGTACTGTTGTATGTATCCAG
CGGCGGCGGCGCGCAACGAAGCTATGTCCAAGCGCAAAATCAAAGAAGAGATGCTCCAGGTCATCGCGCC
GGAGATCTATGGCCCCCCGAAGAAGGAAGAGCAGGATTACAAGCCCCGAAAGCTAAAGCGGGTCAAAAAG
AAAAAGAAAGATGATGATGATGAACTTGACGACGAGGTGGAACTGCTGCACGCTACCGCGCCCAGGCGAC
GGGTACAGTGGAAAGGTCGACGCGTAAAACGTGTTTTGCGACCCGGCACCACCGTAGTCTTTACGCCCGG
TGAGCGCTCCACCCGCACCTACAAGCGCGTGTATGATGAGGTGTACGGCGACGAGGACCTGCTTGAGCAG
GCCAACGAGCGCCTCGGGGAGTTTGCCTACGGAAAGCGGCATAAGGACATGCTGGCGTTGCCGCTGGACG
AGGGCAACCCAACACCTAGCCTAAAGCCCGTAACACTGCAGCAGGTGCTGCCCGCGCTTGCACCGTCCGA
AGAAAAGCGCGGCCTAAAGCGCGAGTCTGGTGACTTGGCACCCACCGTGCAGCTGATGGTACCCAAGCGC
CAGCGACTGGAAGATGTCTTGGAAAAAATGACCGTGGAACCTGGGCTGGAGCCCGAGGTCCGCGTGCGGC
CAATCAAGCAGGTGGCGCCGGGACTGGGCGTGCAGACCGTGGACGTTCAGATACCCACTACCAGTAGCAC
CAGTATTGCCACCGCCACAGAGGGCATGGAGACACAAACGTCCCCGGTTGCCTCAGCGGTGGCGGATGCC

GCGGTGCAGGCGGTCGCTGCGGCCGCGTCCAAGACCTCTACGGAGGTGCAAACGGACCCGTGGATGTTTC
GCGTTTCAGCCCCCCGGCGCCCGCGCGGTTCGAGGAAGTACGGCGCCGCCAGCGCGCTACTGCCCGAATA
TGCCCTACATCCTTCCATTGCGCCTACCCCCGGCTATCGTGGCTACACCTACCGCCCCAGAAGACGAGCA
ACTACCCGACGCCGAACCACCACTGGAACCCGCCGCCGCCGTCGCCGTCGCCAGCCCGTGCTGGCCCCGA
TTTCCGTGCGCAGGGTGGCTCGCGAAGGAGGCAGGACCCTGGTGCTGCCAACAGCGCGCTACCACCCCAG
CATCGTTTAAAAGCCGGTCTTTGTGGTTCTTGCAGATATGGCCCTCACCTGCCGCCTCCGTTTCCCGGTG
CCGGGATTCCGAGGAAGAATGCACCGTAGGAGGGGCATGGCCGGCCACGGCCTGACGGGCGGCATGCGTC
GTGCGCACCACCGGCGGCGGCGCGCGTCGCACCGTCGCATGCGCGGCGGTATCCTGCCCCTCCTTATTCC
ACTGATCGCCGCGGCGATTGGCGCCGTGCCCGGAATTGCATCCGTGGCCTTGCAGGCGCAGAGACACTGA
TTAAAAACAAGTTGCATGTGGAAAAATCAAAATAAAAAGTCTGGACTCTCACGCTCGCTTGGTCCTGTAA
CTATTTTGTAGAATGGAAGACATCAACTTTGCGTCTCTGGCCCCGCGACACGGCTCGCGCCCGTTCATGG
GAAACTGGCAAGATATCGGCACCAGCAATATGAGCGGTGGCGCCTTCAGCTGGGGCTCGCTGTGGAGCGG
CATTAAAAATTTCGGTTCCACCGTTAAGAACTATGGCAGCAAGGCCTGGAACAGCAGCACAGGCCAGATG
CTGAGGGATAAGTTGAAAGAGCAAAATTTCCAACAAAAGGTGGTAGATGGCCTGGCCTCTGGCATTAGCG
GGGTGGTGGACCTGGCCAACCAGGCAGTGCAAAATAAGATTAACAGTAAGCTTGATCCCCGCCCTCCCGT
AGAGGAGCCTCCACCGGCCGTGGAGACAGTGTCTCCAGAGGGGCGTGGCGAAAAGCGTCCGCGCCCCGAC
AGGGAAGAAACTCTGGTGACGCAAATAGACGAGCCTCCCTCGTACGAGGAGGCACTAAAGCAAGGCCTGC
CCACCACCCGTCCCATCGCGCCCATGGCTACCGGAGTGCTGGGCCAGCACACACCCGTAACGCTGGACCT
GCCTCCCCCCGCCGACACCCAGCAGAAACCTGTGCTGCCAGGCCCGACCGCCGTTGTTGTAACCCGTCCT
AGCCGCGCGTCCCTGCGCCGCGCCGCCAGCGGTCCGCGATCGTTGCGGCCCGTAGCCAGTGGCAACTGGC
AAAGCACACTGAACAGCATCGTGGGTCTGGGGGTGCAATCCCTGAAGCGCCGACGATGCTTCTGAATAGC
TAACGTGTCGTATGTGTGTCATGTATGCGTCCATGTCGCCGCCAGAGGAGCTGCTGAGCCGCCGCGCGCC
CGCTTTCCAAGATGGCTACCCCTTCGATGATGCCGCAGTGGTCTTACATGCACATCTCGGGCCAGGACGC
CTCGGAGTACCTGAGCCCCGGGCTGGTGCAGTTTGCCCGCGCCACCGAGACGTACTTCAGCCTGAATAAC
AAGTTTAGAAACCCCACGGTGGCGCCTACGCACGACGTGACCACAGACCGGTCCCAGCGTTTGACGCTGC
GGTTCATCCCTGTGGACCGTGAGGATACTGCGTACTCGTACAAGGCGCGGTTCACCCTAGCTGTGGGTGA
TAACCGTGTGCTGGACATGGCTTCCACGTACTTTGACATCCGCGGCGTGCTGGACAGGGGCCCTACTTTT
AAGCCCTACTCTGGCACTGCCTACAACGCCCTGGCTCCCAAGGGTGCCCCAAATCCTTGCGAATGGGATG
AAGCTGCTACTGCTCTTGAAATAAACCTAGAAGAAGAGGACGATGACAACGAAGACGAAGTAGACGAGCA
AGCTGAGCAGCAAAAAACTCACGTATTTGGGCAGGCGCCTTATTCTGGTATAAATATTACAAAGGAGGGT
ATTCAAATAGGTGTCGAAGGTCAAACACCTAAATATGCCGATAAAACATTTCAACCTGAACCTCAAATAG
GAGAATCTCAGTGGTACGAAACTGAAATTAATCATGCAGCTGGGAGAGTCCTTAAAAAGACTACCCCAAT
GAAACCATGTTACGGTTCATATGCAAAACCCACAAATGAAAATGGAGGGCAAGGCATTCTTGTAAAGCAA
CAAAATGGAAAGCTAGAAAGTCAAGTGGAAATGCAATTTTTCTCAACTACTGAGGCGACCGCAGGCAATG
GTGATAACTTGACTCCTAAAGTGGTATTGTACAGTGAAGATGTAGATATAGAAACCCCAGACACTCATAT
TTCTTACATGCCCACTATTAAGGAAGGTAACTCACGAGAACTAATGGGCCAACAATCTATGCCCAACAGG
CCTAATTACATTGCTTTTAGGGACAATTTTATTGGTCTAATGTATTACAACAGCACGGGTAATATGGGTG
TTCTGGCGGGCCAAGCATCGCAGTTGAATGCTGTTGTAGATTTGCAAGACAGAAACACAGAGCTTTCATA
CCAGCTTTTGCTTGATTCCATTGGTGATAGAACCAGGTACTTTTCTATGTGGAATCAGGCTGTTGACAGC
TATGATCCAGATGTTAGAATTATTGAAAATCATGGAACTGAAGATGAACTTCCAAATTACTGCTTTCCAC
TGGGAGGTGTGATTAATACAGAGACTCTTACCAAGGTAAAACCTAAAACAGGTCAGGAAAATGGATGGGA
AAAAGATGCTACAGAATTTTCAGATAAAAATGAAATAAGAGTTGGAAATAATTTTGCCATGGAAATCAAT
CTAAATGCCAACCTGTGGAGAAATTTCCTGTACTCCAACATAGCGCTGTATTTGCCCGACAAGCTAAAGT
ACAGTCCTTCCAACGTAAAAATTTCTGATAACCCAAACACCTACGACTACATGAACAAGCGAGTGGTGGC

TCCCGGGTTAGTGGACTGCTACATTAACCTTGGAGCACGCTGGTCCCTTGACTATATGGACAACGTCAAC
CCATTTAACCACCACCGCAATGCTGGCCTGCGCTACCGCTCAATGTTGCTGGGCAATGGTCGCTATGTGC
CCTTCCACATCCAGGTGCCTCAGAAGTTCTTTGCCATTAAAAACCTCCTTCTCCTGCCGGGCTCATACAC
CTACGAGTGGAACTTCAGGAAGGATGTTAACATGGTTCTGCAGAGCTCCCTAGGAAATGACCTAAGGGTT
GACGGAGCCAGCATTAAGTTTGATAGCATTTGCCTTTACGCCACCTTCTTCCCCATGGCCCACAACACCG
CCTCCACGCTTGAGGCCATGCTTAGAAACGACACCAACGACCAGTCCTTTAACGACTATCTCTCCGCCGC
CAACATGCTCTACCCTATACCCGCCAACGCTACCAACGTGCCCATATCCATCCCCTCCCGCAACTGGGCG
GCTTTCCGCGGCTGGGCCTTCACGCGCCTTAAGACTAAGGAAACCCCATCACTGGGCTCGGGCTACGACC
CTTATTACACCTACTCTGGCTCTATACCCTACCTAGATGGAACCTTTTACCTCAACCACACCTTTAAGAA
GGTGGCCATTACCTTTGACTCTTCTGTCAGCTGGCCTGGCAATGACCGCCTGCTTACCCCCAACGAGTTT
GAAATTAAGCGCTCAGTTGACGGGGAGGGTTACAACGTTGCCCAGTGTAACATGACCAAAGACTGGTTCC
TGGTACAAATGCTAGCTAACTACAACATTGGCTACCAGGGCTTCTATATCCCAGAGAGCTACAAGGACCG
CATGTACTCCTTCTTTAGAAACTTCCAGCCCATGAGCCGTCAGGTGGTGGATGATACTAAATACAAGGAC
TACCAACAGGTGGGCATCCTACACCAACACAACAACTCTGGATTTGTTGGCTACCTTGCCCCCACCATGC
GCGAAGGACAGGCCTACCCTGCTAACTTCCCCTATCCGCTTATAGGCAAGACCGCAGTTGACAGCATTAC
CCAGAAAAAGTTTCTTTGCGATCGCACCCTTTGGCGCATCCCATTCTCCAGTAACTTTATGTCCATGGGC
GCACTCACAGACCTGGGCCAAAACCTTCTCTACGCCAACTCCGCCCACGCGCTAGACATGACTTTTGAGG
TGGATCCCATGGACGAGCCCACCCTTCTTTATGTTTTGTTTGAAGTCTTTGACGTGGTCCGTGTGCACCG
GCCGCACCGCGGCGTCATCGAAACCGTGTACCTGCGCACGCCCTTCTCGGCCGGCAACGCCACAACATAA
AGAAGCAAGCAACATCAACAACAGCTGCCGCCATGGGCTCCAGTGAGCAGGAACTGAAAGCCATTGTCAA
AGATCTTGGTTGTGGGCCATATTTTTTGGGCACCTATGACAAGCGCTTTCCAGGCTTTGTTTCTCCACAC
AAGCTCGCCTGCGCCATAGTCAATACGGCCGGTCGCGAGACTGGGGGCGTACACTGGATGGCCTTTGCCT
GGAACCCGCACTCAAAAACATGCTACCTCTTTGAGCCCTTTGGCTTTTCTGACCAGCGACTCAAGCAGGT
TTACCAGTTTGAGTACGAGTCACTCCTGCGCCGTAGCGCCATTGCTTCTTCCCCCGACCGCTGTATAACG
CTGGAAAAGTCCACCCAAAGCGTACAGGGGCCCAACTCGGCCGCCTGTGGACTATTCTGCTGCATGTTTC
TCCACGCCTTTGCCAACTGGCCCCAAACTCCCATGGATCACAACCCCCACCATGAACCTTATTACCGGGGT
ACCCAACTCCATGCTCAACAGTCCCCAGGTACAGCCCACCCTGCGTCGCAACCAGGAACAGCTCTACAGC
TTCCTGGAGCGCCACTCGCCCTACTTCCGCAGCCACAGTGCGCAGATTAGGAGCGCCACTTCTTTTTGTC
ACTTGAAAAACATGTAAAAATAATGTACTAGAGACACTTTCAATAAAGGCAAATGCTTTTATTTGTACAC
TCTCGGGTGATTATTTACCCCCACCCTTGCCGTCTGCGCCGTTTAAAAATCAAAGGGGTTCTGCCGCGCA
TCGCTATGCGCCACTGGCAGGGACACGTTGCGATACTGGTGTTTAGTGCTCCACTTAAACTCAGGCACAA
CCATCCGCGGCAGCTCGGTGAAGTTTTCACTCCACAGGCTGCGCACCATCACCAACGCGTTTAGCAGGTC
GGGCGCCGATATCTTGAAGTCGCAGTTGGGGCCTCCGCCCTGCGCGCGCGAGTTGCGATACACAGGGTTG
CAGCACTGGAACACTATCAGCGCCGGGTGGTGCACGCTGGCCAGCACGCTCTTGTCGGAGATCAGATCCG
CGTCCAGGTCCTCCGCGTTGCTCAGGGCGAACGGAGTCAACTTTGGTAGCTGCCTTCCCAAAAAGGGCGC
GTGCCCAGGCTTTGAGTTGCACTCGCACCGTAGTGGCATCAAAAGGTGACCGTGCCCGGTCTGGGCGTTA
GGATACAGCGCCTGCATAAAAGCCTTGATCTGCTTAAAAGCCACCTGAGCCTTTGCGCCTTCAGAGAAGA
ACATGCCGCAAGACTTGCCGGAAAACTGATTGGCCGGACAGGCCGCGTCGTGCACGCAGCACCTTGCGTC
GGTGTTGGAGATCTGCACCACATTTCGGCCCCACCGGTTCTTCACGATCTTGGCCTTGCTAGACTGCTCC
TTCAGCGCGCGCTGCCCGTTTTCGCTCGTCACATCCATTTCAATCACGTGCTCCTTATTTATCATAATGC
TTCCGTGTAGACACTTAAGCTCGCCTTCGATCTCAGCGCAGCGGTGCAGCCACAACGCGCAGCCCGTGGG
CTCGTGATGCTTGTAGGTCACCTCTGCAAACGACTGCAGGTACGCCTGCAGGAATCGCCCCATCATCGTC
ACAAAGGTCTTGTTGCTGGTGAAGGTCAGCTGCAACCCGCGGTGCTCCTCGTTCAGCCAGGTCTTGCATA
CGGCCGCCAGAGCTTCCACTTGGTCAGGCAGTAGTTTGAAGTTCGCCTTTAGATCGTTATCCACGTGGTA

CTTGTCCATCAGCGCGCGCGCAGCCTCCATGCCCTTCTCCCACGCAGACACGATCGGCACACTCAGCGGG
TTCATCACCGTAATTTCACTTTCCGCTTCGCTGGGCTCTTCCTCTTCCTCTTGCGTCCGCATACCACGCG
CCACTGGGTCGTCTTCATTCAGCCGCCGCACTGTGCGCTTACCTCCTTTGCCATGCTTGATTAGCACCGG
TGGGTTGCTGAAACCCACCATTTGTAGCGCCACATCTTCTCTTTCTTCCTCGCTGTCCACGATTACCTCT
GGTGATGGCGGGCGCTCGGGCTTGGGAGAAGGGCGCTTCTTTTTCTTCTTGGGCGCAATGGCCAAATCCG
CCGCCGAGGTCGATGGCCGCGGGCTGGGTGTGCGCGGCACCAGCGCGTCTTGTGATGAGTCTTCCTCGTC
CTCGGACTCGATACGCCGCCTCATCCGCTTTTTTGGGGGCGCCCGGGGAGGCGGCGGCGACGGGGACGGG
GACGACACGTCCTCCATGGTTGGGGGACGTCGCGCCGCACCGCGTCCGCGCTCGGGGGTGGTTTCGCGCT
GCTCCTCTTCCCGACTGGCCATTTCCTTCTCCTATAGGCAGAAAAAGATCATGGAGTCAGTCGAGAAGAA
GGACAGCCTAACCGCCCCCTCTGAGTTCGCCACCACCGCCTCCACCGATGCCGCCAACGCGCCTACCACC
TTCCCCGTCGAGGCACCCCCGCTTGAGGAGGAGGAAGTGATTATCGAGCAGGACCCAGGTTTTGTAAGCG
AAGACGACGAGGACCGCTCAGTACCAACAGAGGATAAAAAGCAAGACCAGGACAACGCAGAGGCAAACGA
GGAACAAGTCGGGCGGGGGGACGAAAGGCATGGCGACTACCTAGATGTGGGAGACGACGTGCTGTTGAAG
CATCTGCAGCGCCAGTGCGCCATTATCTGCGACGCGTTGCAAGAGCGCAGCGATGTGCCCCTCGCCATAG
CGGATGTCAGCCTTGCCTACGAACGCCACCTATTCTCACCGCGCGTACCCCCCAAACGCCAAGAAAACGG
CACATGCGAGCCCAACCCGCGCCTCAACTTCTACCCCGTATTTGCCGTGCCAGAGGTGCTTGCCACCTAT
CACATCTTTTTCCAAAACTGCAAGATACCCCTATCCTGCCGTGCCAACCGCAGCCGAGCGGACAAGCAGC
TGGCCTTGCGGCAGGGCGCTGTCATACCTGATATCGCCTCGCTCAACGAAGTGCCAAAAATCTTTGAGGG
TCTTGGACGCGACGAGAAGCGCGCGGCAAACGCTCTGCAACAGGAAAACAGCGAAAATGAAAGTCACTCT
GGAGTGTTGGTGGAACTCGAGGGTGACAACGCGCGCCTAGCCGTACTAAAACGCAGCATCGAGGTCACCC
ACTTTGCCTACCCGGCACTTAACCTACCCCCCAAGGTCATGAGCACAGTCATGAGTGAGCTGATCGTGCG
CCGTGCGCAGCCCCTGGAGAGGGATGCAAATTTGCAAGAACAAACAGAGGAGGGCCTACCCGCAGTTGGC
GACGAGCAGCTAGCGCGCTGGCTTCAAACGCGCGAGCCTGCCGACTTGGAGGAGCGACGCAAACTAATGA
TGGCCGCAGTGCTCGTTACCGTGGAGCTTGAGTGCATGCAGCGGTTCTTTGCTGACCCGGAGATGCAGCG
CAAGCTAGAGGAAACATTGCACTACACCTTTCGACAGGGCTACGTACGCCAGGCCTGCAAGATCTCCAAC
GTGGAGCTCTGCAACCTGGTCTCCTACCTTGGAATTTTGCACGAAAACCGCCTTGGGCAAAACGTGCTTC
ATTCCACGCTCAAGGGCGAGGCGCGCCGCGACTACGTCCGCGACTGCGTTTACTTATTTCTATGCTACAC
CTGGCAGACGGCCATGGGCGTTTGGCAGCAGTGCTTGGAGGAGTGCAACCTCAAGGAGCTGCAGAAACTG
CTAAAGCAAAACTTGAAGGACCTATGGACGGCCTTCAACGAGCGCTCCGTGGCCGCGCACCTGGCGGACA
TCATTTTCCCCGAACGCCTGCTTAAAACCCTGCAACAGGGTCTGCCAGACTTCACCAGTCAAAGCATGTT
GCAGAACTTTAGGAACTTTATCCTAGAGCGCTCAGGAATCTTGCCCGCCACCTGCTGTGCACTTCCTAGC
GACTTTGTGCCCATTAAGTACCGCGAATGCCCTCCGCCGCTTTGGGGCCACTGCTACCTTCTGCAGCTAG
CCAACTACCTTGCCTACCACTCTGACATAATGGAAGACGTGAGCGGTGACGGTCTACTGGAGTGTCACTG
TCGCTGCAACCTATGCACCCCGCACCGCTCCCTGGTTTGCAATTCGCAGCTGCTTAACGAAAGTCAAATT
ATCGGTACCTTTGAGCTGCAGGGTCCCTCGCCTGACGAAAAGTCCGCGGCTCCGGGGTTGAAACTCACTC
CGGGGCTGTGGACGTCGGCTTACCTTCGCAAATTTGTACCTGAGGACTACCACGCCCACGAGATTAGGTT
CTACGAAGACCAATCCCGCCCGCCAAATGCGGAGCTTACCGCCTGCGTCATTACCCAGGGCCACATTCTT
GGCCAATTGCAAGCCATCAACAAAGCCCGCCAAGAGTTTCTGCTACGAAAGGGACGGGGGGTTTACTTGG
ACCCCCAGTCCGGCGAGGAGCTCAACCCAATCCCCCCGCCGCCGCAGCCCTATCAGCAGCAGCCGCGGGC
CCTTGCTTCCCAGGATGGCACCCAAAAAGAAGCTGCAGCTGCCGCCGCCACCCACGGACGAGGAGGAATA
CTGGGACAGTCAGGCAGAGGAGGTTTTGGACGAGGAGGAGGAGGACATGATGGAAGACTGGGAGAGCCTA
GACGAGGAAGCTTCCGAGGTCGAAGAGGTGTCAGACGAAACACCGTCACCCTCGGTCGCATTCCCCTCGC
CGGCGCCCCAGAAATCGGCAACCGGTTCCAGCATGGCTACAACCTCCGCTCCTCAGGCGCCGCCGGCACT
GCCCGTTCGCCGACCCAACCGTAGATGGGACACCACTGGAACCAGGGCCGGTAAGTCCAAGCAGCCGCCG

CCGTTAGCCCAAGAGCAACAACAGCGCCAAGGCTACCGCTCATGGCGCGGGCACAAGAACGCCATAGTTG
CTTGCTTGCAAGACTGTGGGGGCAACATCTCCTTCGCCCGCCGCTTTCTTCTCTACCATCACGGCGTGGC
CTTCCCCCGTAACATCCTGCATTACTACCGTCATCTCTACAGCCCATACTGCACCGGCGGCAGCGGCAGC
GGCAGCAACAGCAGCGGCCACACAGAAGCAAAGGCGACCGGATAGCAAGACTCTGACAAAGCCCAAGAAA
TCCACAGCGGCGGCAGCAGCAGGAGGAGGAGCGCTGCGTCTGGCGCCCAACGAACCCGTATCGACCCGCG
AGCTTAGAAACAGGATTTTTCCCACTCTGTATGCTATATTTCAACAGAGCAGGGGCCAAGAACAAGAGCT
GAAAATAAAAAACAGGTCTCTGCGATCCCTCACCCGCAGCTGCCTGTATCACAAAAGCGAAGATCAGCTT
CGGCGCACGCTGGAAGACGCGGAGGCTCTCTTCAGTAAATACTGCGCGCTGACTCTTAAGGACTAGTTTC
GCGCCCTTTCTCAAATTTAAGCGCGAAAACTACGTCATCTCCAGCGGCCACACCCGGCGCCAGCACCTGT
CGTCAGCGCCATTATGAGCAAGGAAATTCCCACGCCCTACATGTGGAGTTACCAGCCACAAATGGGACTT
GCGGCTGGAGCTGCCCAAGACTACTCAACCCGAATAAACTACATGAGCGCGGGACCCCACATGATATCCC
GGGTCAACGGAATCCGCGCCCACCGAAACCGAATTCTCTTGGAACAGGCGGCTATTACCACCACACCTCG
TAATAACCTTAATCCCCGTAGTTGGCCCGCTGCCCTGGTGTACCAGGAAAGTCCCGCTCCCACCACTGTG
GTACTTCCCAGAGACGCCCAGGCCGAAGTTCAGATGACTAACTCAGGGGCGCAGCTTGCGGGCGGCTTTC
GTCACAGGGTGCGGTCGCCCGGGCAGGGTATAACTCACCTGACAATCAGAGGGCGAGGTATTCAGCTCAA
CGACGAGTCGGTGAGCTCCTCGCTTGGTCTCCGTCCGGACGGGACATTTCAGATCGGCGGCGCCGGCCGT
CCTTCATTCACGCCTCGTCAGGCAATCCTAACTCTGCAGACCTCGTCCTCTGAGCCGCGCTCTGGAGGCA
TTGGAACTCTGCAATTTATTGAGGAGTTTGTGCCATCGGTCTACTTTAACCCCTTCTCGGGACCTCCCGG
CCACTATCCGGATCAATTTATTCCTAACTTTGACGCGGTAAAGGACTCGGCGGACGGCTACGACTGAATG
TTAAGTGGAGAGGCAGAGCAACTGCGCCTGAAACACCTGGTCCACTGTCGCCGCCACAAGTGCTTTGCCC
GCGACTCCGGTGAGTTTTGCTACTTTGAATTGCCCGAGGATCATATCGAGGGCCCGGCGCACGGCGTCCG
GCTTACCGCCCAGGGAGAGCTTGCCCGTAGCCTGATTCGGGAGTTTACCCAGCGCCCCCTGCTAGTTGAG
CGGGACAGGGGACCCTGTGTTCTCACTGTGATTTGCAACTGTCCTAACCTTGGATTACATCAAGATCTTT
GTTGCCATCTCTGTGCTGAGTATAATAAATACAGAAATTAAAATATACTGGGGCTCCTATCGCCATCCTG
TAAACGCCACCGTCTTCACCCGCCCAAGCAAACCAAGGCGAACCTTACCTGGTACTTTTAACATCTCTCC
CTCTGTGATTTACAACAGTTTCAACCCAGACGGAGTGAGTCTACGAGAGAACCTCTCCGAGCTCAGCTAC
TCCATCAGAAAAAACACCACCCTCCTTACCTGCCGGGAACGTACGAGTGCGTCACCGGCCGCTGCACCAC
ACCTACCGCCTGACCGTAAACCAGACTTTTTCCGGACAGACCTCAATAACTCTGTTTACCAGAACAGGAG
GTGAGCTTAGAAAACCCTTAGGGTATTAGGCCAAAGGCGCAGCTACTGTGGGGTTTATGAACAATTCAAG
CAACTCTACGGGCTATTCTAATTCAGGTTTCTCTAGAATCGGGGTTGGGGTTATTCTCTGTCTTGTGATT
CTCTTTATTCTTATACTAACGCTTCTCTGCCTAAGGCTCGCCGCCTGCTGTGTGCACATTTGCATTTATT
GTCAGCTTTTTAAACGCTGGGGTCGCCACCCAAGATGATTAGGTACATAATCCTAGGTTTACTCACCCTT
GCGTCAGCCCACGGTACCACCCAAAAGGTGGATTTTAAGGAGCCAGCCTGTAATGTTACATTCGCAGCTG
AAGCTAATGAGTGCACCACTCTTATAAAATGCACCACAGAACATGAAAAGCTGCTTATTCGCCACAAAAA
CAAAATTGGCAAGTATGCTGTTTATGCTATTTGGCAGCCAGGTGACACTACAGAGTATAATGTTACAGTT
TTCCAGGGTAAAAGTCATAAAACTTTTATGTATACTTTTCCATTTTATGAAATGTGCGACATTACCATGT
ACATGAGCAAACAGTATAAGTTGTGGCCCCCACAAAATTGTGTGGAAAACACTGGCACTTTCTGCTGCAC
TGCTATGCTAATTACAGTGCTCGCTTTGGTCTGTACCCTACTCTATATTAAATACAAAAGCAGACGCAGC
TTTATTGAGGAAAAGAAAATGCCTTAATTTACTAAGTTACAAAGCTAATGTCACCACTAACTGCTTTACT
CGCTGCTTGCAAAACAAATTCAAAAAGTTAGCATTATAATTAGAATAGGATTTAAACCCCCCGGTCATTT
CCTGCTCAATACCATTCCCCTGAACAATTGACTCTATGTGGGATATGCTCCAGCGCTACAACCTTGAAGT
CAGGCTTCCTGGATGTCAGCATCTGACTTTGGCCAGCACCTGTCCCGCGGATTTGTTCCAGTCCAACTAC
AGCGACCCACCCTAACAGAGATGACCAACACAACCAACGCGGCCGCCGCTACCGGACTTACATCTACCAC
AAATACACCCCAAGTTTCTGCCTTTGTCAATAACTGGGATAACTTGGGCATGTGGTGGTTCTCCATAGCG

CTTATGTTTGTATGCCTTATTATTATGTGGCTCATCTGCTGCCTAAAGCGCAAACGCGCCCGACCACCCA
TCTATAGTCCCATCATTGTGCTACACCCAAACAATGATGGAATCCATAGATTGGACGGACTGAAACACAT
GTTCTTTTCTCTTACAGTATGATTAAATGAGACATGATTCCTCGAGTTTTTATATTACTGACCCTTGTTG
CGCTTTTTTGTGCGTGCTCCACATTGGCTGCGGTTTCTCACATCGAAGTAGACTGCATTCCAGCCTTCAC
AGTCTATTTGCTTTACGGATTTGTCACCCTCACGCTCATCTGCAGCCTCATCACTGTGGTCATCGCCTTT
ATCCAGTGCATTGACTGGGTCTGTGTGCGCTTTGCATATCTCAGACACCATCCCCAGTACAGGGACAGGA
CTATAGCTGAGCTTCTTAGAATTCTTTAATTATGAAATTTACTGTGACTTTTCTGCTGATTATTTGCACC
CTATCTGCGTTTTGTTCCCCGACCTCCAAGCCTCAAAGACATATATCATGCAGATTCACTCGTATATGGA
ATATTCCAAGTTGCTACAATGAAAAAAGCGATCTTTCCGAAGCCTGGTTATATGCAATCATCTCTGTTAT
GGTGTTCTGCAGTACCATCTTAGCCCTAGCTATATATCCCTACCTTGACATTGGCTGGAAACGAATAGAT
GCCATGAACCACCCAACTTTCCCCGCGCCCGCTATGCTTCCACTGCAACAAGTTGTTGCCGGCGGCTTTG
TCCCAGCCAATCAGCCTCGCCCCACTTCTCCCACCCCCACTGAAATCAGCTACTTTAATCTAACAGGAGG
AGATGACTGACACCCTAGATCTAGAAATGGACGGAATTATTACAGAGCAGCGCCTGCTAGAAAGACGCAG
GGCAGCGGCCGAGCAACAGCGCATGAATCAAGAGCTCCAAGACATGGTTAACTTGCACCAGTGCAAAAGG
GGTATCTTTTGTCTGGTAAAGCAGGCCAAAGTCACCTACGACAGTAATACCACCGGACACCGCCTTAGCT
ACAAGTTGCCAACCAAGCGTCAGAAATTGGTGGTCATGGTGGGAGAAAAGCCCATTACCATAACTCAGCA
CTCGGTAGAAACCGAAGGCTGCATTCACTCACCTTGTCAAGGACCTGAGGATCTCTGCACCCTTATTAAG
ACCCTGTGCGGTCTCAAAGATCTTATTCCCTTTAACTAATAAAAAAAAATAATAAAGCATCACTTACTTA
AAATCAGTTAGCAAATTTCTGTCCAGTTTATTCAGCAGCACCTCCTTGCCCTCCTCCCAGCTCTGGTATT
GCAGCTTCCTCCTGGCTGCAAACTTTCTCCACAATCTAAATGGAATGTCAGTTTCCTCCTGTTCCTGTCC
ATCCGCACCCACTATCTTCATGTTGTTGCAGATGAAGCGCGCAAGACCGTCTGAAGATACCTTCAACCCC
GTGTATCCATATGACACGGAAACCGGTCCTCCAACTGTGCCTTTTCTTACTCCTCCCTTTGTATCCCCCA
ATGGGTTTCAAGAGAGTCCCCCTGGGGTACTCTCTTTGCGCCTATCCGAACCTCTAGTTACCTCCAATGG
CATGCTTGCGCTCAAAATGGGCAACGGCCTCTCTCTGGACGAGGCCGGCAACCTTACCTCCCAAAATGTA
ACCACTGTGAGCCCACCTCTCAAAAAAACCAAGTCAAACATAAACCTGGAAATATCTGCACCCCTCACAG
TTACCTCAGAAGCCCTAACTGTGGCTGCCGCCGCACCTCTAATGGTCGCGGGCAACACACTCACCATGCA
ATCACAGGCCCCGCTAACCGTGCACGACTCCAAACTTAGCATTGCCACCCAAGGACCCCTCACAGTGTCA
GAAGGAAAGCTAGCCCTGCAAACATCAGGCCCCCTCACCACCACCGATAGCAGTACCCTTACTATCACTG
CCTCACCCCCTCTAACTACTGCCACTGGTAGCTTGGGCATTGACTTGAAAGAGCCCATTTATACACAAAA
TGGAAAACTAGGACTAAAGTACGGGGCTCCTTTGCATGTAACAGACGACCTAAACACTTTGACCGTAGCA
ACTGGTCCAGGTGTGACTATTAATAATACTTCCTTGCAAACTAAAGTTACTGGAGCCTTGGGTTTTGATT
CACAAGGCAATATGCAACTTAATGTAGCAGGAGGACTAAGGATTGATTCTCAAAACAGACGCCTTATACT
TGATGTTAGTTATCCGTTTGATGCTCAAAACCAACTAAATCTAAGACTAGGACAGGGCCCTCTTTTTATA
AACTCAGCCCACAACTTGGATATTAACTACAACAAAGGCCTTTACTTGTTTACAGCTTCAAACAATTCCA
AAAAGCTTGAGGTTAACCTAAGCACTGCCAAGGGGTTGATGTTTGACGCTACAGCCATAGCCATTAATGC
AGGAGATGGGCTTGAATTTGGTTCACCTAATGCACCAAACACAAATCCCCTCAAAACAAAAATTGGCCAT
GGCCTAGAATTTGATTCAAACAAGGCTATGGTTCCTAAACTAGGAACTGGCCTTAGTTTTGACAGCACAG
GTGCCATTACAGTAGGAAACAAAAATAATGATAAGCTAACTTTGTGGACCACACCAGCTCCATCTCCTAA
CTGTAGACTAAATGCAGAGAAAGATGCTAAACTCACTTTGGTCTTAACAAAATGTGGCAGTCAAATACTT
GCTACAGTTTCAGTTTTGGCTGTTAAAGGCAGTTTGGCTCCAATATCTGGAACAGTTCAAAGTGCTCATC
TTATTATAAGATTTGACGAAAATGGAGTGCTACTAAACAATTCCTTCCTGGACCCAGAATATTGGAACTT
TAGAAATGGAGATCTTACTGAAGGCACAGCCTATACAAACGCTGTTGGATTTATGCCTAACCTATCAGCT
TATCCAAAATCTCACGGTAAAACTGCCAAAAGTAACATTGTCAGTCAAGTTTACTTAAACGGAGACAAAA
CTAAACCTGTAACACTAACCATTACACTAAACGGTACACAGGAAACAGGAGACACAACTCCAAGTGCATA

CTCTATGTCATTTTCATGGGACTGGTCTGGCCACAACTACATTAATGAAATATTTGCCACATCCTCTTAC
ACTTTTTCATACATTGCCCAAGAATAAAGAATCGTTTGTGTTATGTTTCAACGTGTTTATTTTTCAATTG
CAGAAAATTTCAAGTCATTTTTCATTCAGTAGTATAGCCCCACCACCACATAGCTTATACAGATCACCGT
ACCTTAATCAAACTCACAGAACCCTAGTATTCAACCTGCCACCTCCCTCCCAACACACAGAGTACACAGT
CCTTTCTCCCCGGCTGGCCTTAAAAAGCATCATATCATGGGTAACAGACATATTCTTAGGTGTTATATTC
CACACGGTTTCCTGTCGAGCCAAACGCTCATCAGTGATATTAATAAACTCCCCGGGCAGCTCACTTAAGT
TCATGTCGCTGTCCAGCTGCTGAGCCACAGGCTGCTGTCCAACTTGCGGTTGCTTAACGGGCGGCGAAGG
AGAAGTCCACGCCTACATGGGGGTAGAGTCATAATCGTGCATCAGGATAGGGCGGTGGTGCTGCAGCAGC
GCGCGAATAAACTGCTGCCGCCGCCGCTCCGTCCTGCAGGAATACAACATGGCAGTGGTCTCCTCAGCGA
TGATTCGCACCGCCCGCAGCATAAGGCGCCTTGTCCTCCGGGCACAGCAGCGCACCCTGATCTCACTTAA
ATCAGCACAGTAACTGCAGCACAGCACCACAATATTGTTCAAAATCCCACAGTGCAAGGCGCTGTATCCA
AAGCTCATGGCGGGGACCACAGAACCCACGTGGCCATCATACCACAAGCGCAGGTAGATTAAGTGGCGAC
CCCTCATAAACACGCTGGACATAAACATTACCTCTTTTGGCATGTTGTAATTCACCACCTCCCGGTACCA
TATAAACCTCTGATTAAACATGGCGCCATCCACCACCATCCTAAACCAGCTGGCCAAAACCTGCCCGCCG
GCTATACACTGCAGGGAACCGGGACTGGAACAATGACAGTGGAGAGCCCAGGACTCGTAACCATGGATCA
TCATGCTCGTCATGATATCAATGTTGGCACAACACAGGCACACGTGCATACACTTCCTCAGGATTACAAG
CTCCTCCCGCGTTAGAACCATATCCCAGGGAACAACCCATTCCTGAATCAGCGTAAATCCCACACTGCAG
GGAAGACCTCGCACGTAACTCACGTTGTGCATTGTCAAAGTGTTACATTCGGGCAGCAGCGGATGATCCT
CCAGTATGGTAGCGCGGGTTTCTGTCTCAAAAGGAGGTAGACGATCCCTACTGTACGGAGTGCGCCGAGA
CAACCGAGATCGTGTTGGTCGTAGTGTCATGCCAAATGGAACGCCGGACGTAGTCATATTTCCTGAAGCA
AAACCAGGTGCGGGCGTGACAAACAGATCTGCGTCTCCGGTCTCGCCGCTTAGATCGCTCTGTGTAGTAG
TTGTAGTATATCCACTCTCTCAAAGCATCCAGGCGCCCCCTGGCTTCGGGTTCTATGTAAACTCCTTCAT
GCGCCGCTGCCCTGATAACATCCACCACCGCAGAATAAGCCACACCCAGCCAACCTACACATTCGTTCTG
CGAGTCACACACGGGAGGAGCGGGAAGAGCTGGAAGAACCATGTTTTTTTTTTTTATTCCAAAAGATTATC
CAAAACCTCAAAATGAAGATCTATTAAGTGAACGCGCTCCCCTCCGGTGGCGTGGTCAAACTCTACAGCC
AAAGAACAGATAATGGCATTTGTAAGATGTTGCACAATGGCTTCCAAAAGGCAAACGGCCCTCACGTCCA
AGTGGACGTAAAGGCTAAACCCTTCAGGGTGAATCTCCTCTATAAACATTCCAGCACCTTCAACCATGCC
CAAATAATTCTCATCTCGCCACCTTCTCAATATATCTCTAAGCAAATCCCGAATATTAAGTCCGGCCATT
GTAAAAATCTGCTCCAGAGCGCCCTCCACCTTCAGCCTCAAGCAGCGAATCATGATTGCAAAAATTCAGG
TTCCTCACAGACCTGTATAAGATTCAAAAGCGGAACATTAACAAAAATACCGCGATCCCGTAGGTCCCTT
CGCAGGGCCAGCTGAACATAATCGTGCAGGTCTGCACGGACCAGCGCGGCCACTTCCCCGCCAGGAACCA
TGACAAAAGAACCCACACTGATTATGACACGCATACTCGGAGCTATGCTAACCAGCGTAGCCCCGATGTA
AGCTTGTTGCATGGGCGGCGATATAAAATGCAAGGTGCTGCTCAAAAAATCAGGCAAAGCCTCGCGCAAA
AAAGAAAGCACATCGTAGTCATGCTCATGCAGATAAAGGCAGGTAAGCTCCGGAACCACCACAGAAAAAG
ACACCATTTTTCTCTCAAACATGTCTGCGGGTTTCTGCATAAACACAAAATAAAATAACAAAAAAACATT
TAAACATTAGAAGCCTGTCTTACAACAGGAAAAACAACCCTTATAAGCATAAGACGGACTACGGCCATGC
CGGCGTGACCGTAAAAAAACTGGTCACCGTGATTAAAAAGCACCACCGACAGCTCCTCGGTCATGTCCGG
AGTCATAATGTAAGACTCGGTAAACACATCAGGTTGATTCACATCGGTCAGTGCTAAAAAGCGACCGAAA
TAGCCCGGGGGAATACATACCCGCAGGCGTAGAGACAACATTACAGCCCCCATAGGAGGTATAACAAAAT
TAATAGGAGAGAAAAACACATAAACACCTGAAAAACCCTCCTGCCTAGGCAAAATAGCACCCTCCCGCTC
CAGAACAACATACAGCGCTTCCACAGCGGCAGCCATAACAGTCAGCCTTACCAGTAAAAAAGAAAACCTA
TTAAAAAAACACCACTCGACACGGCACCAGCTCAATCAGTCACAGTGTAAAAAAGGGCCAAGTGCAGAGC
GAGTATATATAGGACTAAAAAATGACGTAACGGTTAAAGTCCACAAAAAACACCCAGAAAACCGCACGCG
AACCTACGCCCAGAAACGAAAGCCAAAAAACCCACAACTTCCTCAAATCGTCACTTCCGTTTTCCCACGT

TACGTAACTTCCCATTTTAAGAAAACTACAATTCCCAACACATACAAGTTACTCCGCCCTAAAACCTACG
TCACCCGCCCCGTTCCCACGCCCCGCGCCACGTCACAAACTCCACCCCCTCATTATCATATTGGCTTCAA
TCCAAAATAAGGTATATTATTGATGATG

[00267] Human adenovirus 35, complete genome

NCBI Reference Sequence: AC_000019.1 (SEQ ID NO: 41)

CATCATCAATAATATACCTTATAGATGGAATGGTGCCAATATGTAAATGAGGTGATTTTA
AAAAGTGTGGGCCGTGTGGTGATTGGCTGTGGGGTTAACGGTTAAAAGGGGCGGCGCGGC
CGTGGGAAAATGACGTTTTATGGGGGTGGAGTTTTTTTGCAAGTTGTCGCGGGAAATGTT
ACGCATAAAAAGGCTTCTTTTCTCACGGAACTACTTAGTTTTCCCACGGTATTTAACAGG
AAATGAGGTAGTTTTGACCGGATGCAAGTGAAAATTGCTGATTTTCGCGCGAAAACTGAA
TGAGGAAGTGTTTTTCTGAATAATGTGGTATTTATGGCAGGGTGGAGTATTTGTTCAGGG
CCAGGTAGACTTTGACCCATTACGTGGAGGTTTCGATTACCGTGTTTTTTACCTGAATTT
CCGCGTACCGTGTCAAAGTCTTCTGTTTTTACGTAGGTGTCAGCTGATCGCTAGGGTATT
TATACCTCAGGGTTTGTGTCAAGAGGCCACTCTTGAGTGCCAGCGAGAAGAGTTTTCTCC
TCTGCGCCGGCAGTTTAATAATAAAAAAATGAGAGATTTGCGATTTCTGCCTCAGGAAAT
AATCTCTGCTGAGACTGGAAATGAAATATTGGAGCTTGTGGTGCACGCCCTGATGGGAGA
CGATCCGGAGCCACCTGTGCAGCTTTTTGAGCCTCCTACGCTTCAGGAACTGTATGATTT
AGAGGTAGAGGGATCGGAGGATTCTAATGAGGAAGCTGTGAATGGCTTTTTTACCGATTC
TATGCTTTTAGCTGCTAATGAAGGATTAGAATTAGATCCGCCTTTGGACACTTTCAATAC
TCCAGGGGTGATTGTGGAAAGCGGTACAGGTGTAAGAAAATTACCTGATTTGAGTTCCGT
GGACTGTGATTTGCACTGCTATGAAGACGGGTTTCCTCCGAGTGATGAGGAGGACCATGA
AAAGGAGCAGTCCATGCAGACTGCAGCGGGTGAGGGAGTGAAGGCTGCCAATGTTGGTTT
TCAGTTGGATTGCCCGGAGCTTCCTGGACATGGCTGTAAGTCTTGTGAATTTCACAGGAA
AAATACTGGAGTAAAGGAACTGTTATGTTCGCTTTGTTATATGAGAACGCACTGCCACTT
TATTTACAGTAAGTGTGTTTAAGTTAAAATTTAAAGGAATATGCTGTTTTTCACATGTAT
ATTGAGTGTGAGTTTTGTGCTTCTTATTATAGGTCCTGTGTCTGATGCTGATGAATCACC
ATCTCCTGATTCTACTACCTCACCTCCTGATATTCAAGCACCTGTTCCTGTGGACGTGCG
CAAGCCCATTCCTGTGAAGCTTAAGCCTGGGAAACGTCCAGCAGTGGAGAAACTTGAGGA
CTTGTTACAGGGTGGGGACGGACCTTTGGACTTGAGTACACGGAAACGTCCAAGACAATA
AGTGTTCCATATCCGTGTTTACTTAAGGTGACGTCAATATTTGTGTGAGAGTGCAATGTA
ATAAAAATATGTTAACTGTTCACTGGTTTTTATTGCTTTTTGGGCGGGGACTCAGGTATA
TAAGTAGAAGCAGACCTGTGTGGTTAGCTCATAGGAGCTGGCTTTCATCCATGGAGGTTT
GGGCCATTTTGGAAGACCTTAGGAAGACTAGGCAACTGTTAGAGAGCGCTTCGGACGGAG
TCTCCGGTTTTTGGAGATTCTGGTTCGCTAGTGAATTAGCTAGGGTAGTTTTTAGGATAA
AACAGGACTATAAACAAGAATTTGAAAAGTTGTTGGTAGATTGCCCAGGACTTTTTGAAG
CTCTTAATTTGGGCCATCAGGTTCACTTTAAAGAAAAAGTTTTATCAGTTTTAGACTTTT
CAACCCCAGGTAGAACTGCTGCTGCTGTGGCTTTTCTTACTTTTATATTAGATAAATGGA
TCCCGCAGACTCATTTCAGCAGGGGATACGTTTTGGATTTCATAGCCACAGCATTGTGGA
GAACATGGAAGGTTCGCAAGATGAGGACAATCTTAGGTTACTGGCCAGTGCAGCCTTTGG
GTGTAGCGGGAATCCTGAGGCATCCACCGGTCATGCCAGCGGTTCTGGAGGAGGAACAGC
AAGAGGACAACCCGAGAGCCGGCCTGGACCCTCCAGTGGAGGAGGCGGAGTAGCTGACTT
GTCTCCTGAACTGCAACGGGTGCTTACTGGATCTACGTCCACTGGACGGGATAGGGGCGT
TAAGAGGGAGAGGGCATCCAGTGGTACTGATGCTAGATCTGAGTTGGCTTTAAGTTTAAT
GAGTCGCAGACGTCCTGAAACCATTTGGTGGCATGAGGTTCAGAAAGAGGGAAGGGATGA
AGTTTCTGTATTGCAGGAGAAATATTCACTGGAACAGGTGAAAACATGTTGGTTGGAGCC
AGAGGATGATTGGGCGGTGGCCATTAAAAATTATGCCAAGATAGCTTTGAGGCCTGATAA
ACAGTATAAGATCAGTAGACGGATTAATATCCGGAATGCTTGTTACATATCTGGAAATGG
GGCTGAGGTGGTAATAGATACTCAAGACAAGACAGTTATTAGATGCTGCATGATGGATAT
GTGGCCTGGAGTAGTCGGTATGGAAGCAGTCACTTTTGTAAATGTTAAGTTTAGGGGAGA
TGGTTATAATGGAATAGTGTTTATGGCCAATACCAAACTTATATTGCATGGTTGTAGCTT
TTTTGGTTTCAACAATACCTGTGTAGATGCCTGGGGACAGGTTAGTGTACGGGGGTGTAG
TTTCTATGCGTGTTGGATTGCCACAGCTGGCAGAACCAAGAGTCAATTGTCTCTGAAGAA
ATGCATATTCCAAAGATGTAACCTGGGCATTCTGAATGAAGGCGAAGCAAGGGTCCGTCA
CTGCGCTTCTACAGATACTGGATGTTTTATTTTAATTAAGGGAAATGCCAGCGTAAAGCA
TAACATGATTTGTGGTGCTTCCGATGAGAGGCCTTATCAAATGCTCACTTGTGCTGGTGG

GCATTGTAATATGCTGGCTACTGTGCATATTGTTTCCCATCAACGCAAAAAATGGCCTGT
TTTTGATCACAATGTGTTGACCAAGTGCACCATGCATGCAGGTGGGCGTAGAGGAATGTT
TATGCCTTACCAGTGTAACATGAATCATGTGAAAGTGTTGTTGGAACCAGATGCCTTTTC
CAGAATGAGCCTAACAGGAATCTTTGACATGAACACGCAAATCTGGAAGATCCTGAGGTA
TGATGATACGAGATCGAGGGTGCGCGCATGCGAATGCGGAGGCAAGCATGCCAGGTTCCA
GCCGGTGTGTGTAGATGTGACCGAAGATCTCAGACCGGATCATTTGGTTATTGCCCGCAC
TGGAGCAGAGTTCGGATCCAGTGGAGAAGAAACTGACTAAGGTGAGTATTGGGAAAACTT
TGGGGTGGGATTTTCAGATGGACAGATTGAGTAAAAATTTGTTTTTTCTGTCTTGCAGCT
GACATGAGTGGAAATGCTTCTTTTAAGGGGGGAGTCTTCAGCCCTTATCTGACAGGGCGT
CTCCCATCCTGGGCAGGAGTTCGTCAGAATGTTATGGGATCTACTGTGGATGGAAGACCC
GTTCAACCCGCCAATTCTTCAACGCTGACCTATGCTACTTTAAGTTCTTCACCTTTGGAC
GCAGCTGCAGCCGCTGCCGCCGCCTCTGTCGCCGCTAACACTGTGCTTGGAATGGGTTAC
TATGGAAGCATCGTGGCTAATTCCACTTCCTCTAATAACCCTTCTACACTGACTCAGGAC
AAGTTACTTGTCCTTTTGGCCCAGCTGGAGGCTTTGACCCAACGTCTGGGTGAACTTTCT
CAGCAGGTGGCCGAGTTGCGAGTACAAACTGAGTCTGCTGTCGGCACGGCAAAGTCTAAA
TAAAAAAAATTCCAGAATCAATGAATAAATAAACGAGCTTGTTGTTGATTTAAAATCAAG
TGTTTTTATTTCATTTTTCGCGCACGGTATGCCCTGGACCACCGATCTCGATCATTGAGA
ACTCGGTGGATTTTTTCCAGAATCCTATAGAGGTGGGATTGAATGTTTAGATACATGGGC
ATTAGGCCGTCTTTGGGGTGGAGATAGCTCCATTGAAGGGATTCATGCTCCGGGGTAGTG
TTGTAAATCACCCAGTCATAACAAGGTCGCAGTGCATGGTGTTGCACAATATCTTTTAGA
AGTAGGCTGATTGCCACAGATAAGCCCTTGGTGTAGGTGTTTACAAACCGGTTGAGCTGG
GAGGGGTGCATTCGAGGTGAAATTATGTGCATTTTGGATTGGATTTTTAAGTTGGCAATA
TTGCCGCCAAGATCCCGTCTTGGGTTCATGTTATGAAGGACTACCAAGACGGTGTATCCG
GTACATTTAGGAAATTTATCGTGCAGCTTGGATGGAAAAGCGTGGAAAAATTTGGAGACA
CCCTTGTGTCCTCCGAGATTTTCCATGCACTCATCCATGATAATAGCAATGGGGCCGTGG
GCAGCGGCGCGGGCAAACACGTTCCGTGGGTCTGACACATCATAGTTATGTTCCTGAGTT
AAATCATCATAAGCCATTTTAATGAATTTGGGGCGGAGCGTACCAGATTGGGGTATGAAT
GTTCCTTCGGGCCCCGGAGCATAGTTCCCCTCACAGATTTGCATTTCCCAAGCTTTCAGT
TCTGAGGGTGGAATCATGTCCACCTGGGGGGCTATGAAGAACACCGTTTCGGGGGCGGGG
GTGATTAGTTGGGATGATAGCAAGTTTCTGAGCAATTGAGATTTGCCACATCCGGTGGGG
CCATAAATAATTCCGATTACAGGTTGCAGGTGGTAGTTTAGGGAACGGCAACTGCCGTCT
TCTCGAAGCAAGGGGGCCACCTCGTTCATCATTTCCCTTACATGCATATTTTCCCGCACC
AAATCCATTAGGAGGCGCTCTCCTCCTAGTGATAGAAGTTCTTGTAGTGAGGAAAAGTTT
TTCAGCGGTTTTAGACCGTCAGCCATGGGCATTTTGGAAAGAGTTTGCTGCAAAAGTTCT
AGTCTGTTCCACAGTTCAGTGATGTGTTCTATGGCATCTCGATCCAGCAGACCTCCTCGT
TTCGCGGGTTTGGACGGCTCCTGGAGTAGGGTATGAGACGATGGGCGTCCAGCGCTGCCA
GGGTTCGGTCCTTCCAGGGTCTCAGTGTTCGAGTCAGGGTTGTTTCCGTCACAGTGAAGG
GGTGTGCGCCTGCTTGGGCGCTTGCCAGGGTGCGCTTCAGACTCATTCTGCTGGTGGAGA
ACTTCTGTCGCTTGGCGCCCTGTATGTCGGCCAAGTAGCAGTTTACCATGAGTTCGTAGT
TGAGCGCCTCGGCTGCGTGGCCTTTGGCGCGGAGCTTACCTTTGGAAGTTTTCTTGCATA
CCGGGCAGTATAGGCATTTCAGCGCATACAGCTTGGGCGCAAGGAAAATGGATTCTGGGG
AGTATGCATCCGCGCCGCAGGAGGCGCAAACAGTTTCACATTCCACCAGCCAGGTTAAAT
CCGGTTCATTGGGGTCAAAAACAAGTTTTCCGCCATATTTTTTGATGCGTTTCTTACCTT
TGGTCTCCATAAGTTCGTGTCCTCGTTGAGTGACAAACAGGCTGTCCGTATCTCCGTAGA
CTGATTTTACAGGCCTCTTCTCCAGTGGAGTGCCTCGGTCTTCTTCGTACAGGAACTCTG
ACCACTCTGATACAAAGGCGCGCGTCCAGGCCAGCACAAAGGAGGCTATGTGGGAGGGGT
AGCGATCGTTGTCAACCAGGGGGTCCACCTTTTCCAAAGTATGCAAACACATGTCACCCT
CTTCAACATCCAGGAATGTGATTGGCTTGTAGGTGTATTTCACGTGACCTGGGGTCCCCG
CTGGGGGGGTATAAAAGGGGGCGGTTCTTTGCTCTTCCTCACTGTCTTCCGGATCGCTGT
CCAGGAACGTCAGCTGTTGGGGTAGGTATTCCCTCTCGAAGGCGGGCATGACCTCTGCAC
TCAGGTTGTCAGTTTCTAAGAACGAGGAGGATTTGATATTGACAGTGCCGGTTGAGATGC
CTTTCATGAGGTTTTCGTCCATTTGGTCAGAAAACACAATTTTTTTATTGTCAAGTTTGG
TGGCAAATGATCCATACAGGGCGTTGGATAAAAGTTTGGCAATGGATCGCATGGTTTGGT
TCTTTTCCTTGTCCGCGCGCTCTTTGGCGGCGATGTTGAGTTGGACATACTCGCGTGCCA
GGCACTTCCATTCGGGGAAGATAGTTGTTAATTCATCTGGCACGATTCTCACTTGCCACC
CTCGATTATGCAAGGTAATTAAATCCACACTGGTGGCCACCTCGCCTCGAAGGGGTTCAT
TGGTCCAACAGAGCCTACCTCCTTTCCTAGAACAGAAAGGGGGAAGTGGGTCTAGCATAA
GTTCATCGGGAGGGTCTGCATCCATGGTAAAGATTCCCGGAAGTAAATCCTTATCAAAAT
AGCTGATGGGAGTGGGGTCATCTAAGGCCATTTGCCATTCTCGAGCTGCCAGTGCGCGCT
CATATGGGTTAAGGGGACTGCCCCAGGGCATGGGATGGGTGAGAGCAGAGGCATACATGC

CACAGATGTCATAGACGTAGATGGGATCCTCAAAGATGCCTATGTAGGTTGGATAGCATC
GCCCCCCTCTGATACTTGCTCGCACATAGTCATATAGTTCATGTGATGGCGCTAGCAGCC
CCGGACCCAAGTTGGTGCGATTGGGTTTTTCTGTTCTGTAGACGATCTGGCGAAAGATGG
CGTGAGAATTGGAAGAGATGGTGGGTCTTTGAAAAATGTTGAAATGGGCATGAGGTAGAC
CTACAGAGTCTCTGACAAAGTGGGCATAAGATTCTTGAAGCTTGGTTACCAGTTCGGCGG
TGACAAGTACGTCTAGGGCGCAGTAGTCAAGTGTTTCTTGAATGATGTCATAACCTGGTT
GGTTTTTCTTTTCCCACAGTTCGCGGTTGAGAAGGTATTCTTCGCGATCCTTCCAGTACT
CTTCTAGCGGAAACCCGTCTTTGTCTGCACGGTAAGATCCTAGCATGTAGAACTGATTAA
CTGCCTTGTAAGGGCAGCAGCCCTTCTCTACGGGTAGAGAGTATGCTTGAGCAGCTTTTC
GTAGCGAAGCGTGAGTAAGGGCAAAGGTGTCTCTGACCATGACTTTGAGAAATTGGTATT
TGAAGTCCATGTCGTCACAGGCTCCCTGTTCCCAGAGTTGGAAGTCTACCCGTTTCTTGT
AGGCGGGGTTGGGCAAAGCGAAAGTAACATCATTGAAGAGAATCTTACCGGCTCTGGGCA
TAAAATTGCGAGTGATGCGGAAAGGCTGTGGTACTTCCGCTCGATTGTTGATCACCTGGG
CAGCTAGGACGATTTCGTCGAAACCGTTGATGTTGTGTCCTACGATGTATAATTCTATGA
AACGCGGCGTGCCTCTGACGTGAGGTAGCTTACTGAGCTCATCAAAGGTTAGGTCTGTGG
GGTCAGATAAGGCGTAGTGTTCGAGAGCCCATTCGTGCAGGTGAGGATTTGCATGTAGGA
ATGATGACCAAAGATCTACCGCCAGTGCTGTTTGTAACTGGTCCCGATACTGACGAAAAT
GCCGGCCAATTGCCATTTTTTCTGGAGTGACACAGTAGAAGGTTCTGGGGTCTTGTTGCC
ATCGATCCCACTTGAGTTTAATGGCTAGATCGTGGGCCATGTTGACGAGACGCTCTTCTC
CTGAGAGTTTCATGACCAGCATGAAAGGAACTAGTTGTTTGCCAAAGGATCCCATCCAGG
TGTAAGTTTCCACATCGTAGGTCAGGAAGAGTCTTTCTGTGCGAGGATGAGAGCCGATCG
GGAAGAACTGGATTTCCTGCCACCAGTTGGAGGATTGGCTGTTGATGTGATGGAAGTAGA
AGTTTCTGCGGCGCGCCGAGCATTCGTGTTTGTGCTTGTACAGACGGCCGCAGTAGTCGC
AGCGTTGCACGGGTTGTATCTCGTGAATGAGCTGTACCTGGCTTCCCTTGACGAGAAATT
TCAGTGGGAAGCCGAGGCCTGGCGATTGTATCTCGTGCTCTTCTATATTCGCTGTATCGG
CCTGTTCATCTTCTGTTTCGATGGTGGTCATGCTGACGAGCCCCCGCGGGAGGCAAGTCC
AGACCTCGGCGCGGGAGGGGCGGAGCTGAAGGACGAGAGCGCGCAGGCTGGAGCTGTCCA
GAGTCCTGAGACGCTGCGGACTCAGGTTAGTAGGTAGGGACAGAAGATTAACTTGCATGA
TCTTTTCCAGGGCGTGCGGGAGGTTCAGATGGTACTTGATTTCCACAGGTTCGTTTGTAG
AGACGTCAATGGCTTGCAGGGTTCCGTGTCCTTTGGGCGCCACTACCGTACCTTTGTTTT
TTCTTTTGATCGGTGGTGGCTCTCTTGCTTCTTGCATGCTCAGAAGCGGTGACGGGGACG
CGCGCCGGGCGGCAGCGGTTGTTCCGGACCCGGGGGCATGGCTGGTAGTGGCACGTCGGC
GCCGCGCACGGGCAGGTTCTGGTATTGCGCTCTGAGAAGACTTGCGTGCGCCACCACGCG
TCGATTGACGTCTTGTATCTGACGTCTCTGGGTGAAAGCTACCGGCCCCGTGAGCTTGAA
CCTGAAAGAGAGTTCAACAGAATCAATTTCGGTATCGTTAACGGCAGCTTGTCTCAGTAT
TTCTTGTACGTCACCAGAGTTGTCCTGGTAGGCGATCTCCGCCATGAACTGCTCGATTTC
TTCCTCCTGAAGATCTCCGCGACCCGCTCTTTCGACGGTGGCCGCGAGGTCATTGGAGAT
ACGGCCCATGAGTTGGGAGAATGCATTCATGCCCGCCTCGTTCCAGACGCGGCTGTAAAC
CACGGCCCCCTCGGAGTCTCTTGCGCGCATCACCACCTGAGCGAGGTTAAGCTCCACGTG
TCTGGTGAAGACCGCATAGTTGCATAGGCGCTGAAAAAGGTAGTTGAGTGTGGTGGCAAT
GTGTTCGGCGACGAAGAAATACATGATCCATCGTCTCAGCGGCATTTCGCTAACATCGCC
CAGAGCTTCCAAGCGCTCCATGGCCTCGTAGAAGTCCACGGCAAAATTAAAAAACTGGGA
GTTTCGCGCGGACACGGTCAATTCCTCCTCGAGAAGACGGATGAGTTCGGCTATGGTGGC
CCGTACTTCGCGTTCGAAGGCTCCCGGGATCTCTTCTTCCTCTTCTATCTCTTCTTCCAC
TAACATCTCTTCTTCGTCTTCAGGCGGGGGCGGAGGGGGCACGCGGCGACGTCGACGGCG
CACGGGCAAACGGTCGATGAATCGTTCAATGACCTCTCCGCGGCGGCGGCGCATGGTTTC
AGTGACGGCGCGGCCGTTCTCGCGCGGTCGCAGAGTAAAAACACCGCCGCGCATCTCCTT
AAAGTGGTGACTGGGAGGTTCTCCGTTTGGGAGGGAGAGGGCGCTGATTATACATTTTAT
TAATTGGCCCGTAGGGACTGCGCGCAGAGATCTGATCGTGTCAAGATCCACGGGATCTGA
AAACCTTTCGACGAAAGCGTCTAACCAGTCACAGTCACAAGGTAGGCTGAGTACGGCTTC
TTGTGGGCGGGGGTGGTTATGTGTTCGGTCTGGGTCTTCTGTTTCTTCTTCATCTCGGGA
AGGTGAGACGATGCTGCTGGTGATGAAATTAAAGTAGGCAGTTCTAAGACGGCGGATGGT
GGCGAGGAGCACCAGGTCTTTGGGTCCGGCTTGCTGGATACGCAGGCGATTGGCCATTCC
CCAAGCATTATCCTGACATCTAGCAAGATCTTTGTAGTAGTCTTGCATGAGCCGTTCTAC
GGGCACTTCTTCCTCACCCGTTCTGCCATGCATACGTGTGAGTCCAAATCCGCGCATTGG
TTGTACCAGTGCCAAGTCAGCTACGACTCTTTCGGCGAGGATGGCTTGCTGTACTTGGGT
AAGGGTGGCTTGAAAGTCATCAAAATCCACAAAGCGGTGGTAAGCCCCTGTATTAATGGT
GTAAGCACAGTTGGCCATGACTGACCAGTTAACTGTCTGGTGACCAGGGCGCACGAGCTC
GGTGTATTTAAGGCGCGAATAGGCGCGGGTGTCAAAGATGTAATCGTTGCAGGTGCGCAC
CAGATACTGGTACCCTATAAGAAAATGCGGCGGTGGTTGGCGGTAGAGAGGCCATCGTTC

TGTAGCTGGAGCGCCAGGGGCGAGGTCTTCCAACATAAGGCGGTGATAGCCGTAGATGTA
CCTGGACATCCAGGTGATTCCTGCGGCGGTAGTAGAAGCCCGAGGAAACTCGCGTACGCG
GTTCCAAATGTTGCGTAGCGGCATGAAGTAGTTCATTGTAGGCACGGTTTGACCAGTGAG
GCGCGCGCAGTCATTGATGCTCTATAGACACGGAGAAAATGAAAGCGTTCAGCGACTCGA
CTCCGTAGCCTGGAGGAACGTGAACGGGTTGGGTCGCGGTGTACCCCGGTTCGAGACTTG
TACTCGAGCCGGCCGGAGCCGCGGCTAACGTGGTATTGGCACTCCCGTCTCGACCCAGCC
TACAAAAATCCAGGATACGGAATCGAGTCGTTTTGCTGGTTTCCGAATGGCAGGGAAGTG
AGTCCTATTTTTTTTTTTTTTTTGCCGCTCAGATGCATCCCGTGCTGCGACAGATGCGCC
CCCAACAACAGCCCCCCTCGCAGCAGCAGCAGCAGCAACCACAAAAGGCTGTCCCTGCAA
CTACTGCAACTGCCGCCGTGAGCGGTGCGGGACAGCCCGCCTATGATCTGGACTTGGAAG
AGGGCGAAGGACTGGCACGTCTAGGTGCGCCTTCGCCCGAGCGGCATCCGCGAGTTCAAC
TGAAAAAAGATTCTCGCGAGGCGTATGTGCCCCAACAGAACCTATTTAGAGACAGAAGCG
GCGAGGAGCCGGAGGAGATGCGAGCTTCCCGCTTTAACGCGGGTCGTGAGCTGCGTCACG
GTTTGGACCGAAGACGAGTGTTGCGAGACGAGGATTTCGAAGTTGATGAAGTGACAGGGA
TCAGTCCTGCCAGGGCACACGTGGCTGCAGCCAACCTTGTATCGGCTTACGAGCAGACAG
TAAAGGAAGAGCGTAACTTCCAAAAGTCTTTTAATAATCATGTGCGAACCCTGATTGCCC
GCGAAGAAGTTACCCTTGGTTTGATGCATTTGTGGGATTTGATGGAAGCTATCATTCAGA
ACCCTACTAGCAAACCTCTGACCGCCCAGCTGTTTCTGGTGGTGCAACACAGCAGAGACA
ATGAGGCTTTCAGAGAGGCGCTGCTGAACATCACCGAACCCGAGGGGAGATGGTTGTATG
ATCTTATCAACATTCTACAGAGTATCATAGTGCAGGAGCGGAGCCTGGGCCTGGCCGAGA
AGGTAGCTGCCATCAATTACTCGGTTTTGAGCTTGGGAAAATATTACGCTCGCAAAATCT
ACAAGACTCCATACGTTCCCATAGACAAGGAGGTGAAGATAGATGGGTTCTACATGCGCA
TGACGCTCAAGGTCTTGACCCTGAGCGATGATCTTGGGGTGTATCGCAATGACAGAATGC
ATCGCGCGGTTAGCGCCAGCAGGAGGCGCGAGTTAAGCGACAGGGAACTGATGCACAGTT
TGCAAAGAGCTCTGACTGGAGCTGGAACCGAGGGTGAGAATTACTTCGACATGGGAGCTG
ACTTGCAGTGGCAGCCTAGTCGCAGGGCTCTGAGCGCCGCGACGGCAGGATGTGAGCTTC
CTTACATAGAAGAGGCGGATGAAGGCGAGGAGGAAGAGGGCGAGTACTTGGAAGACTGAT
GGCACAACCCGTGTTTTTTGCTAGATGGAACAGCAAGCACCGGATCCCGCAATGCGGGCG
GCGCTGCAGAGCCAGCCGTCCGGCATTAACTCCTCGGACGATTGGACCCAGGCCATGCAA
CGTATCATGGCGTTGACGACTCGCAACCCCGAAGCCTTTAGACAGCAACCCCAGGCCAAC
CGTCTATCGGCCATCATGGAAGCTGTAGTGCCTTCCCGATCTAATCCCACTCATGAGAAG
GTCCTGGCCATCGTGAACGCGTTGGTGGAGAACAAAGCTATTCGTCCAGATGAGGCCGGA
CTGGTATACAACGCTCTCTTAGAACGCGTGGCTCGCTACAACAGTAGCAATGTGCAAACC
AATTTGGACCGTATGATAACAGATGTACGCGAAGCCGTGTCTCAGCGCGAAAGGTTCCAG
CGTGATGCCAACCTGGGTTCGCTGGTGGCGTTAAATGCTTTCTTGAGTACTCAGCCTGCT
AATGTGCCGCGTGGTCAACAGGATTATACTAACTTTTTAAGTGCTTTGAGACTGATGGTA
TCAGAAGTACCTCAGAGCGAAGTGTATCAGTCCGGTCCTGATTACTTCTTTCAGACTAGC
AGACAGGGCTTGCAGACGGTAAATCTGAGCCAAGCTTTTAAAAACCTTAAAGGTTTGTGG
GGAGTGCATGCCCCGGTAGGAGAAAGAGCAACCGTGTCTAGCTTGTTAACTCCGAACTCC
CGCCTGTTATTACTGTTGGTAGCTCCTTTCACCGACAGCGGTAGCATCGACCGTAATTCC
TATTTGGGTTACCTACTAAACCTGTATCGCGAAGCCATAGGGCAAAGTCAGGTGGACGAG
CAGACCTATCAAGAAATTACCCAAGTCAGTCGCGCTTTGGGACAGGAAGACACTGGCAGT
TTGGAAGCCACTCTGAACTTCTTGCTTACCAATCGGTCTCAAAAGATCCCTCCTCAATAT
GCTCTTACTGCGGAGGAGGAGAGGATCCTTAGATATGTGCAGCAGAGCGTGGGATTGTTT
CTGATGCAAGAGGGGGCAACTCCGACTGCAGCACTGGACATGACAGCGCGAAATATGGAG
CCCAGCATGTATGCCAGTAACCGACCTTTCATTAACAAACTGCTGGACTACTTGCACAGA
GCTGCCGCTATGAACTCTGATTATTTCACCAATGCCATCTTAAACCCGCACTGGCTGCCC
CCACCTGGTTTCTACACGGGCGAATATGACATGCCCGACCCTAATGACGGATTTCTGTGG
GACGACGTGGACAGCGATGTTTTTTCACCTCTTTCTGATCATCGCACGTGGAAAAAGGAA
GGCGGTGATAGAATGCATTCTTCTGCATCGCTGTCCGGGGTCATGGGTGCTACCGCGGCT
GAGCCCGAGTCTGCAAGTCCTTTTCCTAGTCTACCCTTTTCTCTACACAGTGTACGTAGC
AGCGAAGTGGGTAGAATAAGTCGCCCGAGTTTAATGGGCGAAGAGGAGTACCTAAACGAT
TCCTTGCTCAGACCGGCAAGAGAAAAAAATTTCCCAAACAATGGAATAGAAAGTTTGGTG
GATAAAATGAGTAGATGGAAGACTTATGCTCAGGATCACAGAGACGAGCCTGGGATCATG
GGGACTACAAGTAGAGCGAGCCGTAGACGCCAGCGCCATGACAGACAGAGGGGTCTTGTG
TGGGACGATGAGGATTCGGCCGATGATAGCAGCGTGTTGGACTTGGGTGGGAGAGGAAGG
GGCAACCCGTTTGCTCATTTGCGCCCTCGCTTGGGTGGTATGTTGTGAAAAAAAATAAAA
AAGAAAAACTCACCAAGGCCATGGCGACGAGCGTACGTTCGTTCTTCTTTATTATCTGTG
TCTAGTATAATGAGGCGAGTCGTGCTAGGCGGAGCGGTGGTGTATCCGGAGGGTCCTCCT
CCTTCGTACGAGAGCGTGATGCAGCAGCAGCAGGCGACGGCGGTGATGCAATCCCCACTG

GAGGCTCCCTTTGTGCCTCCGCGATACCTGGCACCTACGGAGGGCAGAAACAGCATTCGT
TACTCGGAACTGGCACCTCAGTACGATACCACCAGGTTGTATCTGGTGGACAACAAGTCG
GCGGACATTGCTTCTCTGAACTATCAGAATGACCACAGCAACTTCTTGACCACGGTGGTG
CAGAACAATGACTTTACCCCTACGGAAGCCAGCACCCAGACCATTAACTTTGATGAACGA
TCGCGGTGGGGCGGTCAGCTAAAGACCATCATGCATACTAACATGCCAAACGTGAACGAG
TATATGTTTAGTAACAAGTTCAAAGCGCGTGTGATGGTGTCCAGAAAACCTCCCGACGGT
GCTGCAGTTGGGGATACTTATGATCACAAGCAGGATATTTTGGAATATGAGTGGTTCGAG
TTTACTTTGCCAGAAGGCAACTTTTCAGTTACTATGACTATTGATTTGATGAACAATGCC
ATCATAGATAATTACTTGAAAGTGGGTAGACAGAATGGAGTGCTTGAAAGTGACATTGGT
GTTAAGTTCGACACCAGGAACTTCAAGCTGGGATGGGATCCCGAAACCAAGTTGATCATG
CCTGGAGTGTATACGTATGAAGCCTTCCATCCTGACATTGTCTTACTGCCTGGCTGCGGA
GTGGATTTTACCGAGAGTCGTTTGAGCAACCTTCTTGGTATCAGAAAAAAACAGCCATTT
CAAGAGGGTTTTAAGATTTTGTATGAAGATTTAGAAGGTGGTAATATTCCGGCCCTCTTG
GATGTAGATGCCTATGAGAACAGTAAGAAAGAACAAAAAGCCAAAATAGAAGCTGCTACA
GCTGCTGCAGAAGCTAAGGCAAACATAGTTGCCAGCGACTCTACAAGGGTTGCTAACGCT
GGAGAGGTCAGAGGAGACAATTTTGCGCCAACACCTGTTCCGACTGCAGAATCATTATTG
GCCGATGTGTCTGAAGGAACGGACGTGAAACTCACTATTCAACCTGTAGAAAAAGATAGT
AAGAATAGAAGCTATAATGTGTTGGAAGACAAAATCAACACAGCCTATCGCAGTTGGTAT
CTTTCGTACAATTATGGCGATCCCGAAAAAGGAGTGCGTTCCTGGACATTGCTCACCACC
TCAGATGTCACCTGCGGAGCAGAGCAGGTTTACTGGTCGCTTCCAGACATGATGAAGGAT
CCTGTCACTTTCCGCTCCACTAGACAAGTCAGTAACTACCCTGTGGTGGGTGCAGAGCTT
ATGCCCGTCTTCTCAAAGAGCTTCTACAACGAACAAGCTGTGTACTCCCAGCAGCTCCGC
CAGTCCACCTCGCTTACGCACGTCTTCAACCGCTTTCCTGAGAACCAGATTTTAATCCGT
CCGCCGGCGCCCACCATTACCACCGTCAGTGAAAACGTTCCTGCTCTCACAGATCACGGG
ACCCTGCCGTTGCGCAGCAGTATCCGGGGAGTCCAACGTGTGACCGTTACTGACGCCAGA
CGCCGCACCTGTCCCTACGTGTACAAGGCACTGGGCATAGTCGCACCGCGCGTCCTTTCA
AGCCGCACTTTCTAAAAAAAAAAAAATGTCCATTCTTATCTCGCCCAGTAATAACACCGGT
TGGGGTCTGCGCGCTCCAAGCAAGATGTACGGAGGCGCACGCAAACGTTCTACCCAACAT
CCCGTGCGTGTTCGCGGACATTTTCGCGCTCCATGGGGTGCCCTCAAGGGCCGCACTCGC
GTTCGAACCACCGTCGATGATGTAATCGATCAGGTGGTTGCCGACGCCCGTAATTATACT
CCTACTGCGCCTACATCTACTGTGGATGCAGTTATTGACAGTGTAGTGGCTGACGCTCGC
AACTATGCTCGACGTAAGAGCCGGCGAAGGCGCATTGCCAGACGCCACCGAGCTACCACT
GCCATGCGAGCCGCAAGAGCTCTGCTACGAAGAGCTAGACGCGTGGGGCGAAGAGCCATG
CTTAGGGCGGCCAGACGTGCAGCTTCGGGCGCCAGCGCCGGCAGGTCCCGCAGGCAAGCA
GCCGCTGTCGCAGCGGCGACTATTGCCGACATGGCCCAATCGCGAAGAGGCAATGTATAC
TGGGTGCGTGACGCTGCCACCGGTCAACGTGTACCCGTGCGCACCCGTCCCCCTCGCACT
TAGAAGATACTGAGCAGTCTCCGATGTTGTGTCCCAGCGGCGAGGATGTCCAAGCGCAAA
TACAAGGAAGAAATGCTGCAGGTTATCGCACCTGAAGTCTACGGCCAACCGTTGAAGGAT
GAAAAAAAACCCCGCAAAATCAAGCGGGTTAAAAAGGACAAAAAAGAAGAGGAAGATGGC
GATGATGGGCTGGCGGAGTTTGTGCGCGAGTTTGCCCCACGGCGACGCGTGCAATGGCGT
GGGCGCAAAGTTCGACATGTGTTGAGACCTGGAACTTCGGTGGTCTTTACACCCGGCGAG
CGTTCAAGCGCTACTTTTAAGCGTTCCTATGATGAGGTGTACGGGGATGATGATATTCTT
GAGCAGGCGGCTGACCGATTAGGCGAGTTTGCTTATGGCAAGCGTAGTAGAATAACTTCC
AAGGATGAGACAGTGTCAATACCCTTGGATCATGGAAATCCCACCCCTAGTCTTAAACCG
GTCACTTTGCAGCAAGTGTTACCCGTAACTCCGCGAACAGGTGTTAAACGCGAAGGTGAA
GATTTGTATCCCACTATGCAACTGATGGTACCCAAACGCCAGAAGTTGGAGGACGTTTTG
GAGAAAGTAAAAGTGGATCCAGATATTCAACCTGAGGTTAAAGTGAGACCCATTAAGCAG
GTAGCGCCTGGTCTGGGGGTACAAACTGTAGACATTAAGATTCCCACTGAAAGTATGGAA
GTGCAAACTGAACCCGCAAAGCCTACTGCCACCTCCACTGAAGTGCAAACGGATCCATGG
ATGCCCATGCCTATTACAACTGACGCCGCCGGTCCCACTCGAAGATCCCGACGAAAGTAC
GGTCCAGCAAGTCTGTTGATGCCCAATTATGTTGTACACCCATCTATTATTCCTACTCCT
GGTTACCGAGGCACTCGCTACTATCGCAGCCGAAACAGTACCTCCCGCCGTCGCCGCAAG
ACACCTGCAAATCGCAGTCGTCGCCGTAGACGCACAAGCAAACCGACTCCCGGCGCCCTG
GTGCGGCAAGTGTACCGCAATGGTAGTGCGGAACCTTTGACACTGCCGCGTGCGCGTTAC
CATCCGAGTATCATCACTTAATCAATGTTGCCGCTGCCTCCTTGCAGATATGGCCCTCAC
TTGTCGCCTTCGCGTTCCCATCACTGGTTACCGAGGAAGAAACTCGCGCCGTAGAAGAGG
GATGTTGGGACGCGGAATGCGACGCTACAGGCGACGGCGTGCTATCCGCAAGCAATTGCG
GGGTGGTTTTTTACCAGCCTTAATTCCAATTATCGCTGCTGCAATTGGCGCGATACCAGG
CATAGCTTCCGTGGCGGTTCAGGCCTCGCAACGACATTGACATTGGAAAAAAAACGTATA
AATAAAAAAAAATACAATGGACTCTGACACTCCTGGTCCTGTGACTATGTTTTCTTAGAG

ATGGAAGACATCAATTTTTCATCCTTGGCTCCGCGACACGGCACGAAGCCGTACATGGGC
ACCTGGAGCGACATCGGCACGAGCCAACTGAACGGGGGCGCCTTCAATTGGAGCAGTATC
TGGAGCGGGCTTAAAAATTTTGGCTCAACCATAAAAACATACGGGAACAAAGCTTGGAAC
AGCAGTACAGGACAGGCGCTTAGAAATAAACTTAAAGACCAGAACTTCCAACAAAAAGTA
GTCGATGGGATAGCTTCCGGCATCAATGGAGTGGTAGATTTGGCTAACCAGGCTGTGCAG
AAAAAGATAAACAGTCGTTTGGACCCGCCGCCAGCAACCCCAGGTGAAATGCAAGTGGAG
GAAGAAATTCCTCCGCCAGAAAAACGAGGCGACAAGCGTCCGCGTCCCGATTTGGAAGAG
ACGCTGGTGACGCGCGTAGATGAACCGCCTTCTTATGAGGAAGCAACGAAGCTTGGAATG
CCCACCACTAGACCGATAGCCCCAATGGCCACCGGGGGTGATGAAACCTTCTCAGTTGCAT
CGACCCGTCACCTTGGATTTGCCCCCTCCCCCTGCTGCTACTGCTGTACCCGCTTCTAAG
CCTGTCGCTGCCCCGAAACCAGTCGCCGTAGCCAGGTCACGTCCCGGGGGCGCTCCTCGT
CCAAATGCGCACTGGCAAAATACTCTGAACAGCATCGTGGGTCTAGGCGTGCAAAGTGTA
AAACGCCGTCGCTGCTTTTAATTAAATATGGAGTAGCGCTTAACTTGCCTATCTGTGTAT
ATGTGTCATTACACGCCGTCACAGCAGCAGAGGAAAAAAGGAAGAGGTCGTGCGTCGACG
CTGAGTTACTTTCAAGATGGCCACCCCATCGATGCTGCCCCAATGGGCATACATGCACAT
CGCCGGACAGGATGCTTCGGAGTACCTGAGTCCGGGTCTGGTGCAGTTCGCCCGCGCCAC
AGACACCTACTTCAATCTGGGAAATAAGTTTAGAAATCCCACCGTAGCGCCGACCCACGA
TGTGACCACCGACCGTAGCCAGCGGCTCATGTTGCGCTTCGTGCCCGTTGACCGGGAGGA
CAATACATACTCTTACAAAGTGCGGTACACCCTGGCCGTGGGCGACAACAGAGTGCTGGA
TATGGCCAGCACGTTCTTTGACATTAGGGGCGTGTTGGACAGAGGTCCCAGTTTCAAACC
CTATTCTGGTACGGCTTACAACTCTCTGGCTCCTAAAGGCGCTCCAAATGCATCTCAATG
GATTGCAAAAGGCGTACCAACTGCAGCAGCCGCAGGCAATGGTGAAGAAGAACATGAAAC
AGAGGAGAAAACTGCTACTTACACTTTTGCCAATGCTCCTGTAAAAGCCGAGGCTCAAAT
TACAAAAGAGGGCTTACCAATAGGTTTGGAGATTTCAGCTGAAAACGAATCTAAACCCAT
CTATGCAGATAAACTTTATCAGCCAGAACCTCAAGTGGGAGATGAAACTTGGACTGACCT
AGACGGAAAAACCGAAGAGTATGGAGGCAGGGCTCTAAAGCCTACTACTAACATGAAACC
CTGTTACGGGTCCTATGCGAAGCCTACTAATTTAAAAGGTGGTCAGGCAAAACCGAAAAA
CTCGGAACCGTCGAGTGAAAAAATTGAATATGATATTGACATGGAATTTTTTGATAACTC
ATCGCAAAGAACAAACTTCAGTCCTAAAATTGTCATGTATGCAGAAAATGTAGGTTTGGA
AACGCCAGACACTCATGTAGTGTACAAACCTGGAACAGAAGACACAAGTTCCGAAGCTAA
TTTGGGACAACAGTCTATGCCCAACAGACCCAACTACATTGGCTTCAGAGATAACTTTAT
TGGACTCATGTACTATAACAGTACTGGTAACATGGGGGTGCTGGCTGGTCAAGCGTCTCA
GTTAAATGCAGTGGTTGACTTGCAGGACAGAAACACAGAACTTTCTTACCAACTCTTGCT
TGACTCTCTGGGCGACAGAACCAGATACTTTAGCATGTGGAATCAGGCTGTGGACAGTTA
TGATCCTGATGTACGTGTTATTGAAAATCATGGTGTGGAAGATGAACTTCCCAACTATTG
TTTTCCACTGGACGGCATAGGTGTTCCAACAACCAGTTACAAATCAATAGTTCCAAATGG
AGAAGATAATAATAATTGGAAAGAACCTGAAGTAAATGGAACAAGTGAGATCGGACAGGG
TAATTTGTTTGCCATGGAAATTAACCTTCAAGCCAATCTATGGCGAAGTTTCCTTTATTC
CAATGTGGCTCTGTATCTCCCAGACTCGTACAAATACACCCCGTCCAATGTCACTCTTCC
AGAAAACAAAAACACCTACGACTACATGAACGGGCGGGTGGTGCCGCCATCTCTAGTAGA
CACCTATGTGAACATTGGTGCCAGGTGGTCTCTGGATGCCATGGACAATGTCAACCCATT
CAACCACCACCGTAACGCTGGCTTGCGTTACCGATCTATGCTTCTGGGTAACGGACGTTA
TGTGCCTTTCCACATACAAGTGCCTCAAAAATTCTTCGCTGTTAAAAACCTGCTGCTTCT
CCCAGGCTCCTACACTTATGAGTGGAACTTTAGGAAGGATGTGAACATGGTTCTACAGAG
TTCCCTCGGTAACGACCTGCGGGTAGATGGCGCCAGCATCAGTTTCACGAGCATCAACCT
CTATGCTACTTTTTTCCCCATGGCTCACAACACCGCTTCCACCCTTGAAGCCATGCTGCG
GAATGACACCAATGATCAGTCATTCAACGACTACCTATCTGCAGCTAACATGCTCTACCC
CATTCCTGCCAATGCAACCAATATTCCCATTTCCATTCCTTCTCGCAACTGGGCGGCTTT
CAGAGGCTGGTCATTTACCAGACTGAAAACCAAAGAAACTCCCTCTTTGGGGTCTGGATT
TGACCCCTACTTTGTCTATTCTGGTTCTATTCCCTACCTGGATGGTACCTTCTACCTGAA
CCACACTTTTAAGAAGGTTTCCATCATGTTTGACTCTTCAGTGAGCTGGCCTGGAAATGA
CAGGTTACTATCTCCTAACGAATTTGAAATAAAGCGCACTGTGGATGGCGAAGGCTACAA
CGTAGCCCAATGCAACATGACCAAAGACTGGTTCTTGGTACAGATGCTCGCCAACTACAA
CATCGGCTATCAGGGCTTCTACATTCCAGAAGGATACAAAGATCGCATGTATTCATTTTT
CAGAAACTTCCAGCCCATGAGCAGGCAGGTGGTTGATGAGGTCAATTACAAAGACTTCAA
GGCCGTCGCCATACCCTACCAACACAACAACTCTGGCTTTGTGGGTTACATGGCTCCGAC
CATGCGCCAAGGTCAACCCTATCCCGCTAACTATCCCTATCCACTCATTGGAACAACTGC
CGTAAATAGTGTTACGCAGAAAAAGTTCTTGTGTGACAGAACCATGTGGCGCATACCGTT
CTCGAGCAACTTCATGTCTATGGGGGCCCTTACAGACTTGGGACAGAATATGCTCTATGC
CAACTCAGCTCATGCTCTGGACATGACCTTTGAGGTGGATCCCATGGATGAGCCCACCCT

GCTTTATCTTCTCTTCGAAGTTTTCGACGTGGTCAGAGTGCATCAGCCACACCGCGGCAT
CATCGAGGCAGTCTACCTGCGTACACCGTTCTCGGCCGGTAACGCTACCACGTAAGAAGC
TTCTTGCTTCTTGCAAATAGCAGCTGCAACCATGGCCTGCGGATCCCAAAACGGCTCCAG
CGAGCAAGAGCTCAGAGCCATTGTCCAAGACCTGGGTTGCGGACCCTATTTTTTGGGAAC
CTACGATAAGCGCTTCCCGGGGTTCATGGCCCCCGATAAGCTCGCCTGTGCCATTGTAAA
TACGGCCGGACGTGAGACGGGGGGAGAGCACTGGTTGGCTTTCGGTTGGAACCCACGTTC
TAACACCTGCTACCTTTTTGATCCTTTTGGATTCTCGGATGATCGTCTCAAACAGATTTA
CCAGTTTGAATATGAGGGTCTCCTGCGCCGCAGCGCTCTTGCTACCAAGGACCGCTGTAT
TACGCTGGAAAAATCTACCCAGACCGTGCAGGGCCCCCGTTCTGCCGCCTGCGGACTTTT
CTGCTGCATGTTCCTTCACGCCTTTGTGCACTGGCCTGACCGTCCCATGGACGGAAACCC
CACCATGAAATTGCTAACTGGAGTGCCAAACAACATGCTTCATTCTCCTAAAGTCCAGCC
CACCCTGTGTGACAATCAAAAAGCACTCTACCATTTTCTTAATACCCATTCGCCTTATTT
TCGCTCTCATCGTACACACATCGAAAGGGCCACTGCGTTCGACCGTATGGATGTTCAATA
ATGACTCATGTAAACAACGTGTTCAATAAACATCACTTTATTTTTTTACATGTATCAAGG
CTCTGGATTACTTATTTATTTACAAGTCGAATGGGTTCTGACGAGAATCAGAATGACCCG
CAGGCAGTGATACGTTGCGGAACTGATACTTGGGTTGCCACTTGAATTCGGGAATCACCA
ACTTGGGAACCGGTATATCGGGCAGGATGTCACTCCACAGCTTTCTGGTCAGCTGCAAAG
CTCCAAGCAGGTCAGGAGCCGAAATCTTGAAATCACAATTAGGACCAGTGCTCTGAGCGC
GAGAGTTGCGGTACACCGGATTGCAGCACTGAAACACCATCAGCGACGGATGTCTCACGC
TTGCCAGCACGGTGGGATCTGCAATCATGCCCACATCCAGATCTTCAGCATTGGCAATGC
TGAACGGGGTCATCTTGCAGGTCTGCCTACCCATGGCGGGCACCCAATTAGGCTTGTGGT
TGCAATCGCAGTGCAGGGGGATCAGTATCATCTTGGCCTGATCCTGTCTGATTCCTGGAT
ACACGGCTCTCATGAAAGCATCATATTGCTTGAAAGCCTGCTGGGCTTTACTACCCTCGG
TATAAAACATCCCGCAGGACCTGCTCGAAAACTGGTTAGCTGCACAGCCGGCATCATTCA
CACAGCAGCGGGCGTCATTGTTGGCTATTTGCACCACACTTCTGCCCCAGCGGTTTTGGG
TGATTTTGGTTCGCTCGGGATTCTCCTTTAAGGCTCGTTGTCCGTTCTCGCTGGCCACAT
CCATCTCGATAATCTGCTCCTTCTGAATCATAATATTGCCATGCAGGCACTTCAGCTTGC
CCTCATAATCATTGCAGCCATGAGGCCACAACGCACAGCCTGTACATTCCCAATTATGGT
GGGCGATCTGAGAAAAAGAATGTATCATTCCCTGCAGAAATCTTCCCATCATCGTGCTCA
GTGTCTTGTGACTAGTGAAAGTTAACTGGATGCCTCGGTGCTCTTCGTTTACGTACTGGT
GACAGATGCGCTTGTATTGTTCGTGTTGCTCAGGCATTAGTTTAAAACAGGTTCTAAGTT
CGTTATCCAGCCTGTACTTCTCCATCAGCAGACACATCACTTCCATGCCTTTCTCCCAAG
CAGACACCAGGGGCAAGCTAATCGGATTCTTAACAGTGCAGGCAGCAGCTCCTTTAGCCA
GAGGGTCATCTTTAGCGATCTTCTCAATGCTTCTTTTGCCATCCTTCTCAACGATGCGCA
CGGGCGGGTAGCTGAAACCCACTGCTACAAGTTGCGCCTCTTCTCTTTCTTCTTCGCTGT
CTTGACTGATGTCTTGCATGGGGATATGTTTGGTCTTCCTTGGCTTCTTTTTGGGGGGTA
TCGGAGGAGGAGGACTGTCGCTCCGTTCCGGAGACAGGGAGGATTGTGACGTTTCGCTCA
CCATTACCAACTGACTGTCGGTAGAAGAACCTGACCCCACACGGCGACAGGTGTTTTTCT
TCGGGGGCAGAGGTGGAGGCGATTGCGAAGGGCTGCGGTCCGACCTGGAAGGCGGATGAC
TGGCAGAACCCCTTCCGCGTTCGGGGGTGTGCTCCCTGTGGCGGTCGCTTAACTGATTTC
CTTCGCGGCTGGCCATTGTGTTCTCCTAGGCAGAGAAACAACAGACATGGAAACTCAGCC
ATTGCTGTCAACATCGCCACGAGTGCCATCACATCTCGTCCTCAGCGACGAGGAAAAGGA
GCAGAGCTTAAGCATTCCACCGCCCAGTCCTGCCACCACCTCTACCCTAGAAGATAAGGA
GGTCGACGCATCTCATGACATGCAGAATAAAAAAGCGAAAGAGTCTGAGACAGACATCGA
GCAAGACCCGGGCTATGTGACACCGGTGGAACACGAGGAAGAGTTGAAACGCTTTCTAGA
GAGAGAGGATGAAAACTGCCCAAAACAGCGAGCAGATAACTATCACCAAGATGCTGGAAA
TAGGGATCAGAACACCGACTACCTCATAGGGCTTGACGGGGAAGACGCGCTCCTTAAACA
TCTAGCAAGACAGTCGCTCATAGTCAAGGATGCATTATTGGACAGAACTGAAGTGCCCAT
CAGTGTGGAAGAGCTCAGCTGCGCCTACGAGCTTAACCTTTTTTCACCTCGTACTCCCCC
CAAACGTCAGCCAAACGGCACCTGCGAGCCAAATCCTCGCTTAAACTTTTATCCAGCTTT
TGCTGTGCCAGAAGTACTGGCTACCTATCACATCTTTTTTAAAAATCAAAAAATTCCAGT
CTCCTGCCGCGCTAATCGCACCCGCGCCGATGCCCTACTCAATCTGGGACCTGGTTCACG
CTTACCTGATATAGCTTCCTTGGAAGAGGTTCCAAAGATCTTCGAGGGTCTGGGCAATAA
TGAGACTCGGGCCGCAAATGCTCTGCAAAAGGGAGAAAATGGCATGGATGAGCATCACAG
CGTTCTGGTGGAATTGGAAGGCGATAATGCCAGACTCGCAGTACTCAAGCGAAGCGTCGA
GGTCACACACTTCGCATATCCCGCTGTCAACCTGCCCCCTAAAGTCATGACGGCGGTCAT
GGACCAGTTACTCATTAAGCGCGCAAGTCCCCTTTCAGAAGACATGCATGACCCAGATGC
CTGTGATGAGGGTAAACCAGTGGTCAGTGATGAGCAGCTAACCCGATGGCTGGGCACCGA
CTCTCCCCGGGATTTGGAAGAGCGTCGCAAGCTTATGATGGCCGTGGTGCTGGTTACCGT
AGAACTAGAGTGTCTCCGACGTTTCTTTACCGATTCAGAAACCTTGCGCAAACTCGAAGA

GAATCTGCACTACACTTTTAGACACGGCTTTGTGCGGCAGGCATGCAAGATATCTAACGT
GGAACTCACCAACCTGGTTTCCTACATGGGTATTCTGCATGAGAATCGCCTAGGACAAAG
CGTGCTGCACAGCACCCTTAAGGGGGAAGCCCGCCGTGATTACATCCGCGATTGTGTCTA
TCTCTACCTGTGCCACACGTGGCAAACCGGCATGGGTGTATGGCAGCAATGTTTAGAAGA
ACAGAACTTGAAAGAGCTTGACAAGCTCTTACAGAAATCTCTTAAGGTTCTGTGGACAGG
GTTCGACGAGCGCACCGTCGCTTCCGACCTGGCAGACCTCATCTTCCCAGAGCGTCTCAG
GGTTACTTTGCGAAACGGATTGCCTGACTTTATGAGCCAGAGCATGCTTAACAATTTTCG
CTCTTTCATCCTGGAACGCTCCGGTATCCTGCCCGCCACCTGCTGCGCACTGCCCTCCGA
CTTTGTGCCTCTCACCTACCGCGAGTGCCCCCCGCCGCTATGGAGTCACTGCTACCTGTT
CCGTCTGGCCAACTATCTCTCCTACCACTCGGATGTGATCGAGGATGTGAGCGGAGACGG
CTTGCTGGAGTGCCACTGCCGCTGCAATCTGTGCACGCCCCACCGGTCCCTAGCTTGCAA
CCCCCAGTTGATGAGCGAAACCCAGATAATAGGCACCTTTGAATTGCAAGGCCCCAGCAG
CCAAGGCGATGGGTCTTCTCCTGGGCAAAGTTTAAAACTGACCCCGGGACTGTGGACCTC
CGCCTACTTGCGCAAGTTTGCTCCGGAAGATTACCACCCCTATGAAATCAAGTTCTATGA
GGACCAATCACAGCCTCCAAAGGCCGAACTTTCGGCTTGCGTCATCACCCAGGGGGCAAT
TCTGGCCCAATTGCAAGCCATCCAAAAATCCCGCCAAGAATTTCTACTGAAAAAGGGTAA
GGGGGTCTACCTTGACCCCCAGACCGGCGAGGAACTCAACACAAGGTTCCCTCAGGATGT
CCCAACGACGAGAAAACAAGAAGTTGAAGGTGCAGCCGCCGCCCCCAGAAGATATGGAGG
AAGATTGGGACAGTCAGGCAGAGGAGGCGGAGGAGGACAGTCTGGAGGACAGTCTGGAGG
AAGACAGTTTGGAGGAGGAAAACGAGGAGGCAGAGGAGGTGGAAGAAGTAACCGCCGACA
AACAGTTATCCTCGGCTGCGGAGACAAGCAACAGCGCTACCATCTCCGCTCCGAGTCGAG
GAACCCGGCGGCGTCCCAGCAGTAGATGGGACGAGACCGGACGCTTCCCGAACCCAACCA
GCGCTTCCAAGACCGGTAAGAAGGATCGGCAGGGATACAAGTCCTGGCGGGGGCATAAGA
ATGCCATCATCTCCTGCTTGCATGAGTGCGGGGGCAACATATCCTTCACGCGGCGCTACT
TGCTATTCCACCATGGGGTGAACTTTCCGCGCAATGTTTTGCATTACTACCGTCACCTCC
ACAGCCCCTACTATAGCCAGCAAATCCCGACAGTCTCGACAGATAAAGACAGCGGCGGCG
ACCTCCAACAGAAAACCAGCAGCGGCAGTTAGAAAATACACAACAAGTGCAGCAACAGGA
GGATTAAAGATTACAGCCAACGAGCCAGCGCAAACCCGAGAGTTAAGAAATCGGATCTTT
CCAACCCTGTATGCCATCTTCCAGCAGAGTCGGGGTCAAGAGCAGGAACTGAAAATAAAA
AACCGATCTCTGCGTTCGCTCACCAGAAGTTGTTTGTATCACAAGAGCGAAGATCAACTT
CAGCGCACTCTCGAGGACGCCGAGGCTCTCTTCAACAAGTACTGCGCGCTGACTCTTAAA
GAGTAGGCAGCGACCGCGCTTATTCAAAAAAGGCGGGAATTACATCATCCTCGACATGAG
TAAAGAAATTCCCACGCCTTACATGTGGAGTTATCAACCCCAAATGGGATTGGCAGCAGG
CGCCTCCCAGGACTACTCCACCCGCATGAATTGGCTCAGCGCCGGGCCTTCTATGATTTC
TCGAGTTAATGATATACGCGCCTACCGAAACCAAATACTTTTGGAACAGTCAGCTCTTAC
CACCACGCCCCGCCAACACCTTAATCCCAGAAATTGGCCCGCCGCCCTAGTGTACCAGGA
AAGTCCCGCTCCCACCACTGTATTACTTCCTCGAGACGCCCAGGCCGAAGTCCAAATGAC
TAATGCAGGTGCGCAGTTAGCTGGCGGCTCCACCCTATGTCGTCACAGGCCTCGGCATAA
TATAAAACGCCTGATGATCAGAGGCCGAGGTATCCAGCTCAACGACGAGTCGGTGAGCTC
TCCGCTTGGTCTACGACCAGACGGAATCTTTCAGATTGCCGGCTGCGGGAGATCTTCCTT
CACCCCTCGTCAGGCTGTTCTGACTTTGGAAAGTTCGTCTTCGCAACCCCGCTCGGGCGG
AATCGGGACCGTTCAATTTGTAGAGGAGTTTACTCCCTCTGTCTACTTCAACCCCTTCTC
CGGATCTCCTGGGCACTACCCGGACGAGTTCATACCGAACTTCGACGCGATTAGCGAGTC
AGTGGACGGCTACGATTGATGTCTGGTGACGCGGCTGAGCTATCTCGGCTGCGACATCTA
GACCACTGCCGCCGCTTTCGCTGCTTTGCCCGGGAACTTATTGAGTTCATCTACTTCGAA
CTCCCCAAGGATCACCCTCAAGGTCCGGCCCACGGAGTGCGGATTACTATCGAAGGCAAA
ATAGACTCTCGCCTGCAACGAATTTTCTCCCAGCGGCCCGTGCTGATCGAGCGAGACCAG
GGAAACACCACGGTTTCCATCTACTGCATTTGTAATCACCCCGGATTGCATGAAAGCCTT
TGCTGTCTTATGTGTACTGAGTTTAATAAAAACTGAATTAAGACTCTCCTACGGACTGCC
GCTTCTTCAACCCGGATTTTACAACCAGAAGAACAAAACTTTTCCTGTCGTCCAGGACTC
TGTTAACTTCACCTTTCCTACTCACAAACTAGAAGCTCAACGACTACACCGCTTTTCCAG
AAGCATTTTCCCTACTAATACTACTTTCAAAACCGGAGGTGAGCTCCACGGTCTCCCTAC
AGAAAACCCTTGGGTGGAAGCGGGCCTTGTAGTACTAGGAATTCTTGCGGGTGGGCTTGT
GATTATTCTTTGCTACCTATACACACCTTGCTTCACTTTCCTAGTGGTGTTGTGGTATTG
GTTTAAAAAATGGGGCCCATACTAGTCTTGCTTGTTTTACTTTCGCTTTTGGAACCGGGT
TCTGCCAATTACGATCCATGTCTAGACTTTGACCCAGAAAACTGCACACTTACTTTTGCA
CCCGACACAAGCCGCATCTGTGGAGTTCTTATTAAGTGCGGATGGGAATGCAGGTCCGTT
GAAATTACACACAATAACAAAACCTGGAACAATACCTTATCCACCACATGGGAGCCAGGA
GTTCCCGAGTGGTACACTGTCTCTGTCCGAGGTCCTGACGGTTCCATCCGCATTAGTAAC
AACACTTTCATTTTTTCTGAAATGTGCGATCTGGCCATGTTCATGAGCAAACAGTATTCT

CTATGGCCTCCTAGCAAGGACAACATCGTAACGTTCTCCATTGCTTATTGCTTGTGCGCT
TGCCTTCTTACTGCTTTACTGTGCGTATGCATACACCTGCTTGTAACCACTCGCATCAAA
AACGCCAATAACAAAGAAAAAATGCCTTAACCTCTTTCTGTTTACAGACATGGCTTCTCT
TACATCTCTCATATTTGTCAGCATTGTCACTGCCGCTCACGGACAAACAGTCGTCTCTAT
CCCACTAGGACATAATTACACTCTCATAGGACCCCCAATCACTTCAGAGGTCATCTGGAC
CAAACTGGGAAGCGTTGATTACTTTGATATAATCTGTAACAAAACAAAACCAATAATAGT
AACTTGCAACATACAAAATCTTACATTGATTAATGTTAGCAAAGTTTACAGCGGTTACTA
TTATGGTTATGACAGATACAGTAGTCAATATAGAAATTACTTGGTTCGTGTTACCCAGTT
GAAAACCACGAAAATGCCAAATATGGCAAAGATTCGATCCGATGACAATTCTCTAGAAAC
TTTTACATCTCCCACCACACCCGACGAAAAAAACATCCCAGATTCAATGATTGCAATTGT
TGCAGCGGTGGCAGTGGTGATGGCACTAATAATAATATGCATGCTTTTATATGCTTGTCG
CTACAAAAAGTTTCATCCTAAAAAACAAGATCTCCTACTAAGGCTTAACATTTAATTTCT
TTTTATACAGCCATGGTTTCCACTACCACATTCCTTATGCTTACTAGTCTCGCAACTCTG
ACTTCTGCTCGCTCACACCTCACTGTAACTATAGGCTCAAACTGCACACTAAAAGGACCT
CAAGGTGGTCATGTCTTTTGGTGGAGAATATATGACAATGGATGGTTTACAAAACCATGT
GACCAACCTGGTAGATTTTTCTGCAACGGCAGAGACCTAACCATTATCAACGTGACAGCA
AATGACAAAGGCTTCTATTATGGAACCGACTATAAAAGTAGTTTAGATTATAACATTATT
GTACTGCCATCTACCACTCCAGCACCCCGCACAACTACTTTCTCTAGCAGCAGTGTCGCT
AACAATACAATTTCCAATCCAACCTTTGCCGCGCTTTTAAAACGCACTGTGAATAATTCT
ACAACTTCACATACAACAATTTCCACTTCAACAATCAGCATCATCGCTGCAGTGACAATT
GGAATATCTATTCTTGTTTTTACCATAACCTACTACGCCTGCTGCTATAGAAAAGACAAA
CATAAAGGTGATCCATTACTTAGATTTGATATTTAATTTGTTCTTTTTTTTTTATTTACAG
TATGGTGAACACCAATCATGGTACCTAGAAATTTCTTCTTCACCATACTCATCTGTGCTT
TTAATGTTTGCGCTACTTTCACAGCAGTAGCCACAGCAACCCCAGACTGTATAGGAGCAT
TTGCTTCCTATGCACTTTTTGCTTTTGTTACTTGCATCTGCGTATGTAGCATAGTCTGCC
TGGTTATTAATTTTTTCCAACTTCTAGACTGGATCCTTGTGCGAATTGCCTACCTGCGCC
ACCATCCCGAATACCGCAACCAAAATATCGCGGCACTTCTTAGACTCATCTAAAACCATG
CAGGCTATACTACCAATATTTTTGCTTCTATTGCTTCCCTACGCTGTCTCAACCCCAGCT
GCCTATAGTACTCCACCAGAACACCTTAGAAAATGCAAATTCCAACAACCGTGGTCATTT
CTTGCTTGCTATCGAGAAAAATCAGAAATCCCCCCAAATTTAATAATGATTGCTGGAATA
ATTAATATAATCTGTTGCACCATAATTTCATTTTTGATATACCCCCTATTTGATTTTGGC
TGGAATGCTCCCAATGCACATGATCATCCACAAGACCCAGAGGAACACATTCCCCCACAA
AACATGCAACATCCAATAGCGCTAATAGATTACGAAAGTGAACCACAACCCCCACTACTC
CCTGCTATTAGTTACTTCAACCTAACCGGCGGAGATGACTGAAACACTCACCACCTCCAA
TTCCGCCGAGGATCTGCTCGATATGGACGGCCGCGTCTCAGAACAACGACTTGCCCAACT
ACGCATCCGCCAGCAGCAGGAACGCGTGGCCAAAGAGCTCAGAGATGTCATCCAAATTCA
CCAATGCAAAAAAGGCATATTCTGTTTGGTAAAACAAGCCAAGATATCCTACGAGATCAC
CGCTACTGACCATCGCCTCTCTTACGAACTTGGCCCCCAACGACAAAAATTTACCTGCAT
GGTGGGAATCAACCCCATAGTTATCACCCAACAAAGTGGAGATACTAAGGGTTGCATTCA
CTGCTCCTGCGATTCCATCGAGTGCACCTACACCCTGCTGAAGACCCTATGCGGCCTAAG
AGACCTGCTACCAATGAATTAAAAAAAAAATGATTAATAAAAAATCACTTACTTGAAATCA
GCAATAAGGTCTCTGTTGAAATTTTCTCCCAGCAGCACCTCACTTCCCTCTTCCCAACTC
TGGTATTCTAAACCCCGTTCAGCGGCATACTTTCTCCATACTTTAAAGGGGATGTCAAAT
TTTAGCTCCTCTCCTGTACCCACAATCTTCATGTCTTTCTTCCCAGATGACCAAGAGAGT
CCGGCTCAGTGACTCCTTCAACCCTGTCTACCCCTATGAAGATGAAAGCACCTCCCAACA
CCCCTTTATAAACCCAGGGTTTATTTCCCCAAATGGCTTCACACAAAGCCCAGACGGAGT
TCTTACTTTAAAATGTTTAACCCCACTAACAACCACAGGCGGATCTCTACAGCTAAAAGT
GGGAGGGGGACTTACAGTGGATGACACTGATGGTACCTTACAAGAAAACATACGTGCTAC
AGCACCCATTACTAAAAATAATCACTCTGTAGAACTATCCATTGGAAATGGATTAGAAAC
TCAAAACAATAAACTATGTGCCAAATTGGGAAATGGGTTAAAATTTAACAACGGTGACAT
TTGTATAAAGGATAGTATTAACACCTTATGGACTGGAATAAACCCTCCACCTAACTGTCA
AATTGTGGAAAACACTAATACAAATGATGGCAAACTTACTTTAGTATTAGTAAAAAATGG
AGGGCTTGTTAATGGCTACGTGTCTCTAGTTGGTGTATCAGACACTGTGAACCAAATGTT
CACACAAAAGACAGCAAACATCCAATTAAGATTATATTTTGACTCTTCTGGAAATCTATT
AACTGAGGAATCAGACTTAAAAATTCCACTTAAAAATAAATCTTCTACAGCGACCAGTGA
AACTGTAGCCAGCAGCAAAGCCTTTATGCCAAGTACTACAGCTTATCCCTTCAACACCAC
TACTAGGGATAGTGAAAACTACATTCATGGAATATGTTACTACATGACTAGTTATGATAG
AAGTCTATTTCCCTTGAACATTTCTATAATGCTAAACAGCCGTATGATTTCTTCCAATGT
TGCCTATGCCATACAATTTGAATGGAATCTAAATGCAAGTGAATCTCCAGAAAGCAACAT
AGCTACGCTGACCACATCCCCCTTTTTCTTTTCTTACATTACAGAAGACGACAACTAAAA

TAAAGTTTAAGTGTTTTTATTTAAAATCACAAAATTCGAGTAGTTATTTTGCCTCCACCT
TCCCATTTGACAGAATACACCAATCTCTCCCCACGCACAGCTTTAAACATTTGGATACCA
TTAGAGATAGACATTGTTTTAGATTCCACATTCCAAACAGTTTCAGAGCGAGCCAATCTG
GGGTCAGTGATAGATAAAAATCCATCGCGATAGTCTTTTAAAGCGCTTTCACAGTCCAAC
TGCTGCGGATGCGACTCCGGAGTTTGGATCACGGTCATCTGGAAGAAGAACGATGGGAAT
CATAATCCGAAAACGGTATCGGACGATTGTGTCTCATCAAACCCACAAGCAGCCGCTGTC
TGCGTCGCTCCGTGCGACTGCTGTTTATGGGATCAGGGTCCACAGTTTCCTGAAGCATGA
TTTTAATAGCCCTTAACATCAACTTTCTGGTGCGATGCGCGCAGCAACGCATTCTGATTT
CACTCAAATCTTTGCAGTAGGTACAACACATTATTACAATATTGTTTAATAAACCATAAT
TAAAAGCGCTCCAGCCAAAACTCATATCTGATATAATCGCCCCTGCATGACCATCATACC
AAAGTTTAATATAAATTAAATGACGTTCCCTCAAAAACACACTACCCACATACATGATCT
CTTTTGGCATGTGCATATTAACAATCTGTCTGTACCATGGACAACGTTGGTTAATCATGC
AACCCAATATAACCTTCCGGAACCACACTGCCAACACCGCTCCCCCAGCCATGCATTGAA
GTGAACCCTGCTGATTACAATGACAATGAAGAACCCAATTCTCTCGACCGTGAATCACTT
GAGAATGAAAAATATCTATAGTGGCACAACATAGACATAAATGCATGCATCTTCTCATAA
TTTTTAACTCCTCAGGATTTAGAAACATATCCCAGGGAATAGGAAGCTCTTGCAGAACAG
TAAAGCTGGCAGAACAAGGAAGACCACGAACACAACTTACACTATGCATAGTCATAGTAT
CACAATCTGGCAACAGCGGGTGGTCTTCAGTCATAGAAGCTCGGGTTTCATTTTCCTCAC
AACGTGGTAACTGGGCTCTGGTGTAAGGGTGATGTCTGGCGCATGATGTCGAGCGTGCGC
GCAACCTTGTCATAATGGAGTTGCTTCCTGACATTCTCGTATTTTGTATAGCAAAACGCG
GCCCTGGCAGAACACACTCTTCTTCGCCTTCTATCCTGCCGCTTAGCGTGTTCCGTGTGA
TAGTTCAAGTACAGCCACACTCTTAAGTTGGTCAAAAGAATGCTGGCTTCAGTTGTAATC
AAAACTCCATCGCATCTAATTGTTCTGAGGAAATCATCCACGGTAGCATATGCAAATCCC
AACCAAGCAATGCAACTGGATTGCGTTTCAAGCAGGAGAGGAGAGGGAAGAGACGGAAGA
ACCATGTTAATTTTTATTCCAAACGATCTCGCAGTACTTCAAATTGTAGATCGCGCAGAT
GGCATCTCTCGCCCCCACTGTGTTGGTGAAAAAGCACAGCTAAATCAAAAGAAATGCGAT
TTTCAAGGTGCTCAACGGTGGCTTCCAACAAAGCCTCCACGCGCACATCCAAGAACAAAA
GAATACCAAAAGAAGGAGCATTTTCTAACTCCTCAATCATCATATTACATTCCTGCACCA
TTCCCAGATAATTTTCAGCTTTCCAGCCTTGAATTATTCGTGTCAGTTCTTGTGGTAAAT
CCAATCCACACATTACAAACAGGTCCCGGAGGGCGCCCTCCACCACCATTCTTAAACACA
CCCTCATAATGACAAAATATCTTGCTCCTGTGTCACCTGTAGCGAATTGAGAATGGCAAC
ATCAATTGACATGCCCTTGGCTCTAAGTTCTTCTTTAAGTTCTAGTTGTAAAAACTCTCT
CATATTATCACCAAACTGCTTAGCCAGAAGCCCCCCGGGAACAAGAGCAGGGGACGCTAC
AGTGCAGTACAAGCGCAGACCTCCCCAATTGGCTCCAGCAAAAACAAGATTGGAATAAGC
ATATTGGGAACCACCAGTAATATCATCGAAGTTGCTGGAAATATAATCAGGCAGAGTTTC
TTGTAGAAATTGAATAAAAGAAAAATTTGCCAAAAAAACATTCAAAACCTCTGGGATGCA
AATGCAATAGGTTACCGCGCTGCGCTCCAACATTGTTAGTTTTGAATTAGTCTGCAAAAA
TAAAAAAAAAACAAGCGTCATATCATAGTAGCCTGACGAACAGGTGGATAAATCAGTCTT
TCCATCACAAGACAAGCCACAGGGTCTCCAGCTCGACCCTCGTAAAACCTGTCATCGTGA
TTAAACAACAGCACCGAAAGTTCCTCGCGGTGACCAGCATGAATAAGTCTTGATGAAGCA
TACAATCCAGACATGTTAGCATCAGTTAAGGAGAAAAAACAGCCAACATAGCCTTTGGGT
ATAATTATGCTTAATCGTAAGTATAGCAAAGCCACCCCTCGCGGATACAAAGTAAAAGGC
ACAGGAGAATAAAAAATATAATTATTTCTCTGCTGCTGTTTAGGCAACGTCGCCCCCGGT
CCCTCTAAATACACATACAAAGCCTCATCAGCCATGGCTTACCAGAGAAAGTACAGCGGG
CACACAAACCACAAGCTCTAAAGTCACTCTCCAACCTCTCCACAATATATATACACAAGC
CCTAAACTGACGTAATGGGACTAAAGTGTAAAAAATCCCGCCAAACCCAACACACACCCC
GAAACTGCGTCACCAGGGAAAAGTACAGTTTCACTTCCGCAATCCCAACAAGCGTCACTT
CCTCTTTCTCACGGTACGTCACATCCCATTAACTTACAACGTCATTTTCCCACGGCCGCG
CCGCCCCTTTTAACCGTTAACCCCACAGCCAATCACCACACGGCCCACACTTTTTAAAAT
CACCTCATTTACATATTGGCACCATTCCATCTATAAGGTATATTATTGATGATG

INCORPORATION BY REFERENCE

[00268] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[00269] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and the range of equivalency of the claims are intended to be embraced therein.

CLAIMS:

1. A recombinant adenovirus comprising a nucleotide sequence inserted in an insertion site, wherein the insertion site is located between the stop codon of a first viral transcription unit and the stop codon of a second viral transcription unit,
wherein the stop codon of the first viral transcription unit is nearer to the stop codon of the second viral transcription unit than the start site of the first viral transcription unit is to the stop codon of the second viral transcription unit,
wherein the stop codon of the second viral transcription unit is nearer to the stop codon of the first viral transcription unit than the start site of the second viral transcription unit is to the stop codon of the first viral transcription unit, and
wherein there is no viral transcription unit between the first viral transcription unit and the second viral transcription unit before the nucleotide sequence is inserted.

2. The recombinant adenovirus of claim 1, wherein the first viral transcription unit is adenovirus IX gene and the second viral transcription unit is adenovirus IVa2 gene.

3. The recombinant adenovirus of claim 1, wherein the first viral transcription unit is adenovirus fiber gene and the second viral transcription unit is ORF6 or ORF6/7 of adenovirus E4 gene.

4. A recombinant adenovirus comprising a nucleotide sequence inserted in an IX-E2 insertion site, wherein the IX-E2 insertion site is located between the stop codon of adenovirus IX gene and the stop codon of adenovirus IVa2 gene.

5. The recombinant adenovirus of any one of claims 1, 2, and 4, wherein the nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4093 of the Ad5 genome (SEQ ID NO: 1).

6. The recombinant adenovirus of any one of claims 1, 2, and 4, wherein the nucleotide sequence is inserted between nucleotides corresponding to 3899 and 3970 of the Ad35 genome (SEQ ID NO: 41).

7. The recombinant adenovirus of any one of claims 1, 2, 4, and 5, wherein the nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4050, nucleotides corresponding to 4050 and 4070, or nucleotides corresponding to 4070 and 4093 of the Ad5 genome (SEQ ID NO: 1).

8. The recombinant adenovirus of any one of claims 1, 2, 4, and 6, wherein the nucleotide sequence is inserted between nucleotides corresponding to 3899 and 3920, nucleotides corresponding to 3920 and 3940, or nucleotides corresponding to 3940 and 3970 of the Ad35 genome (SEQ ID NO: 41).

9. A recombinant adenovirus comprising a nucleotide sequence inserted in an L5-E4 insertion site, wherein the L5-E4 insertion site is located between the stop codon of adenovirus fiber gene and the stop codon of ORF6 or ORF6/7 of adenovirus E4 gene.

10. The recombinant adenovirus of any one of claims 1, 3, and 9, wherein the nucleotide sequence is inserted between nucleotides corresponding to 32785 to 32916 of the Ad5 genome (SEQ ID NO: 1).

11. The recombinant adenovirus of any one of claims 1, 3, 9, and 10, wherein the nucleotide sequence is inserted between nucleotides corresponding to 32785 and 32800, nucleotides corresponding to 32800 and 32820, nucleotides corresponding to 32820 and 32840, nucleotides corresponding to 32840 and 32860, nucleotides corresponding to 32860 and 32880, nucleotides corresponding to 32880 and 32900, or nucleotides corresponding to 32900 and 32916 of the Ad5 genome (SEQ ID NO: 1).

12. The recombinant adenovirus of any one of claims 1, 3, and 9, wherein the nucleotide sequence is inserted between nucleotides corresponding to 31799 and 31821 of the Ad35 genome (SEQ ID NO: 41).

13. The recombinant adenovirus of any one of claims 1, 3, 9, and 12, wherein the nucleotide sequence is inserted between nucleotides corresponding to 31799 and

32810, or nucleotides corresponding to 32810 and 31821 of the Ad35 genome (SEQ ID NO: 41).

14. A recombinant adenovirus comprising a first nucleotide sequence inserted in an IX-E2 insertion site and a second nucleotide sequence inserted in an L5-E4 insertion site, wherein the IX-E2 insertion site is located between the stop codon of adenovirus IX gene and the stop codon of adenovirus IVa2 gene, and wherein the L5-E4 insertion site is located between the stop codon of adenovirus fiber gene and the stop codon of ORF6 or ORF6/7 of adenovirus E4 gene.

15. The recombinant adenovirus of claim 14, wherein the first nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4093 of the Ad5 genome (SEQ ID NO: 1).

16. The recombinant adenovirus of claim 14 or claim 15, wherein the first nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4050, nucleotides corresponding to 4050 and 4070, or nucleotides corresponding to 4070 and 4093 of the Ad5 genome (SEQ ID NO: 1).

17. The recombinant adenovirus of claim 14, wherein the first nucleotide sequence is inserted between nucleotides corresponding to 3899 and 3970 of the Ad35 genome (SEQ ID NO: 41).

18. The recombinant adenovirus of claim 14 or claim 15, wherein the first nucleotide sequence is inserted between nucleotides corresponding to 3899 and 3920, nucleotides corresponding to 3920 and 3940, or nucleotides corresponding to 3940 and 3970 of the Ad35 genome (SEQ ID NO: 41).

19. The recombinant adenovirus of any one of claims 14-18, wherein the second nucleotide sequence is inserted between nucleotides corresponding to 32785 to 32916 of the Ad5 genome (SEQ ID NO: 1).

20. The recombinant adenovirus of any one of claims 14-19, wherein the second nucleotide sequence is inserted between nucleotides corresponding to 32785 and 32800, nucleotides corresponding to 32801 and 32820, nucleotides corresponding to 32821 and 32840, nucleotides corresponding to 32841 and 32860, nucleotides corresponding to 32861 and 32880, nucleotides corresponding to 32881 and 32900, or nucleotides corresponding to 32901 and 32916 of the Ad5 genome (SEQ ID NO: 1).

21. The recombinant adenovirus of any one of claims 14-18, wherein the second nucleotide sequence is inserted between nucleotides corresponding to 31799 and 31821 of the Ad5 genome (SEQ ID NO: 41).

22. The recombinant adenovirus of any one of claims 14-18 and 21, wherein the second nucleotide sequence is inserted between nucleotides corresponding to 31799 and 32810, or nucleotides corresponding to 32810 and 31821 of the Ad35 genome (SEQ ID NO: 41).

23. The recombinant adenovirus of any one of claims 4-8 and 14-22, wherein the IX-E2 insertion site comprises a deletion of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, or 60 nucleotides.

24. The recombinant adenovirus of any one of claims 9-23, wherein the L5-E4 insertion site comprises a deletion of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, or 130 nucleotides.

25. The recombinant adenovirus of any one of claims 1-24, wherein the nucleotide sequence comprises at least one transgene.

26. The recombinant adenovirus of claim 25, wherein the nucleotide sequence further comprises a promoter, wherein the transgene is operably linked to the promoter.

27. The recombinant adenovirus of any one of claims 1-26 comprises, in a 5' to 3' orientation:

(i) a first polyadenylation signal;

(ii) a promoter;

(iii) a transgene;

(iv) a second polyadenylation signal; and

(v) a third polyadenylation signal;

wherein the transgene is operably linked to the promoter, wherein the transgene is inserted between the first polyadenylation signal and the third polyadenylation signal.

28. The recombinant adenovirus of claim 27, wherein the first polyadenylation signal is the polyadenylation signal of the fiber (L5) gene, the second polyadenylation signal is the polyadenylation signal of the transgene, and the third polyadenylation signal is the polyadenylation signal of the ORF6 or ORF6/7 of the adenovirus E4 gene, or the first polyadenylation signal is the polyadenylation signal of the IX gene, the second polyadenylation signal is the polyadenylation signal of the transgene, and the third polyadenylation signal is the polyadenylation signal of the adenovirus IVa2 gene.

29. The recombinant adenovirus of any one of claims 1-26 comprises, in a 5' to 3' orientation:

(i) a first polyadenylation signal;

(ii) a second polyadenylation signal;

(iii) a promoter;

(iv) a transgene;

(v) a third polyadenylation signal; and

(vi) a fourth polyadenylation signal;

wherein the transgene is operably linked to the promoter, wherein the transgene is inserted between the first polyadenylation signal and the fourth polyadenylation signal, and wherein the second polyadenylation signal is in the opposite transcriptional direction of the first polyadenylation signal.

30. The recombinant adenovirus of claim 29, wherein the first polyadenylation signal is the polyadenylation signal of the fiber (L5) gene, the third polyadenylation signal is the polyadenylation signal of the transgene, and the fourth polyadenylation signal is the polyadenylation signal of the ORF6 or ORF6/7 of the adenovirus E4 gene, or the first polyadenylation signal is the polyadenylation signal of the IX gene, the third

polyadenylation signal is the polyadenylation signal of the transgene, and the fourth polyadenylation signal is the polyadenylation signal of the adenovirus IVa2 gene.

31. The recombinant adenovirus of any one of claims 26-30, wherein the promoter is a ubiquitous promoter, a tissue-specific promoter, or tumor-specific promoter.

32. The recombinant adenovirus of any one of claims 1-31, wherein the recombinant adenovirus further comprises a nucleotide sequence inserted in an E1b-19K insertion site, an E3 insertion site, or an E4 insertion site.

33. The recombinant adenovirus of any one of claims 1-32, wherein the nucleotide sequence comprises a first nucleotide sequence comprising a first transgene and a second nucleotide sequence comprising a second transgene, wherein the first nucleotide sequence and the second nucleotide sequence are separated by a linker.

34. The recombinant adenovirus of claim 33, wherein the linker encodes a peptide cleavable by a protease or proteases.

35. The recombinant adenovirus of claim 33 or claim 34, wherein the linker encodes an internal ribosome entry site (IRES) or a self-cleaving 2A peptide.

36. The recombinant adenovirus of claim 32, wherein the E1b-19K insertion site is located between the start site of Elb-19K and the stop codon of Elb-19K.

37. The recombinant adenovirus of claim 32 or claim 36, wherein the E1b-19K insertion site comprises a deletion of from about 100 to about 305, about 100 to about 300, about 100 to about 250, about 100 to about 200, about 100 to about 150, about 150 to about 305, about 150 to about 300, about 150 to about 250, or about 150 to about 200 nucleotides adjacent the start site of E1b-19K.

38. The recombinant adenovirus of any one of claims 32, 36, and 37, wherein the E1b-19K insertion site comprises a deletion of about 200 nucleotides adjacent the start site of E1b-19K.

39. The recombinant adenovirus of any one of claims 32, 36, and 37, wherein the E1b-19K insertion site comprises a deletion corresponding to nucleotides 1714-1917 of the Ad5 genome (SEQ ID NO: 1).

40. The recombinant adenovirus of any one of claims 32, 36, and 37, wherein the E1b-19K insertion site comprises a deletion corresponding to nucleotides 1611-2153 of the Ad35 genome (SEQ ID NO: 41).

41. The recombinant adenovirus of claim 32, wherein the E3 insertion site is located between the stop codon of adenovirus pVIII gene and the start site of adenovirus fiber gene.

42. The recombinant adenovirus of claim 32 or claim 41, wherein the E3 insertion site wherein the E3 insertion site is located between the stop codon of E3-10.5K and the stop codon of E3-14.7K.

43. The recombinant adenovirus any one of claims 32, 41, and 42, wherein the E3 insertion site comprises a deletion of from about 500 to about 3185, from about 500 to about 3000, from about 500 to about 2500, from about 500 to about 2000, from about 500 to about 1500, from about 500 to about 1000, from about 1000 to about 3185, from about 1000 to about 3000, from about 1000 to about 2500, from about 1000 to about 2000, from about 1000 to about 1500, from about 1500 to about 3185, from about 1500 to about 3000, from about 1500 to about 2000, from about 2000 to about 3185, from about 2000 to about 3000, from about 2000 to about 2500, from about 2500 to about 3185, from about 2500 to about 3000, or about 3000 to about 3185 nucleotides.

44. The recombinant adenovirus of any one of claims 32 and 41-43, wherein the E3 insertion site comprises a deletion of from about 500 to about 1551, from about 500 to about 1500, from about 500 to about 1000, from about 1000 to about 1551, from about 1000 to about 1500, or from about 1500 to about 1551 nucleotides adjacent the stop codon of E3-10.5K.

45. The recombinant adenovirus of any one of claims 32 and 41-44, wherein the E3 insertion site comprises a deletion of about 1,050 nucleotides adjacent the stop codon of E3-10.5K.

46. The recombinant adenovirus any one of claims 32 and 41-44, wherein the E3 insertion site comprises a deletion of 1,063 nucleotides following the stop codon of E3-10.5K.

47. The recombinant adenovirus any one of claims 32 and 41-43, wherein the E3 insertion site comprises a deletion corresponding to the Ad5 dl309 E3 deletion.

48. The recombinant adenovirus any one of claims 32 and 41-47, wherein the E3 insertion site comprises a deletion corresponding to nucleotides 29773-30836 of the Ad5 genome (SEQ ID NO: 1).

49. The recombinant adenovirus any one of claims 32 and 41-46, wherein the E3 insertion site comprises a deletion corresponding to nucleotides 29119-30622 of the Ad35 genome (SEQ ID NO: 41).

50. The recombinant adenovirus of any one of claims 1-49, wherein the recombinant adenovirus comprises an E1a promoter having one or more deletions of a functional Pea3 binding site.

51. The recombinant adenovirus of claim 50, wherein the deletion comprises a deletion of nucleotides corresponding to about -300 to about -250 upstream of the initiation site of Ela.

52. The recombinant adenovirus of claim 50, wherein the deletion comprises a deletion of nucleotides corresponding to -305 to -255 upstream of the initiation site of Ela.

53. The recombinant adenovirus of claim 50, wherein the deletion comprises a deletion of nucleotides corresponding to 195-244 of the Ad5 genome (SEQ ID NO: 1).

54. The recombinant adenovirus of claim 50, wherein the E1a promoter comprises the sequence GGTGTTTTGG (SEQ ID NO: 4).

55. The recombinant adenovirus of any one of claims 1-54, wherein the recombinant adenovirus comprises one or more Pea3 transcription binding site deletions without one or more E2F transcription binding site deletions in the E1A region.

56. The recombinant adenovirus of any one of claims 1-54, wherein the recombinant adenovirus comprises one or more E2F transcription binding site deletions without one or more Pea3 transcription binding site deletions in the E1A region.

57. The recombinant adenovirus of any one of claims 1-48, wherein the recombinant adenovirus comprises a modified TATA box-based promoter operably linked to a gene, wherein the modified TATA box-based promoter lacks a functional TATA box and permits selective expression of the gene in a hyperproliferative cell and/or a modified CAAT box-based promoter operably linked to a gene, wherein the modified CAAT box-based promoter lacks a functional CAAT box and permits selective expression of the gene in a hyperproliferative cell.

58. The recombinant adenovirus of claim 57, wherein the modified TATA box-based promoter is an early gene promoter.

59. The recombinant adenovirus of claim 58, wherein the modified TATA box-based promoter is an E1a promoter, E1b promoter, or E4 promoter.

60. The recombinant adenovirus of claim 59, wherein the modified TATA box-based promoter is an E1a promoter.

61. The recombinant adenovirus of any one of claims 57-60, wherein the modification included in the modified TATA box-based promoter comprises a deletion of the entire TATA box.

62. The recombinant adenovirus of any one of claims 57-60, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to -27 to -24 of the E1a promoter.

63. The recombinant adenovirus of any one of claims 57-62, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to -31 to -24 of the E1a promoter.

64. The recombinant adenovirus of any one of claims 57-63, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to -44 to +54 of the E1a promoter.

65. The recombinant adenovirus of any one of claims 57-64, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to -146 to +54 of the E1a promoter.

66. The recombinant adenovirus of any one of claims 57-60, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to 472 to 475 of the Ad5 genome (SEQ ID NO: 1).

67. The recombinant adenovirus of claim 66, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to 468 to 475 of the Ad5 genome (SEQ ID NO: 1).

68. The recombinant adenovirus of claim 67, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to 455 to 552 of the Ad5 genome (SEQ ID NO: 1).

69. The recombinant adenovirus of claim 68, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to 353 to 552 of the Ad5 genome (SEQ ID NO: 1).

70. The recombinant adenovirus of any one of claims 57-69, wherein the recombinant adenovirus comprises a polynucleotide deletion that results in a virus comprising the sequence CTAGGACTG (SEQ ID NO: 5), AGTGCCCG (SEQ ID NO: 44) and/or TATTCCCG (SEQ ID NO: 45).

71. The recombinant adenovirus of claim 57, wherein the modified CAAT box-based promoter is an early gene promoter.

72. The recombinant adenovirus of claim 71, wherein the modified CAAT box-based promoter is an E1a promoter, E1b promoter, or E4 promoter.

73. The recombinant adenovirus of claim 72, wherein the modified CAAT box-based promoter is an E1a promoter.

74. The recombinant adenovirus of any one of claims 71-73, wherein the modification included in the modified CAAT box-based promoter comprises a deletion of the entire CAAT box.

75. The recombinant adenovirus of any one of claims 71-74, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to -76 to -68 of the E1a promoter.

76. The recombinant adenovirus of any one of claims 71-74, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to 423 to 431 of the Ad5 genome (SEQ ID NO: 1).

77. The recombinant adenovirus of any one of claims 71-76, wherein the recombinant adenovirus comprises a polynucleotide deletion that results in a virus comprising the sequence TTCCGTGGCG (SEQ ID NO: 46).

78. The recombinant adenovirus of any one of claims 57-80, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to 477 to 484 of the Ad35 genome (SEQ ID NO: 41).

79. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes a monomeric, dimeric, trimeric, tetrameric, or multimeric protein.

80. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes a RNA that has a therapeutic activity.

81. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes a fusion protein comprising at least one binding domain.

82. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes an immunomodulatory molecule.

83. The recombinant adenovirus of claim 82, wherein the immunomodulatory molecule is a costimulatory ligand, a cytokine, or a cytokine receptor.

84. The recombinant adenovirus of claim 82 or claim 83, wherein the immunomodulatory molecule is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-7, IL-10, IL-10 trap, IL-10R, IL-12A/p35, IL-12B/p40, IL-15, IL-23A/p19, IL24, IL-27, IL-33, IL-35, IL-15, an IL-15 receptor fusion protein, TGF-β, a TGF-β trap, an IL-10 trap, VEGF, indoleamin-2,3-dioxygenase (IDO), inducible T-cell co-stimulator ligand (ICOS-L), CD80, CD137L, TNF-α, IFN-α, IFN- β, IFN-γ, or GM-CSF, GITR ligand (GITRL), OX40 ligand (OX40L), CD40 ligand (CD40L), drug-inducible CD40 (iCD40), CD154, CD70, CD86, CD137, CD137L, BORIS/CTCFL, bone morphogenetic protein (BMP), TNFSF9, FGF, ICAM, Podocalyxin, functional fragments thereof, and derivatives thereof.

85. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes an antigen-binding molecule.

86. The recombinant adenovirus of claim 85, wherein the antigen-binding molecule is an anti-PD-1 antibody, an anti-TGF-β antibody, an anti-PD-L1 antibody, and an anti-CTLA-4 antibody, or functional fragments thereof.

87. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes an antigen or a ligand to the antigen.

88. The recombinant adenovirus of claim 87, wherein the antigen is selected from the group consisting of CAIX, CEA, CD5, CD7, CD10, CD19, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD80, CD133, CD135 (Flt3), Flt3I, CD138, a cytomegalovirus (CMV) infected cell antigen, 4-1BB, EGP-2, EGP-40, EpCAM, erbB2, erbB3, erbB4, FBP, Fetal acetylcholine receptor, KRAS, HPV E6, E7, BING-4, EphA3, calcium activated chloride channel-2, cyclin B1, 9D7, Ep-CAM, SAP-1, PRAME, SSX-2, immature laminin receptor, folate receptor-a, telomerase, tyrosinase, melan-A, NY-ESO-1, GD2, GD3, hTERT, IL13R-a2, x-light chain, KDR, LeY, LI cell adhesion molecule, MAGE-A1, MAGE-A3, MART1, MART2, MUC1, Mesothelin, HER-2/neu, EGFRvIII, NKG2D ligands, NY-ES0-1, gp100, TRP-1/-2, TRP-1/-2, P polypeptide, MC1R, prostate specific antigen, BRAF, androgen-receptor, β-catenin, BRCA1/2, CDK4, CML66, fibronectin, p53, TGF-βRII, T cell receptor, oncofetal antigen, 5T4, PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, functional fragments thereof, and derivatives thereof.

89. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes a toxin.

90. The recombinant adenovirus of claim 89, wherein the toxin is pseudomonas exotoxin, ricin toxin, or diphtheria toxin.

91. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes an enzyme.

92. The recombinant adenovirus of claim 91, wherein the enzyme is selected from the group consisting of beta-glucuronidase, beta-galactosidase, beta-glucosidase, carboxypeptidase, beta-lactamase, esterase, metalloproteinase, relaxin, collagenase, streptokinase, arginase, NOS-2, fragments thereof, and derivatives thereof.

93. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes a cell cycle control agent, a growth factor, an anticoagulant, a pro-drug activating gene, a tumor suppressor gene, an apoptotic gene, an anti-platelet agent, a clotting factor, a cystic fibrosis transmembrane conductance regulator (CFTR) protein, fragments thereof, or derivatives thereof.

94. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes angiostatin, endostatin, acetylcholine, DKK1/Wnt, Ox40L, GITRL, secreted flagellin, thymidine kinase, functional fragments thereof, or derivatives thereof.

95. The recombinant adenovirus of any one of claims 1-94, wherein the recombinant adenovirus is a type 5 adenovirus (Ad5) or type 35 adenovirus (Ad35).

96. The recombinant adenovirus of any one of claims 1-95, wherein the recombinant adenovirus is oncolytic.

97. The recombinant adenovirus of any one of claims 1-96, wherein the recombinant adenovirus selectively replicates in a hyperproliferative cell.

98. The recombinant adenovirus of any one of claims 1-97, wherein the recombinant adenovirus selectively expresses a transgene in a hyperproliferative cell.

99. The recombinant adenovirus of claim 97 or claim 98, wherein the hyperproliferative cell is a tumor cell.

100. An isolated nucleotide sequence comprising the recombinant adenovirus of any one of claims 1-99, optionally wherein the nucleotide sequence is cDNA.

101. An isolated vector comprising the nucleotide sequence of claim 100.

102. An isolated cell comprising the nucleotide sequence of claim 100 or the vector of claim 101.

103. A method of inhibiting proliferation of a tumor cell comprising exposing the tumor cell to an effective amount of the recombinant adenovirus of any one of claims 1-99 to inhibit proliferation of the tumor cell.

104. A method of inhibiting tumor growth in a subject in need thereof, wherein the method comprises administering to the subject to an effective amount of the recombinant adenovirus of any one of claims 1-99 to inhibit growth of the tumor.

105. The method of claim 104, wherein the tumor is a HER2/neu positive tumor, and wherein the recombinant adenovirus comprises an E1a promoter having no more than one deletion of a functional Pea3 binding site.

106. The method of claim 105, wherein the HER2/neu positive tumor is from breast cancer, gastric cancer, ovarian cancer, bladder cancer, salivary gland cancer, endometrial cancer, pancreatic cancer, or non-small-cell lung cancer (NSCLC).

107. The method of claim 103 or claim 104, wherein the tumor is selected from the group consisting of melanoma, squamous cell carcinoma of the skin, basal cell carcinoma, head and neck tumor, breast tumor, anal cancer, cervical cancer, non-small cell lung cancer, mesothelioma, small cell lung tumor, renal cell carcinoma, prostate tumor, gastroesophageal tumor, colorectal tumor, testicular tumor, bladder tumor, ovarian tumor, hepatocellular carcinoma, cholangiocarcinoma, brain tumor, endometrial

tumor, neuroendocrine tumor, merkel cell carcinoma, gastrointestinal stromal tumor, a sarcoma, and pancreatic tumor.

108. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of the recombinant adenovirus of any one of claims 1-99 to treat the cancer in the subject.

109. The method of claim 108, wherein the cancer is selected from melanoma, squamous cell carcinoma of the skin, basal cell carcinoma, head and neck cancer, breast cancer, anal cancer, cervical cancer, non-small cell lung cancer, mesothelioma, small cell lung cancer, renal cell carcinoma, prostate cancer, gastroesophageal cancer, colorectal cancer, testicular cancer, bladder cancer, ovarian cancer, hepatocellular carcinoma, cholangiocarcinoma, brain cancer, endometrial cancer, neuroendocrine cancer, merkel cell carcinoma, gastrointestinal stromal tumors, a sarcoma, prostate cancer, leukemia, lymphoma and pancreatic cancer.

110. A method of treating a disease or condition in a subject in need thereof, wherein the method comprising administering to the subject to an effective amount of the recombinant adenovirus of any one of claims 1-99.

111. The method of claim 110, wherein the disease or condition is selected from the group consisting of an infection, diabetic retinopathy, psoriasis, rheumatoid arthritis, endometriosis, macular degenerative disorders and benign growth disorders such as prostate enlargement and lipomas, a vascular disorder, a cardiovascular disease, an infection, cirrhosis of the liver, a connective tissue disorder, a tumor, a vascular lesion, an ulcerative lesion, an inflammation, thrombosis, and neointima formation.

112. The method of any one of claims 104-111, wherein the subject is a mammal.

113. The method of any one of claims 104-112, wherein the subject is a human.

114. The method of claim 113, wherein the subject is a pediatric human.

115. The method of claim 113, wherein the subject is an adult human.

116. The method of any one of claims 104-115, wherein the recombinant adenovirus is administered by intramuscular, intravenous, intraarterial, intratumoral, intradermal, inhalation, transdermal, topical, eye drops, intranasal, transmucosal, or rectal administration.

117. The method of any one of claims 104-116, further comprising administering to the subject one or more therapies selected from the group consisting of surgery, radiation, chemotherapy, immunotherapy, hormone therapy, and virotherapy.

118. The method of any one of claims 104-117, further comprising administering to the subject one or more immune checkpoint modulators.

119. The method of claim 117, wherein the immune checkpoint modulator is an inhibitor, an antagonist, or an agonist of one or more molecules selected from the group consisting of PD-1, PD-L1, PD-L2, 2B4, TIGIT, LAG3, Tim3, BTLA, CD160, GITR, KIR, 4-1BB, and CTLA4.

120. A pharmaceutical composition comprising the recombinant adenovirus of any one of claims 1-99 and at least one pharmaceutically acceptable carrier or diluent.

121. A formulation for adenoviruses comprising:

(a) the recombinant adenovirus any one of claims 1-99;

(b) at least one buffer;

(c) at least one tonicity modifier;

(d) at least one sugar or at least one stabilizing agent, or both; and

wherein the formulation has a pH ranging between about 7.0 and about 9.0.

122. The formulation of claim 121, wherein the stabilizing agent is glycerol.

123. The formulation of claim 121 or claim 122, wherein the stabilizing agent is at about 2% to about 5% (v/v).

124. The formulation of any one of claims 121-123, wherein the buffer is Tris.

125. The formulation of any one of claims 121-123, wherein the buffer is at concentration of about 1mM to about 30 mM.

126. The formulation of any one of claims 119-123, wherein the tonicity modifier is NaCl.

127. The formulation of any one of claims 119-124, wherein the tonicity modifier is at concentration of about 10 mM to about 250 mM.

128. The formulation of any one of claims 119-125, wherein the formulation comprises a first tonicity modifier and a second tonicity modifier, wherein the first tonicity modifier is a monovalent cation, and wherein the second tonicity modifier is a divalent cation.

129. The formulation of any one of claims 119-128, wherein the tonicity modifier or the divalent cation is $MgCl_2$.

130. The formulation of any one of claims 119-129, wherein the tonicity modifier or the divalent cation is at a concentration of about 0.1 mM to about 5 mM.

131. The formulation of any one of claims 119-130, wherein the sugar is sucrose.

132. The formulation of any one of claims 119-131, wherein the sugar is at weight to volume percentage from about 2% to about 8%.

133. The formulation of any one of claims 119-132, further comprising at least one non-ionic surfactant.

134. The formulation of claim 133, wherein the non-ionic surfactant is polysorbate-80 or polysorbate-40.

135. The formulation of claim 133 or claim 134, wherein the non-ionic surfactant is at a concentration of about 0.001% to about 1%.

136. The formulation of any one of claims 119-135, further comprising at least one inhibitor of free radical oxidation.

137. The formulation of claim 136, wherein the inhibitor of free radical oxidation is EDTA.

138. The formulation of claim 136 or claim 137, wherein the inhibitor of free radical oxidation is at a concentration of about 0.01 mM to about 5 mM.

139. The formulation of any one of claims 119-138, further comprising at least one cryoprotectant.

140. The formulation of claim 139, wherein the cryoprotectant is EtOH.

141. The formulation of claim 139 or claim 140, wherein the cryoprotectant is at a concentration of about 0.5%.

142. The formulation of any one of claims 119-141, wherein the formulation has an osmolarity of about 200 mOs/L to about 800 mOs/L.

143. The formulation of any one of claims 119-142, wherein the recombinant adenovirus is at concentration from about 1 x 10^7 vp/mL to 1 x 10^{13} vp/mL.

144. The formulation of any one of claims 119-143, wherein the formulation comprises about 20 mM Tris, about 25 mM NaCl, about 2.5% glycerol, and wherein the formulation has a pH of about 8.0.

145. The formulation of any one of claims 119-144, wherein the formulation comprises about 20 mM Tris, about 25 mM NaCl, about 3-5% sucrose, and wherein the formulation has a pH of about 8.0.

146. The formulation of any one of claims 119-145, wherein the formulation comprises about 10 mM Tris, about 75 mM NaCl, about 5% sucrose, about 0.02% polysorbate-80, about 1 mM $MgCl_2$, about 0.1 mM EDTA, about 0.5% EtOH, and wherein the formulation has a pH of about 8.0.

147. The formulation of any one of claims 119-146, wherein the formulation further comprises at least one immunoadjuvant.

148. The formulation of claim 147, wherein the immunoadjuvant is selected from 1) Alum, 2) Saponins, 3) non-ionic polymer surfactants, 4) monophosphoryl lipid A, 5) muramyl dipeptides, and 6) cytokines.

149. The formulation of any one of claims 119-148, wherein the formulation further comprises at least one dye.

150. The formulation of any one of claims 119-149, wherein the formulation further comprises at least one reversible protease inhibitor.

151. The formulation of claim 150, wherein the reversible protease inhibitor is an inhibitor of an L3/p23 cysteine protease.

152. The formulation of any one of claims 119-151, wherein the formulation further comprises at least one antioxidant.

153. The formulation of claim 152, the antioxidant is vitamin A, vitamin C, vitamin E, vitamin B6, vitamin B12, folic acid, or folate.