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(54) **ADENO-ASSOCIATED VIRUS (AAV) SEROTYPE 9 SEQUENCES, VECTORS CONTAINING SAME, AND USES THEREFOR**

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C12N 7/01	(2006.01)
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(58) **Field of Classification Search** ..... None
See application file for complete search history.

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WO	WO 99/15677	A1	4/1999
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(57) **ABSTRACT**

Sequences of a serotype 9 adeno-associated virus and vectors and host cells containing these sequences are provided. Also described are methods of using such host cells and vectors in production of rAAV particles. AAV9-mediated delivery of therapeutic and immunogenic genes is also provided.

6 Claims, 9 Drawing Sheets

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Fig. 1A

```
cagagaggga gtggccaact ccatcactag gggtaatcgc gaagcgcctc ccacgctgcc     60

gcgtcagcgc tgacgtagat tacgtcatag gggagtggtc ctgtattagc tgtcacgtga    120

gtgcttttgc gacattttgc gacaccacat ggccatttga ggtatatatg gccgagtgag    180

                                                    Rep68/78 start
cgagcaggat ctccattttg accgcgaaat ttgaacgagc agcagccatg ccgggcttct    240

acgagattgt gatcaaggtg ccgagcgacc tggacgagca cctgccgggc atttctgact    300

cttttgtgaa ctgggtggcc gagaaggaat gggagctgcc cccggattct gacatggatc    360

ggaatctgat cgagcaggca cccctgaccg tggccgagaa gctgcagcgc gacttcctgg    420

tccaatggcg ccgcgtgagt aaggccccgg aggccctctt ctttgttcag ttcgagaagg    480

gcgagagcta ctttcacctg cacgttctgg tcgagaccac gggggtcaag tccatggtgc    540

taggccgctt cctgagtcag attcgggaga agctggtcca gaccatctac cgcgggatcg    600

agccgaccct gcccaactgg ttcgcggtga ccaagacgcg taatggcgcc ggcgggggga    660

acaaggtggt ggacgagtgc tacatcccca actacctcct gcccaagact cagccccgagc    720

tgcagtgggc gtggactaac atggaggagt atataagcgc gtgcttgaac ctggccgagc    780

gcaaacggct cgtggcgcag cacctgaccc acgtcagcca gacgcaggag cagaacaagg    840

agaatctgaa ccccaattct gacgcgcccg tgatcaggtc aaaaacctcc gcgcgctaca    900

Rep40/52 start
tggagctggt cgggtggctg gtggaccggg gcatcacctc cgagaagcag tggatccagg    960

aggaccaggc ctcgtacatc tccttcaacg ccgcctccaa ctcgcggtcc cagatcaagg   1020

ccgcgctgga caatgccggc aagatcatgg cgctgaccaa atccgcgccc gactacctgg   1080

taggcccttc acttccggtg gacattacgc agaaccgcat ctaccgcatc ctgcagctca   1140

acggctacga ccctgcctac gccggctccg tctttctcgg ctgggcacaa aagaagttcg   1200

ggaaacgcaa caccatctgg ctgtttgggc cggccaccac gggaaagacc aacatcgcag   1260

aagccattgc ccacgccgtg ccttctacg gctgcgtcaa ctggaccaat gagaactttc   1320

ccttcaacga ttgcgtcgac aagatggtga tctggtggga ggagggcaag atgacggcca   1380

aggtcgtgga gtccgccaag gccattctcg gcggcagcaa ggtgcgcgtg gaccaaaagt   1440

gcaagtcgtc cgcccagatc gaccccactc ccgtgatcgt cacctccaac accaacatgt   1500
```

Fig. 1B

```
gcgccgtgat tgacgggaac agcaccacct tcgagcacca gcagcctctc caggaccgga   1560

tgtttaagtt cgaactcacc cgccgtctgg agcacgactt tggcaaggtg acaaagcagg   1620

aagtcaaaga gttcttccgc tgggccagtg atcacgtgac cgaggtgggcg catgagtttt   1680

acgtcagaaa gggcggagcc agcaaaagac ccgccccga tgacgcggat aaaagcgagc   1740

ccaagcgggc ctgcccctca gtcgcggatc catcgacgtc agacgcggaa ggagctccgg   1800

tggactttgc cgacaggtac caaaacaaat gttctcgtca cgcgggcatg cttcagatgc   1860

tgcttccctg caaaacgtgc gagagaatga atcagaattt caacatttgc ttcacacacg   1920

gggtcagaga ctgctcagag tgtttccccg gcgtgtcaga atctcaaccg gtcgtcagaa   1980

agaggacgta tcggaaactc tgtgcgattc atcatctgct ggggcgggct cccgagattg   2040

                                                        Rep 78 stop
cttgctcggc ctgcgatctg gtcaacgtgg acctggatga ctgtgtttct gagcaataaa   2100

                vp1 start
tgacttaaac caggtatggc tgccgatggt tatcttccag attggctcga ggacaacctc   2160

tctgagggca ttcgcgagtg gtgggacctg aaacctggag ccccgaaacc caaagccaac   2220

cagcaaaagc aggacgacgg ccggggtctg gtgcttcctg gctacaagta cctcggaccc   2280

ttcaacggac tcgacaaggg ggagcccgtc aacgcggcgg acgcagcggc cctcgagcac   2340

gacaaggcct acgaccagca gctcaaagcg ggtgacaatc cgtacctgcg gtataaccac   2400

gccgacgccg agtttcagga gcgtctgcaa gaagatacgt cttttggggg caacctcggg   2460

cgagcagtct tccaggccaa gaagcgggtt ctcgaacctc tcggtctggt tgaggaaggc   2520

      vp2 start
gctaagacgg ctcctggaaa gaagagaccg gtagagcagt caccccaaga accagactca   2580

tcctcgggca tcggcaaatc aggccagcag cccgctaaaa agagactcaa ttttggtcag   2640

actggcgact cagagtcagt ccccgaccca caacctctcg gagaacctcc agaagccccc   2700

                        vp3 start
tcaggtctgg gacctaatac aatggcttca ggcggtggcg ctccaatggc agacaataac   2760

gaaggcgccg acggagtggg taattcctcg ggaaattggc attgcgattc cacatggctg   2820

ggggacagag tcatcaccac cagcacccga acctgggcat tgcccaccta caacaaccac   2880

ctctacaagc aaatctccaa tggaacatcg ggaggaagca ccaacgacaa cacctacttt   2940
```

Fig. 1C

```
ggctacagca ccccctgggg gtattttgac ttcaacagat tccactgcca cttctcacca   3000
cgtgactggc agcgactcat caacaacaac tggggattcc ggccaaagag actcaacttc   3060
aagctgttca acatccaggt caaggaggtt acgacgaacg aaggcaccaa gaccatcgcc   3120
aataacctta ccagcaccgt ccaggtcttt acggactcgg agtaccagct accgtacgtc   3180
ctaggctctg cccaccaagg atgcctgcca ccgtttcctg cagacgtctt catggttcct   3240
cagtacggct acctgacgct caacaatgga agtcaagcgt taggacgttc ttctttctac   3300
tgtctggaat acttcccttc tcagatgctg agaaccggca acaactttca gttcagctac   3360
actttcgagg acgtgccttt ccacagcagc tacgcacaca gccagagtct agatcgactg   3420
atgaaccccc tcatcgacca gtacctatac tacctggtca gaacacagac aactggaact   3480
gggggaactc aaactttggc attcagccaa gcaggcccta gctcaatggc caatcaggct   3540
agaaactggg tacccgggcc ttgctaccgt cagcagcgcg tctccacaac caccaaccaa   3600
aataacaaca gcaactttgc gtggacggga gctgctaaat tcaagctgaa cgggagagac   3660
tcgctaatga atcctggcgt ggctatggca tcgcacaaag acgacgagga ccgcttcttt   3720
ccatcaagtg gcgttctcat atttggcaag caaggagccg ggaacgatgg agtcgactac   3780
agccaggtgc tgattacaga tgaggaagaa attaaagcca ccaaccctgt agccacagag   3840
gaatacggag cagtggccat caacaaccag gccgctaaca cgcaggcgca aactggactt   3900
gtgcataacc agggagttat tcctggtatg gtctggcaga accgggacgt gtacctgcag   3960
ggccctattt gggctaaaat acctcacaca gatggcaact ttcacccgtc tcctctgatg   4020
ggtggatttg gactgaaaca cccacctcca cagattctaa ttaaaaatac accagtgccg   4080
gcagatcctc ctcttacctt caatcaagcc aagctgaact ctttcatcac gcagtacagc   4140
acgggacaag tcagcgtgga aatcgagtgg gagctgcaga aagaaaacag caagcgctgg   4200
aatccagaga tccagtatac ttcaaactac tacaaatcta caaatgtgga ctttgctgtc   4260
aataccgaag gtgtttactc tgagcctcgc cccattggta ctcgttacct cacccgtaat   4320
   vp1-3 stop          polyA
ttgtaattgc ctgttaatca ataaaccggt taattcgttt cagttgaact ttggtctctg   4380
cg                                                                  4382
```

Fig. 2A

```
          1                                                  50
AAV_2     MAADGYLPDW LEDTLSEGIR QWWKLKPGPP PPKPAERHKD DSRGLVLPGY
AAV_8     MAADGYLPDW LEDNLSEGIR EWWALKPGAP KPKANQQKQD DGRGLVLPGY
AAV_1     MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY
AAV_3     MAADGYLPDW LEDNLSEGIR EWWALKPGVP QPKANQQHQD NRRGLVLPGY
AAV_9     MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY

          51                                                 100
AAV_2     KYLGPFNGLD KGEPVNEADA AALEHDKAYD RQLDSGDNPY LKYNHADAEF
AAV_8     KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLQAGDNPY LRYNHADAEF
AAV_1     KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF
AAV_3     KYLGPGNGLD KGEPVNEADA AALEHDKAYD QQLKAGDNPY LKYNHADAEF
AAV_9     KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF

          101                                                150
AAV_2     QERLKEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEPVKTAP GKKRPVEHSP
AAV_8     QERLQEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEGAKTAP GKKRPVEPSP
AAV_1     QERLQEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEGAKTAP GKKRPVEQSP
AAV_3     QERLQEDTSF GGNLGRAVFQ AKKRILEPLG LVEEAAKTAP GKKGAVDQSP
AAV_9     QERLQEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEGAKTAP GKKRPVEQSP

          151                                                200
AAV_2     .VEPDSSSGT GKAGQQPARK RLNFGQTGDA DSVPDPQPLG QPPAAPSGLG
AAV_8     QRSPDSSTGI GKKGQQPARK RLNFGQTGDS ESVPDPQPLG EPPAAPSGVG
AAV_1     .QEPDSSSGI GKTGQQPAKK RLNFGQTGDS ESVPDPQPLG EPPATPAAVG
AAV_3     .QEPDSSSGV GKSGKQPARK RLNFGQTGDS ESVPDPQPLG EPPAAPTSLG
AAV_9     QE.PDSSSGI GKSGQQPAKK RLNFGQTGDS ESVPDPQPLG EPPEAPSGLG

          201                                                250
AAV_2     TNTMATGSGA PMADNNEGAD GVGNSSGNWH CDSTWMGDRV ITTSTRTWAL
AAV_8     PNTMAAGGGA PMADNNEGAD GVGSSSGNWH CDSTWLGDRV ITTSTRTWAL
AAV_1     PTTMASGGGA PMADNNEGAD GVGNASGNWH CDSTWLGDRV ITTSTRTWAL
AAV_3     SNTMASGGGA PMADNNEGAD GVGNSSGNWH CDSQWLGDRV ITTSTRTWAL
AAV_9     PNTMASGGGA PMADNNEGAD GVGNSSGNWH CDSTWLGDRV ITTSTRTWAL

          251                                                300
AAV_2     PTYNNHLYKQ ISSQS--GAS NDNHYFGYST PWGYFDFNRF HCHFSPRDWQ
AAV_8     PTYNNHLYKQ ISNGTSGGAT NDNTYFGYST PWGYFDFNRF HCHFSPRDWQ
AAV_1     PTYNNHLYKQ ISSAST.GAS NDNHYFGYST PWGYFDFNRF HCHFSPRDWQ
AAV_3     PTYNNHLYKQ ISSQS..GAS NDNHYFGYST PWGYFDFNRF HCHFSPRDWQ
AAV_9     PTYNNHLYKQ ISNGTSGGST NDNTYFGYST PWGYFDFNRF HCHFSPRDWQ
```

Fig. 2B

```
        301                                                         350
AAV_2   RLINNNWGFR PKRLNFKLFN IQVKEVTQND GTTTIANNLT STVQVFTDSE
AAV_8   RLINNNWGFR PKRLSFKLFN IQVKEVTQNE GTKTIANNLT STIQVFTDSE
AAV_1   RLINNNWGFR PKRLNFKLFN IQVKEVTTND GVTTIANNLT STVQVFSDSE
AAV_3   RLINNNWGFR PKKLSFKLFN IQVRGVTQND GTTTIANNLT STVQVFTDSE
AAV_9   RLINNNWGFR PKRLNFKLFN IQVKEVTTNE GTKTIANNLT STVQVFTDSE

        351                                                         400
AAV_2   YQLPYVLGSA HQGCLPPFPA DVFMVPQYGY LTLNNGSQAV GRSSFYCLEY
AAV_8   YQLPYVLGSA HQGCLPPFPA DVFMIPQYGY LTLNNGSQAV GRSSFYCLEY
AAV_1   YQLPYVLGSA HQGCLPPFPA DVFMIPQYGY LTLNNGSQAV GRSSFYCLEY
AAV_3   YQLPYVLGSA HQGCLPPFPA DVFMVPQYGY LTLNNGSQAV GRSSFYCLEY
AAV_9   YQLPYVLGSA HQGCLPPFPA DVFMVPQYGY LTLNNGSQAL GRSSFYCLEY

        401                                                         450
AAV_2   FPSQMLRTGN NFTFSYTFED VPFHSSYAHS QSLDRLMNPL IDQYLYYLSR
AAV_8   FPSQMLRTGN NFQFTYTFED VPFHSSYAHS QSLDRLMNPL IDQYLYYLSR
AAV_1   FPSQMLRTGN NFTFSYTFEE VPFHSSYAHS QSLDRLMNPL IDQYLYYLNR
AAV_3   FPSQMLRTGN NFQFSYTFED VPFHSSYAHS QSLDRLMNPL IDQYLYYLNR
AAV_9   FPSQMLRTGN NFQFSYTFED VPFHSSYAHS QSLDRLMNPL IDQYLYYLVR

        451                                                         500
AAV_2   TNTPSG.TTT QSRLQFSQAG ASDIRDQS RNWLPGPCYRQQ RVSKTSADNN
AAV_8   TQTTGG.TAN TQTLGFSQGG PNTMANQA KNWLPGPCYRQQ RVSTTTGQNN
AAV_1   TQ.NQSGSAQ NKDLLFSRGS PAGMSVQP KNWLPGPCYRQQ RVSKTKTDNN
AAV_3   TQGTTSGTTN QSRLLFSQAG PQSMSLQA RNWLPGPCYRQQ RLSKTANDNN
AAV_9   TQTTG..TGG TQTLAFSQAG PSSMANQA RNWVPGPCYRQQ RVSTTTNQNN

        501                                                         550
AAV_2   NSEYSWTGAT KYHLNGRDSL VNPGPAMASH KDDEEKFFPQ SGVLIFGKQG
AAV_8   NSNFAWTAGT KYHLNGRNSL ANPGIAMATH KDDEERFFPS NGILIFGKQN
AAV_1   NSNFTWTGAS KYNLNGRESI INPGTAMASH KDDEDKFFPM SGVMIFGKES
AAV_3   NSNFPWTAAS KYHLNGRDSL VNPGPAMASH KDDEEKFFPM HGNLIFGKEG
AAV_9   NSNFAWTGAA KFKLNGRDSL MNPGVAMASH KDDEDRFFPS SGVLIFGKQG

        551                                                         600
AAV_2   SEKTNVDIEK VMITDEEEIR T TNPVATEQY GSVSTNLQRG NRQAATADVN
AAV_8   AARDNADYSD VMLTSEEEIK T TNPVATEEY GIVADNLQQQ NTAPQIGTVN
AAV_1   AGASNTALDN VMITDEEEIK A TNPVATERF GTVAVNFQSS STDPATGDVH
AAV_3   TTASNAELDN VMITDEEEIR T TNPVATEQY GTVANNLQSS NTAPTTGTVN
AAV_9   AGNDGVDYSQ VLITDEEEIK A TNPVATEEY GAVAINNQAA NTQAQTGLVH
```

Fig. 2C

```
       601                                                  650
AAV_2  TQGVLPGMVW QDRDVYLQGP IWAKIPHTDG HFHPSPLMGG FGLKHPPPQI
AAV_8  SQGALPGMVW QNRDVYLQGP IWAKIPHTDG NFHPSPLMGG FGLKHPPPQI
AAV_1  AMGALPGMVW QDRDVYLQGP IWAKIPHTDG HFHPSPLMGG FGLKNPPPQI
AAV_3  HQGALPGMVW QDRDVYLQGP IWAKIPHTDG HFHPSPLMGG FGLKHPPPQI
AAV_9  NQGVIPGMVW QNRDVYLQGP IWAKIPHTDG NFHPSPLMGG FGLKHPPPQI

       651                                                  700
AAV_2  LIKNTPVPA NPSTTFSAAKF ASFITQYSTG QVSVEIEWEL QKENSKRWNP
AAV_8  LIKNTPVPA DPPTTFNQSKL NSFITQYSTG QVSVEIEWEL QKENSKRWNP
AAV_1  LIKNTPVPA NPPAEFSATKF ASFITQYSTG QVSVEIEWEL QKENSKRWNP
AAV_3  MIKNTPVPA NPPTTFSPAKF ASFITQYSTG QVSVEIEWEL QKENSKRWNP
AAV_9  LIKNTPVPA DPPLTFNQAKL NSFITQYSTG QVSVEIEWEL QKENSKRWNP

       701                                          739
AAV_2  EIQYTSNYNK SVNVDFTVDT NGVYSEPRPI GTRYLTRNL
AAV_8  EIQYTSNYYK STSVDFAVNT EGVYSEPRPI GTRYLTRNL
AAV_1  EVQYTSNYAK SANVDFTVDN NGLYTEPRPI GTRYLTRPL
AAV_3  EIQYTSNYNK SVNVDFTVDT NGVYSEPRPI GTRYLTRNL
AAV_9  EIQYTSNYYK STNVDFAVNT EGVYSEPRPI GTRYLTRNL
```

Fig. 3A

```
Met Pro Gly Phe Tyr Glu Ile Val Ile Lys Val Pro Ser Asp Leu Asp
1               5                  10                  15

Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Asn Trp Val Ala Glu
            20                  25                  30

Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Arg Asn Leu Ile
        35                  40                  45

Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
    50                  55                  60

Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
65                  70                  75                  80

Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Leu His Val Leu Val Glu
                85                  90                  95

Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
            100                 105                 110

Arg Glu Lys Leu Val Gln Thr Ile Tyr Arg Gly Ile Glu Pro Thr Leu
        115                 120                 125

Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
    130                 135                 140

Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
145                 150                 155                 160

Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Glu Tyr Ile
                165                 170                 175

Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
            180                 185                 190

Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Leu Asn
        195                 200                 205
```

Fig. 3B

```
Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
    210                 215                 220

Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
225                 230                 235                 240

Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
                245                 250                 255

Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
            260                 265                 270

Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ser
        275                 280                 285

Leu Pro Val Asp Ile Thr Gln Asn Arg Ile Tyr Arg Ile Leu Gln Leu
    290                 295                 300

Asn Gly Tyr Asp Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
305                 310                 315                 320

Gln Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
                325                 330                 335

Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
            340                 345                 350

Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
        355                 360                 365

Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
    370                 375                 380

Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
385                 390                 395                 400

Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
                405                 410                 415
```

Fig. 3C

```
Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
            420                 425                 430

Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
        435                 440                 445

Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
    450                 455                 460

Glu Val Lys Glu Phe Phe Arg Trp Ala Ser Asp His Val Thr Glu Val
465                 470                 475                 480

Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Ser Lys Arg Pro Ala
                485                 490                 495

Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
            500                 505                 510

Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
        515                 520                 525

Asp Arg Tyr Gln Asn Lys Cys Ser Arg His Ala Gly Met Leu Gln Met
    530                 535                 540

Leu Leu Pro Cys Lys Thr Cys Glu Arg Met Asn Gln Asn Phe Asn Ile
545                 550                 555                 560

Cys Phe Thr His Gly Val Arg Asp Cys Ser Glu Cys Phe Pro Gly Val
                565                 570                 575

Ser Glu Ser Gln Pro Val Val Arg Lys Arg Thr Tyr Arg Lys Leu Cys
            580                 585                 590

Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys Ser Ala
        595                 600                 605

Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu Gln
    610                 615                 620
```

ADENO-ASSOCIATED VIRUS (AAV) SEROTYPE 9 SEQUENCES, VECTORS CONTAINING SAME, AND USES THEREFOR

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a US National Phase of Application No. PCT/US02/33631, filed Nov. 12, 2002, which claims the benefit under 35 USC 119(e) of US Provisional Appln. No. 60/386,132 filed on Jun. 5, 2002 and US Provisional Appln. No. 60/341,150 filed on Dec. 17, 2001.

BACKGROUND OF THE INVENTION

Adeno-associated virus (AAV), a member of the Parvovirus family, is a small nonenveloped, icosahedral virus with single-stranded linear DNA genomes of 4.7 kilobases (kb) to 6 kb. AAV is assigned to the genus, *Dependovirus*, because the virus was discovered as a contaminant in purified adenovirus stocks. AAV's life cycle includes a latent phase at which AAV genomes, after infection, are site specifically integrated into host chromosomes and an infectious phase in which, following either adenovirus or herpes simplex virus infection, the integrated genomes are subsequently rescued, replicated, and packaged into infectious viruses. The properties of non-pathogenicity, broad host range of infectivity, including non-dividing cells, and potential site-specific chromosomal integration make AAV an attractive tool for gene transfer.

Recent studies suggest that AAV vectors may be the preferred vehicle for gene delivery. To date, there have been 6 different serotypes of AAVs isolated from human or non-human primates (NHP) and well characterized. Among them, human serotype 2 is the first AAV that was developed as a gene transfer vector; it has been widely used for efficient gene transfer experiments in different target tissues and animal models. Clinical trials of the experimental application of AAV2 based vectors to some human disease models are in progress, and include such diseases as cystic fibrosis and hemophilia B.

What are desirable are AAV-based constructs for gene delivery.

SUMMARY OF THE INVENTION

In one aspect, the invention provides novel AAV sequences, compositions containing these sequences, and uses therefor. Advantageously, these compositions are particularly well suited for use in compositions requiring re-administration of rAAV for therapeutic or prophylactic purposes.

These and other aspects of the invention will be readily apparent from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1**A, **1**B and **1**C are the nucleic acid sequences of the rep and cap regions of AAV9 [SEQ ID NO:1].

FIGS. **2**A, **2**B and **2**C are an alignment of the amino acid sequences of the vp1 protein of the capsid of the novel AAV9 [SEQ ID NO:2] sequences of the invention as compared to the published sequences of AAV2 [SEQ ID NO:4], AAV1 [SEQ ID NO:5], and AAV3 [SEQ ID NO:6], and of a novel serotype AAV8 [SEQ ID NO:7], which is the subject of a co-pending application. The alignment was performed using the Clustal W program, with the numbering of AAV2 used for reference. Underlining and bold under the AAV9 sequences indicates cassettes of identity within the HVR.

FIGS. **3**A, **3**B and **3**C are the amino acid sequences of the rep proteins of AAV9 [SEQ ID NO:3].

DETAILED DESCRIPTION OF THE INVENTION

The invention provides the nucleic acid sequences and amino acids of a novel AAV serotype, AAV9. Also provided are fragments of these AAV sequences. Each of these fragments may be readily utilized in a variety of vector systems and host cells. Among desirable AAV9 fragments are the cap proteins, including the vp1, vp2, vp3 and hypervariable regions, the rep proteins, including rep 78, rep 68, rep 52, and rep 40, and the sequences encoding these proteins. These fragments may be readily utilized in a variety of vector systems and host cells. Such fragments may be used alone, in combination with other AAV9 sequences or fragments, or in combination with elements from other AAV or non-AAV viral sequences. In one particularly desirable embodiment, a vector contains the AAV9 cap and/or rep sequences of the invention.

The AAV9 sequences and fragments thereof are useful in production of rAAV, and are also useful as antisense delivery vectors, gene therapy vectors, or vaccine vectors. The invention further provides nucleic acid molecules, gene delivery vectors, and host cells which contain the AAV9 sequences of the invention.

Suitable fragments can be determined using the information provided herein. Alignments are performed using any of a variety of publicly or commercially available Multiple Sequence Alignment Programs, such as "Clustal W", accessible through Web. Servers on the internet. Alternatively, Vector NTI utilities are also used. There are also a number of algorithms known in the art which can be used to measure nucleotide sequence identity, including those contained in the programs described above. As another example, polynucleotide sequences can be compared using Fasta, a program in GCG Version 6.1. Fasta provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) as provided in GCG Version 6.1, herein incorporated by reference. Similar programs are available for amino acid sequences, e.g., the "Clustal X" program. Generally, any of these programs are used at default settings, although one of skill in the art can alter these settings as needed. Alternatively, one of skill in the art can utilize another algorithm or computer program which provides at least the level of identity or alignment as that provided by the referenced algorithms and programs.

The term "substantial homology" or "substantial similarity," when referring to a nucleic acid, or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95 to 99% of the aligned sequences. Preferably, the homology is over full-length sequence, or an open reading frame thereof, or another suitable fragment which is at least 15 nucleotides in length. Examples of suitable fragments are described herein.

The term "substantial homology" or "substantial similarity," when referring to amino acids or fragments thereof,

indicates that, when optimally aligned with appropriate amino acid insertions or deletions with another amino acid (or its complementary strand), there is amino acid sequence identity in at least about 95 to 99% of the aligned sequences. Preferably, the homology is over full-length sequence, or a protein thereof, e.g., a cap protein, a rep protein, or a fragment thereof which is at least 8 amino acids, or more desirably, at least 15 amino acids in length. Examples of suitable fragments are described herein.

By the term "highly conserved" is meant at least 80% identity, preferably at least 90% identity, and more preferably, over 97% identity. Identity is readily determined by one of skill in the art by resort to algorithms and computer programs known by those of skill in the art.

The term "percent sequence identity" or "identical" in the context of nucleic acid sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over the full-length of the genome, the full-length of a gene coding sequence, or a fragment of at least about 500 to 5000 nucleotides, is desired. However, identity among smaller fragments, e.g. of at least about nine nucleotides, usually at least about 20 to 24 nucleotides, at least about 28 to 32 nucleotides, at least about 36 or more nucleotides, may also be desired. Similarly, "percent sequence identity" may be readily determined for amino acid sequences, over the full-length of a protein, or a fragment thereof. Suitably, a fragment is at least about 8 amino acids in length, and may be up to about 700 amino acids. Examples of suitable fragments are described herein.

As described herein, the vectors of the invention containing the AAV capsid proteins of the invention are particularly well suited for use in applications in which the neutralizing antibodies diminish the effectiveness of other AAV serotype based vectors, as well as other viral vectors. The rAAV vectors of the invention are particularly advantageous in rAAV readministration and repeat gene therapy.

These and other embodiments and advantages of the invention are described in more detail below. As used throughout this specification and the claims, the term "comprising" is inclusive of other components, elements, integers, steps and the like. Conversely, the term "consisting" and its variants are exclusive of other components, elements, integers, steps and the like.

I. AAV Serotype 9 Sequences

A. Nucleic Acid Sequences

The AAV9 nucleic acid sequences of the invention include the DNA sequences of FIG. **1** [SEQ ID NO: 1], which consists of 4382 nucleotides. The AAV9 nucleic acid sequences of the invention further encompass the strand which is complementary to FIG. **1** [SEQ ID NO: 1], as well as the RNA and cDNA sequences corresponding to FIG. **1** [SEQ ID NO: 1] and its complementary strand. Also included in the nucleic acid sequences of the invention are natural variants and engineered modifications of FIG. **1** [SEQ ID NO: 1] and its complementary strand. Such modifications include, for example, labels which are known in the art, methylation, and substitution of one or more of the naturally occurring nucleotides with a degenerate nucleotide.

Further included in this invention are nucleic acid sequences which are greater than 85%, preferably at least about 90%, more preferably at least about 95%, and most preferably at least about 98 to 99% identical or homologous to FIG. **1** [SEQ ID NO:1]. Also included within the invention are fragments of FIG. **1** [SEQ ID NO: 1], its complementary strand, cDNA and RNA complementary thereto. Suitable fragments are at least 15 nucleotides in length, and encompass functional fragments, i.e., fragments which are of biological interest. Such fragments include the sequences encoding the three variable proteins (vp) of the AAV9 capsid which are alternative splice variants: vp1 [nt 2116 to 4323 of FIG. **1**, SEQ ID NO:1]; vp2 [nt 2527 to 4323 of FIG. **1**, SEQ ID NO:1]; and vp 3 [nt 2725 to 4323 of FIG. **1**, SEQ ID NO:1]. Other suitable fragments of FIG. **1**, SEQ ID NO:1, the fragment which contains the start codon for the AAV9 capsid protein.

Still other fragments include those encoding the rep proteins, including rep 78 [initiation codon at nt 228 of FIG. **1**], rep 68 [initiation codon at nt 228 of FIG. **1**], rep 52 [initiation codon at nt 900 of FIG. **1**], and rep 40 [initiation codon at nt 900 of FIG. **1**]. See, SEQ ID NO:1. Other fragments of interest may include the AAV invented terminal repeats (ITRs), AAV P19 sequences, AAV P40 sequences, the rep binding site, and the terminal resolute site (TRS). Still other suitable fragments will be readily apparent to those of skill in the art.

In addition to including the nucleic acid sequences provided in the figures and Sequence Listing, the present invention includes nucleic acid molecules and sequences which are designed to express the amino acid sequences, proteins and peptides of the AAV serotypes of the invention. Thus, the invention includes nucleic acid sequences which encode the following novel AAV amino acid sequences and artificial AAV serotypes generated using these sequences and/or unique fragments thereof.

As used herein, artificial AAV serotypes include, without limitation, AAV with a non-naturally occurring capsid protein. Such an artificial capsid may be generated by any suitable technique, using a novel AAV sequence of the invention (e.g., a fragment of a vp1 capsid protein) in combination with heterologous sequences which may be obtained from another AAV serotype (known or novel), non-contiguous portions of the same AAV serotype, from a non-AAV viral source, or from a non-viral source. An artificial AAV serotype may be, without limitation, a chimeric AAV capsid, a recombinant AAV capsid, or a "humanized" AAV capsid.

B. AAV9 Amino Acid Sequences, Proteins and Peptides

The invention further provides proteins and fragments thereof which are encoded by the AAV9 nucleic acids of the invention, and AAV9 amino acids which are generated by other methods. The invention further encompasses AAV serotypes generated using sequences of the novel AAV serotype of the invention, which are generated using synthetic, recombinant or other techniques known to those of skill in the art. The invention is not limited to novel AAV amino acid sequences, peptides and proteins expressed from the novel AAV nucleic acid sequences of the invention and encompasses amino acid sequences, peptides and proteins generated by other methods known in the alt, including, e.g., by chemical synthesis, by other synthetic techniques, or by other methods. For example, the sequences of any of be readily generated using a variety of techniques.

Suitable production techniques are well known to those of skill in the art. See, e.g., Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (Cold Spring Harbor, N.Y.). Alternatively, peptides can also be synthesized by the well known solid phase peptide synthesis methods (Merrifield, *J. Am. Chem. Soc.*, 85:2149 (1962); Stewart and Young, Solid Phase Peptide Synthesis (Freeman, San Francisco, 1969) pp. 27–62). These and other

suitable production methods are within the knowledge of those of skill in the art and are not a limitation of the present invention.

Particularly desirable proteins include the AAV9 capsid proteins and AAV9 rep proteins.

Particularly desirable proteins include the AAV capsid proteins, which are encoded by the nucleotide sequences identified above. The AAV capsid is composed of three proteins, vp1, vp2 and vp3, which are alternative splice variants. The full-length sequence provided in FIG. **2** is that of vp1. The AAV9 capsid proteins include vp1 [SEQ ID NO:2], vp2 [aa 138 to 736 of SEQ ID NO:2], and vp3 [aa 203 to 736 of SEQ ID NO: 2] and functional fragments thereof. Other desirable fragments of the capsid protein include the constant and variable regions, located between hypervariable regions (HPV). Other desirable fragments of the capsid protein include the HPV themselves.

An algorithm developed to determine areas of sequence divergence in AAV2 has yielded 12 hypervariable regions (HVR) of which 5 overlap or are part of the four previously described variable regions. [Chiorini et al, *J. Virol,* 73:1309–19 (1999); Rutledge et al, *J. Virol.,* 72:309–319] Using this algorithm and/or the alignment techniques described herein, the HVR of the novel AAV serotypes are determined. For example, with respect to the number of the AAV2 vp1 [SEQ ID NO:4], the HVR are located as follows: HVR1, aa 146–152; HVR2, aa 182–186; HVR3, aa 262–264; HVR4, aa 381–383; HVR5, aa 450–474; HVR6, aa 490–495; HVR7, aa500–504; HVR8, aa 514–522; HVR9, aa 534–555; HVR10, aa 581–594; HVR11, aa 658–667; and HVR12, aa 705–719. Using the alignment provided herein performed using the Clustal X program at default settings, or using other commercially or publicly available alignment programs at default settings, one of skill in the art can readily determine corresponding fragments of the novel AAV capsids of the invention.

Still other desirable fragments of the AAV9 capsid protein include amino acids 1 to 184 of SEQ ID NO: 2, amino acids 199 to 259; amino acids 274 to 446; amino acids 603 to 659; amino acids 670 to 706; amino acids 724 to 736 of SEQ ID NO:2; aa 185–198; aa 260–273; aa447–477; aa495–602; aa660–669; and aa707–723. Additionally, examples of other suitable fragments of AAV capsids include, with respect to the numbering of AAV2 [SEQ ID NO:4], aa 24 to 42, aa 25 to 28; aa 81 to 85; aa133 to 165; aa 171 to 173; aa 413 to 417; aa 449 to 478; aa 494 to 525; aa 534 to 571; aa 5 aa 660 to 671; aa 709 to 723. Still other desirable fragments include, for example, in AAV7, amino acids 1 to 184 of SEQ ID NO:2, amino acids 199 to 259; amino acids 274 to 446; amino acids 603 to 659; amino acids 670 to 706; amino acids 724 to 736; aa 185 to 198; aa 260 to 273; aa447 to 477; aa495 to 602; aa660 to 669; and aa707 to 723. Using the alignment provided herein performed using the Clustal X program at default settings, or using other commercially or publicly available alignment programs at default settings, one of skill in the art can readily determine corresponding fragments of the novel AAV capsids of the invention.

Still other desirable AAV9 proteins include the rep proteins include rep68/78 and rep40/52 [located within SEQ ID NO: 3]. Suitable fragments of the rep proteins may include aa 1 to 102; aa 103 to 140; aa 141 to 173; aa 174 to 226; aa 227 to 275; aa 276 to 374; aa 375 to 383; aa 384 to 446; aa 447 to 542; aa 543 to 555; aa 556 to 623, of SEQ ID NO:3.

Suitably, fragments are at least 8 amino acids in length. However, fragments of other desired lengths may be readily utilized. Such fragments may be produced recombinantly or by other suitable means, e.g., chemical synthesis.

The invention further provides other AAV9 sequences which are identified using the sequence information provided herein. For example, given the AAV9 sequences provided herein, infectious AAV9 may be isolated using genome walking technology (Siebertetal., 1995, *Nucleic Acid Research,* 23:1087–1088, Friezner-Degen et al., 1986, *J. Biol. Chem.* 261:6972–6985, BD Biosciences Clontech, Palo Alto, Calif.). Genome walking is particularly well suited for identifying and isolating the sequences adjacent to the novel sequences identified according to the method of the invention. This technique is also useful for isolating inverted terminal repeat (ITRs) of the novel AAV9 serotype, based upon the novel AAV capsid and rep sequences provided herein.

The sequences, proteins, and fragments of the invention may be produced by any suitable means, including recombinant production, chemical synthesis, or other synthetic means. Such production methods are within the knowledge of those of skill in the art and are not a limitation of the present invention.

IV. Production of rAAV with AAV9 Capsids

The invention encompasses novel, wild-type AAV9, the sequences of which are free of DNA and/or cellular material with these viruses are associated in nature. In another aspect, the present invention provides molecules which utilize the novel AAV sequences of the invention, including fragments thereof, for production of molecules useful in delivery of a heterologous gene or other nucleic acid sequences to a target cell.

In another aspect, the present invention provides molecules which utilize the AAV9 sequences of the invention, including fragments thereof, for production of viral vectors useful in delivery of a heterologous gene or other nucleic acid sequences to a target cell.

The molecules of the invention which contain AAV9 sequences include any genetic element (vector) which may be delivered to a host cell, e.g., naked DNA, a plasmid, phage, transposon, cosmid, episome, a protein in a non-viral delivery vehicle (e.g., a lipid-based carrier), virus, etc. which transfer the sequences carried thereon. The selected vector may be delivered by any suitable method, including transfection, electroporation, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion. The methods used to construct any embodiment of this invention are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. See, e.g., Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

In one embodiment, the vectors of the invention contain, at a minimum, sequences encoding an AAV9 capsid or a fragment thereof. In another embodiment, the vectors of the invention contain, at a minimum, sequences encoding an AAV9 rep protein or a fragment thereof. Optionally, such vectors may contain both AAV cap and rep proteins. In vectors in which both AAV rep and cap are provides, the AAV rep and AAV cap sequences can both be of AAV9 origin. Alternatively, the present invention provides vectors in which the rep sequences are from an AAV serotype which differs from that which is providing the cap sequences. In one embodiment, the rep and cap sequences are expressed from separate sources (e.g., separate vectors, or a host cell and a vector). In another embodiment, these rep sequences are fused in frame to cap sequences of a different AAV serotype to form a chimeric AAV vector. Optionally, the

vectors of the invention further contain a minigene comprising a selected transgene which is flanked by AAV 5' ITR and AAV 3' ITR.

Thus, in one embodiment, the vectors described herein contain nucleic acid sequences encoding an intact AAV capsid which may be from a single AAV serotype (e.g., AAV9). Such a capsid may comprise amino acids 1 to 736 of SEQ ID NO:2. Alternatively, these vectors contain sequences encoding artificial capsids which contain one or more fragments of the AAV9 capsid fused to heterologous AAV or non-AAV capsid proteins (or fragments thereof). These artificial capsid proteins are selected from non-contiguous portions of the AAV9 capsid or from capsids of other AAV serotypes. For example, a rAAV may have a capsid protein comprising one or more of the AAV9 capsid regions selected from the vp2 and/or vp3, or from vp1, or fragments thereof selected from amino acids 1 to 184, amino acids 199 to 259; amino acids 274 to 446; amino acids 603 to 659; amino acids 670 to 706; amino acids 724 to 736 of the AAV9 capsid, SEQ ID NO: 2. In another example, it may be desirable to alter the start codon of the vp3 protein to GTG. Alternatively, the rAAV may contain one or more of the AAV serotype 9 capsid protein hypervariable regions which are identified herein, or other fragment including, without limitation, aa 185 to 198; aa 260–273; aa 447 to 477; aa 495 to 602; aa 660 to 669; and aa 707 to 723 of the AAV9 capsid. See, SEQ ID NO: 2 These modifications may be to increase expression, yield, and/or to improve purification in the selected expression systems, or for another desired purpose (e.g., to change tropism or alter neutralizing antibody epitopes).

The vectors described herein, e.g., a plasmid, are useful for a variety of purposes, but are particularly well suited for use in production of a rAAV containing a capsid comprising AAV sequences or a fragment thereof. These vectors, including rAAV, their elements, construction, and uses are described in detail herein.

In one aspect, the invention provides a method of generating a recombinant adeno-associated virus (AAV) having an AAV serotype 9 capsid, or a portion thereof. Such a method involves culturing a host cell which contains a nucleic acid sequence encoding an adeno-associated virus (AAV) serotype 9 capsid protein, or fragment thereof, as defined herein; a functional rep gene; a minigene composed of, at a minimum, AAV inverted terminal repeats (ITRs) and a transgene; and sufficient helper functions to permit packaging of the minigene into the AAV9 capsid protein.

The components required to be cultured in the host cell to package an AAV minigene in an AAV capsid may be provided to the host cell in trans. Alternatively, any one or more of the required components (e.g., minigene, rep sequences, cap sequences, and/or helper functions) may be provided by a stable host cell which has been engineered to contain one or more of the required components using methods known to those of skill in the art. Most suitably, such a stable host cell will contain the required component(s) under the control of an inducible promoter. However, the required component(s) may be under the control of a constitutive promoter. Examples of suitable inducible and constitutive promoters are provided herein, in the discussion of regulatory elements suitable for use with the transgene. In still another alternative, a selected stable host cell may contain selected component(s) under the control of a constitutive promoter and other selected component(s) under the control of one or more inducible promoters. For example, a stable host cell may be generated which is derived from 293 cells (which contain E1 helper functions under the control of a constitutive promoter), but which contains the rep and/or cap proteins under the control of inducible promoters. Still other stable host cells may be generated by one of skill in the art.

The minigene, rep sequences, cap sequences, and helper functions required for producing the rAAV of the invention may be delivered to the packaging host cell in the form of any genetic element which transfer the sequences carried thereon. The selected genetic element may be delivered by any suitable method, including those described herein. The methods used to construct any embodiment of this invention are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. See, e.g., Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. Similarly, methods of generating rAAV virions are well known and the selection of a suitable method is not a limitation on the present invention. See, e.g., K. Fisher et al. *J. Virol.,* 70:520–532 (1993) and U.S. Pat. No. 5,478,745.

Unless otherwise specified, the AAV ITRs, and other selected AAV components described herein, may be readily selected from among any AAV serotype, including without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, and the novel serotype of the invention, AAV9. These ITRs or other AAV components may be readily isolated using techniques available to those of skill in the art from an AAV serotype. Such AAV may be isolated or obtained from academic, commercial, or public sources (e.g., the American Type Culture Collection, Manassas, Va.). Alternatively, the AAV sequences may be obtained through synthetic or other suitable means by reference to published sequences such as are available in the literature or in databases such as, e.g., GenBank, PubMed, or the like.

A. The Minigene

The minigene is composed of, at a minimum, a transgene and its regulatory sequences, and 5' and 3' AAV inverted terminal repeats (ITRs). In one desirable embodiment, the ITRs of AAV serotype 2 are used. However, ITRs from other suitable serotypes may be selected. It is this minigene which is packaged into a capsid protein and delivered to a selected host cell.

1. The Transgene

The transgene is a nucleic acid sequence, heterologous to the vector sequences flanking the transgene, which encodes a polypeptide, protein, or other product of interest. The nucleic acid coding sequence is operatively linked to regulatory components in a manner which permits transgene transcription, translation, and/or expression in a host cell.

The composition of the transgene sequence will depend upon the use to which the resulting vector will be put. For example, one type of transgene sequence includes a reporter sequence, which upon expression produces a detectable signal. Such reporter sequences include, without limitation, DNA sequences encoding β-lactamase, β-galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), luciferase, membrane bound proteins including, for example, CD2, CD4, CD8, the influenza hemagglutinin protein, and others well known in the art, to which high affinity antibodies directed thereto exist or can be produced by conventional means, and fusion proteins comprising a membrane bound protein appropriately fused to an antigen tag domain from, among others, hemagglutinin or Myc.

These coding sequences, when associated with regulatory elements which drive their expression, provide signals detectable by conventional means, including enzymatic,

radiographic, calorimetric, fluorescence or other spectrographic assays, fluorescent activating cell sorting assays and immunological assays, including enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and immunohistochemistry. For example, where the marker sequence is the LacZ gene, the presence of the vector carrying the signal is detected by assays for beta-galactosidase activity. Where the transgene is green fluorescent protein or luciferase, the vector carrying the signal may be measured visually by color or light production in a luminometer.

However, desirably, the transgene is a non-marker sequence encoding a product which is useful in biology and medicine, such as proteins, peptides, RNA, enzymes, or catalytic RNAs. Desirable RNA molecules include tRNA, dsRNA, ribosomal RNA, catalytic RNAs, and antisense RNAs. One example of a useful RNA sequence is a sequence which extinguishes expression of a targeted nucleic acid sequence in the treated animal.

The transgene may be used to correct or ameliorate gene deficiencies, which may include deficiencies in which normal genes are expressed at less than normal levels or deficiencies in which the functional gene product is not expressed. A preferred type of transgene sequence encodes a therapeutic protein or polypeptide which is expressed in a host cell. The invention further includes using multiple transgenes, e.g., to correct or ameliorate a gene defect caused by a multi-subunit protein. In certain situations, a different transgene may be used to encode each subunit of a protein, or to encode different peptides or proteins. This is desirable when the size of the DNA encoding the protein subunit is large, e.g., for an immunoglobulin, the platelet-derived growth factor, or a dystrophin protein. In order for the cell to produce the multi-subunit protein, a cell is infected with the recombinant virus containing each of the different subunits. Alternatively, different subunits of a protein may be encoded by the same transgene. In this case, a single transgene includes the DNA encoding each of the subunits, with the DNA for each subunit separated by an internal ribozyme entry site (IRES). This is desirable when the size of the DNA encoding each of the subunits is small, e.g., the total size of the DNA encoding the subunits and the IRES is less than five kilobases. As an alternative to an IRES, the DNA may be separated by sequences encoding a 2A peptide, which self-cleaves in a post-translational event. See, e.g., M. L. Donnelly, et al, *J. Gen. Virol.,* 78(Pt 1):13–21 (January 1997); Furler, S., et al, *Gene Ther.,* 8(11):864–873 (June 2001); Klump H., et al., *Gene Ther.,* 8(10):811–817 (May 2001This 2A peptide is significantly smaller than an IRES, making it well suited for use when space is a limiting factor. However, the selected transgene may encode any biologically active product or other product, e.g., a product desirable for study.

Suitable transgenes may be readily selected by one of skill in the art. The selection of the transgene is not considered to be a limitation of this invention.

2. Regulatory Elements

In addition to the major elements identified above for the minigene, the vector also includes conventional control elements necessary which are operably linked to the transgene in a manner which permits its transcription, translation and/or expression in a cell transfected with the plasmid vector or infected with the virus produced by the invention. As used herein, "operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest.

Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. A great number of expression control sequences, including promoters which are native, constitutive, inducible and/or tissue-specific, are known in the art and may be utilized.

Examples of 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 allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, e.g., acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech and Ariad. Many other systems have been described and can be readily selected by one of skill in the art. Examples of inducible promoters regulated by exogenously supplied compounds include, e.g., 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. Sc. 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.

In another embodiment, the native promoter for the transgene will be used. The native promoter may be preferred when it is desired that expression of the transgene 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.

Another embodiment of the transgene includes a transgene operably linked to a tissue-specific promoter. 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 β-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. Res.,* 11:654–64 (1996)), lymphocytes (CD2, Hansal et al., *J. Immuno.,* 161:1063–8 (1998); immunoglobulin heavy chain; T cell receptor αchain), 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.

Optionally, plasmids carrying therapeutically useful transgenes may also include selectable markers or reporter genes may include sequences encoding geneticin, hygromicin or purimycin resistance, among others. Such selectable reporters or marker genes (preferably located outside the viral genome to be rescued by the method of the invention) can be used to signal the presence of the plasmids in bacterial cells, such as ampicillin resistance. Other components of the plasmid may include an origin of replication. Selection of these and other promoters and vector elements are conventional and many such sequences are available [see, e.g., Sambrook et al, and references cited therein].

The combination of the transgene, promoter/enhancer, and 5' and 3' ITRs is referred to as a "minigene" for ease of reference herein. Provided with the teachings of this invention, the design of such a minigene can be made by resort to conventional techniques.

3. Delivery of the Minigene to a Packaging Host Cell

The minigene can be carried on any suitable vector, e.g., a plasmid, which is delivered to a host cell. The plasmids useful in this invention may be engineered such that they are suitable for replication and, optionally, integration in prokaryotic cells, mammalian cells, or both. These plasmids (or other vectors carrying the 5' AAV ITR-heterologous molecule-3'ITR) contain sequences permitting replication of the minigene in eukaryotes and/or prokaryotes and selection markers for these systems. Selectable markers or reporter genes may include sequences encoding geneticin, hygromicin or purimycin resistance, among others. The plasmids may also contain certain selectable reporters or marker genes that can be used to signal the presence of the vector in bacterial cells, such as ampicillin resistance. Other components of the plasmid may include an origin of replication and an amplicon, such as the amplicon system employing the Epstein Barr virus nuclear antigen. This amplicon system, or other similar amplicon components permit high copy episomal replication in the cells. Preferably, the molecule carrying the minigene is transfected into the cell, where it may exist transiently. Alternatively, the minigene (carrying the 5' AAV ITR-heterologous molecule-3' ITR) may be stably integrated into the genome of the host cell, either chromosomally or as an episome. In certain embodiments, the minigene may be present in multiple copies, optionally in head-to-head, head-to-tail, or tail-to-tail concatamers. Suitable transfection techniques are known and may readily be utilized to deliver the minigene to the host cell.

Generally, when delivering the vector comprising the minigene by transfection, the vector is delivered in an amount from about 5 μg to about 100 μg DNA, and preferably about 10 to about 50 μg DNA to about 1×10^4 cells to about 1×10^{13} cells, and preferably about 10^5 cells. However, the relative amounts of vector DNA to host cells may be adjusted, taking into consideration such factors as the selected vector, the delivery method and the host cells selected.

B. Rep and Cap Sequences

In addition to the minigene, the host cell contains the sequences which drive expression of the AAV9 capsid protein (or a capsid protein comprising a fragment of the AAV9 capsid) in the host cell and rep sequences of the same serotype as the serotype of the AAV ITRs found in the minigene, or a cross-complementing serotype. The AAV cap and rep sequences may be independently obtained from an AAV source as described above and may be introduced into the host cell in any manner known to one in the art as described above. Additionally, when pseudotyping an AAV vector in an AAV9 capsid, the sequences encoding each of the essential rep proteins may be supplied by AAV9, or the sequences encoding the rep proteins may be supplied by different AAV serotypes (e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7). For example, the rep78168 sequences may be from AAV2, whereas the rep52/40 sequences may from AAV1.

In one embodiment, the host cell stably contains the capsid protein under the control of a suitable promoter, such as those described above. Most desirably, in this embodiment, the capsid protein is expressed under the control of an inducible promoter. In another embodiment, the capsid protein is supplied to the host cell in trans. When delivered to the host cell in trans, the capsid protein may be delivered via a plasmid which contains the sequences necessary to direct expression of the selected capsid protein in the host cell. Most desirably, when delivered to the host cell in trans, the plasmid carrying the capsid protein also carries other sequences required for packaging the rAAV, e.g., the rep sequences.

In another embodiment, the host cell stably contains the rep sequences under the control of a suitable promoter, such as those described above. Most desirably, in this embodiment, the essential rep proteins are expressed under the control of an inducible promoter. In another embodiment, the rep proteins are supplied to the host cell in trans. When delivered to the host cell in trans, the rep proteins may be delivered via a plasmid which contains the sequences necessary to direct expression of the selected rep proteins in the host cell. Most desirably, when delivered to the host cell in trans, the plasmid carrying the capsid protein also carries other sequences required for packaging the rAAV, e.g., the rep and cap sequences.

Thus, in one embodiment, the rep and cap sequences may be transfected into the host cell on a single nucleic acid molecule and exist stably in the cell as an episome. In another embodiment, the rep and cap sequences are stably integrated into the genome of the cell. Another embodiment has the rep and cap sequences transiently expressed in the host cell. For example, a useful nucleic acid is molecule for such transfection comprises, from 5' to 3', a promoter, an optional spacer interposed between the promoter and the start site of the rep gene sequence, an AAV rep gene sequence, and an AAV cap gene sequence.

Optionally, the rep and/or cap sequences may be supplied on a vector that contains other DNA sequences that are to be introduced into the host cells. For instance, the vector may contain the rAAV construct comprising the minigene. The vector may comprise one or more of the genes encoding the helper functions, e.g., the adenoviral proteins E1, E2a, and E4ORF6, and the gene for VAI RNA.

Preferably, the promoter used in this construct may be any of the constitutive, inducible or native promoters known to

one of skill in the art or as discussed above. In one embodiment, an AAV PS promoter sequence is employed. The selection of the AAV to provide any of these sequences does not limit the invention.

In another preferred embodiment, the promoter for rep is an inducible promoter, many of which are discussed above in connection with the transgene regulatory elements. One preferred promoter for rep expression is the T7 promoter. The vector comprising the rep gene regulated by the T7 promoter and the cap gene, is transfected or transformed into a cell which either constitutively or inducibly expresses the 17 polymerase. See WO 98/10088, published Mar. 12, 1998.

The spacer is an optional element in the design of the vector. The spacer is a DNA sequence interposed between the promoter and the rep gene ATG start site. The spacer may have any desired design; that is, it may be a random sequence of nucleotides, or alternatively, it may encode a gene product, such as a marker gene. The spacer may contain genes which typically incorporate start/stop and polyA sites. The spacer may be a non-coding DNA sequence from a prokaryote or eukaryote, a repetitive non-coding sequence, a coding sequence without transcriptional controls or a coding sequence with transcriptional controls. Two exemplary sources of spacer sequences are the λ phage ladder sequences or yeast ladder sequences, which are available commercially, e.g., from Gibco or Invitrogen, among others. The spacer may be of any size sufficient to reduce expression of the rep78 and rep68 gene products, leaving the rep52, rep40 and cap gene products expressed at normal levels. The length of the spacer may therefore range from about 10 bp to about 10.0 kbp, preferably in the range of about 100 bp to about 8.0 kbp. To reduce the possibility of recombination, the spacer is preferably less than 2 kbp in length; however, the invention is not so limited.

Although the molecule(s) providing rep and cap may exist in the host cell transiently (i.e., through transfection), it is preferred that one or both of the rep and cap proteins and the promoter(s) controlling their expression be stably expressed in the host cell, e.g., as an episome or by integration into the chromosome of the host cell. The methods employed for constructing embodiments of this invention are conventional genetic engineering or recombinant engineering techniques such as those described in the references above. While this specification provides illustrative examples of specific constructs, using the information provided herein, one of skill in the art may select and design other suitable constructs, using a choice of spacers, P5 promoters, and other elements, including at least one translational start and stop signal, and the optional addition of polyadenylation sites.

In another embodiment of this invention, the rep or cap protein may be provided stably by a host cell.

C. The Helper Functions

The packaging host cell also requires helper functions in order to package the rAAV of the invention. Optionally, these functions may be supplied by a herpesvirus. Most desirably, the necessary helper functions are each provided from a human or non-human primate adenovirus source, such as are described herein or and which are available from a variety of sources, including the ATCC. In one currently preferred embodiment, the host cell is provided with and/or contains an E1a gene product, an E1b gene product, an E2a gene product, and/or an E4 ORF6 gene product. The host cell may contain other adenoviral genes such as VAI RNA, but these genes are not required. In a preferred embodiment, no other adenovirus genes or gene functions are present in the host cell.

By "adenoviral DNA which expresses the E1a gene product", it is meant any adenovirus sequence encoding E1a or any functional E1a portion. Adenoviral DNA which expresses the E2a gene product and adenoviral DNA which expresses the E4 ORF6 gene products are defined similarly. Also included are any alleles or other modifications of the adenoviral gene or functional portion thereof. Such modifications may be deliberately introduced by resort to conventional genetic engineering or mutagenic techniques to enhance the adenoviral function in some manner, as well as naturally occurring allelic variants thereof. Such modifications and methods for manipulating DNA to achieve these adenovirus gene functions are known to those of skill in the art.

The adenovirus E1a, E1b, E2a, and/or E4ORF6 gene products, as well as any other desired helper functions, can be provided using any means that allows their expression in a cell. Each of the sequences encoding these products may be on a separate vector, or one or more genes may be on the same vector. The vector may be any vector known in the art or disclosed above, including plasmids, cosmids and viruses. Introduction into the host cell of the vector may be achieved by any means known in the art or as disclosed above, including transfection, infection, electroporation, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion, among others. One or more of the adenoviral genes may be stably integrated into the genome of the host cell, stably expressed as episomes, or expressed transiently. The gene products may all be expressed transiently, on an episome or stably integrated, or some of the gene products may be expressed stably while others are expressed transiently. Furthermore, the promoters for each of the adenoviral genes may be selected independently from a constitutive promoter, an inducible promoter or a native adenoviral promoter. The promoters may be regulated by a specific physiological state of the organism or cell (i.e., by the differentiation state or in replicating or quiescent cells) or by exogenously-added factors, for example.

D. Host Cells And Packaging Cell Lines

The host cell itself may be selected from any biological organism, including prokaryotic (e.g., bacterial) cells, and eukaryotic cells, including, insect cells, yeast cells and mammalian cells. Particularly desirable host cells are selected from among any mammalian species, including, without limitation, cells such as A549, WEHI, 3T3, 10T1/2, BHK, MDCK, COS 1, COS 7, BSC 1, BSC 40, BMT 10, VERO, WI38, HeLa, 293 cells (which express functional adenoviral E1), Saos, C2C12, L cells, HT1080, HepG2 and primary fibroblast, hepatocyte and myoblast cells derived from mammals including human, monkey, mouse, rat, rabbit, and hamster. The selection of the mammalian species providing the cells is not a limitation of this invention; nor is the type of mammalian cell, i.e., fibroblast, hepatocyte, tumor cell, etc. The requirements for the cell used is that it not carry any adenovirus gene other than E1, E2a and/or E4 ORF6; it not contain any other virus gene which could result in homologous recombination of a contaminating virus during the production of rAAV; and it is capable of infection or transfection of DNA and expression of the transfected DNA. In a preferred embodiment, the host cell is one that has rep and cap stably transfected in the cell.

One host cell useful in the present invention is a host cell stably transformed with the sequences encoding rep and cap, and which is transfected with the adenovirus E1, E2a, and E40RF6 DNA and a construct carrying the minigene as described above. Stable rep and/or cap expressing cell lines,

such as B-50 (PCT/US98/19463), or those described in U.S. Pat. No. 5,658,785, may also be similarly employed. Another desirable host cell contains the minimum adenoviral DNA which is sufficient to express E4 ORF6. Yet other cell lines can be constructed using the AAV9 rep and/or AAV9 cap sequences of the invention.

The preparation of a host cell according to this invention involves techniques such as assembly of selected DNA sequences. This assembly may be accomplished utilizing conventional techniques. Such techniques include cDNA and genomic cloning, which are well known and are described in Sambrook et al., cited above, use of overlapping oligonucleotide sequences of the adenovirus and AAV genomes, combined with polymerase chain reaction, synthetic methods, and any other suitable methods which provide the desired nucleotide sequence.

Introduction of the molecules (as plasmids or viruses) into the host cell may also be accomplished using techniques known to the skilled artisan and as discussed throughout the specification. In one embodiment, standard transfection techniques are used, e.g., $CaPO_4$ transfection or electroporation, and/or infection by hybrid adenovirus/AAV vectors into cell lines such as the human embryonic kidney cell line HEK 293 (a human kidney cell line containing functional adenovirus E1 genes which provides trans-acting E1 proteins).

The AAV9 based gene therapy vectors which are generated by one of skill in the art are beneficial for gene delivery to selected host cells and gene therapy patients since no neutralization antibodies to AAV9 have been found in the human population. One of skill in the art may readily prepare other rAAV viral vectors containing the AAV9 capsid proteins provided herein using a variety of techniques known to those of skill in the art. One may similarly prepare still other rAAV viral vectors containing AAV9 sequence and AAV capsids of another serotype.

Thus, one of skill in the art will readily understand that the AAV9 sequences of the invention can be readily adapted for use in these and other viral vector systems for in vitro, ex vivo or in vivo gene delivery. Similarly, one of skill in the art can readily select other fragments of the AAV9 genome of the invention for use in a variety of rAAV and non-rAAV vector systems. Such vectors systems may include, e.g., lentiviruses, retroviruses, poxviruses, vaccinia viruses, and adenoviral systems, among others. Selection of these vector systems is not a limitation of the present invention.

Thus, the invention further provides vectors generated using the nucleic acid and amino acid sequences of the novel AAV of the invention. Such vectors are useful for a variety of purposes, including for delivery of therapeutic molecules and for use in vaccine regimens. Particularly desirable for delivery of therapeutic molecules are recombinant AAV containing capsids of the novel AAV of the invention. These, or other vector constructs containing novel AAV sequences of the invention may be used in vaccine regimens, e.g., for co-delivery of a cytokine, or for delivery of the immunogen itself.

V. Recombinant Viruses and Uses Therefor

Using the techniques described herein, one of skill in the art can generate a rAAV having a capsid of a serotype 8 of the invention or having a capsid containing one or more fragments of AAV9. In one embodiment, a full-length capsid from a single serotype, e.g., AAV9 [SEQ ID NO: 2] can be utilized. In another embodiment, a full-length capsid may be generated which contains one or more fragments of AAV9 fused in frame with sequences from another selected AAV serotype, or from heterologous portions of AAV9. For example, a rAAV may contain one or more of the novel hypervariable region sequences of AAV9. Alternatively, the unique AAV9 sequences of the invention may be used in constructs containing other viral or non-viral sequences. Optionally, a recombinant virus may carry AAV9 rep sequences encoding one or more of the AAV9 rep proteins.

A. Delivery of Viruses

In another aspect, the present invention provides a method for delivery of a transgene to a host which involves transfecting or infecting a selected host cell with a recombinant viral vector generated with the AAV9 sequences (or functional fragments thereof) of the invention. Methods for delivery are well known to those of skill in the art and are not a limitation of the present invention.

In one desirable embodiment, the invention provides a method for AAV9-mediated delivery of a transgene to a host. This method involves transfecting or infecting a selected host cell with a recombinant viral vector containing a selected transgene under the control of sequences which direct expression thereof and AAV9 capsid proteins.

Optionally, a sample from the host may be first assayed for the presence of antibodies to a selected AAV serotype. A variety of assay formats for detecting neutralizing antibodies are well known to those of skill in the art. The selection of such an assay is not a limitation of the present invention. See, e.g., Fisher et al, *Nature Med.*, 3(3):306–312 (March 1997) and W. C. Manning et al, *Human Gene Therapy*, 9:477–485 (Mar. 1, 1998). The results of this assay may be used to determine which AAV vector containing capsid proteins of a particular serotype are preferred for delivery, e.g., by the absence of neutralizing antibodies specific for that capsid serotype.

In one aspect of this method, the delivery of vector with AAV9 capsid proteins may precede or follow delivery of a gene via a vector with a different serotype AAV capsid protein. Thus, gene delivery via rAAV vectors may be used for repeat gene delivery to a selected host cell. Desirably, subsequently administered rAAV vectors carry the same transgene as the first rAAV vector, but the subsequently administered vectors contain capsid proteins of serotypes which differ from the first vector. For example, if a first vector has AAV9 capsid proteins, subsequently administered vectors may have capsid proteins selected from among the other serotypes, including, without limitation, AAV1, AAV2, AAV3, AAV4, AAV8, AAV6, AAV7, and AAV8.

The above-described recombinant vectors may be delivered to host cells according to published methods. The rAAV, preferably suspended in a physiologically compatible carrier, may be administered to a human or non-human mammalian patient. Suitable carriers may be readily selected by one of skill in the art in view of the indication for which the transfer virus is directed. For example, one suitable carrier includes saline, which may be formulated with a variety of buffering solutions (e.g., phosphate buffered saline). Other exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, sesame oil, and water. The selection of the carrier is not a limitation of the present invention.

Optionally, the compositions of the invention may contain, in addition to the rAAV and carrier(s), other conventional pharmaceutical ingredients, such as preservatives, or chemical stabilizers. Suitable exemplary preservatives include chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, and parachlorophenol. Suitable chemical stabilizers include gelatin and albumin.

The viral vectors are administered in sufficient amounts to transfect the cells and to provide sufficient levels of gene transfer and expression to provide a therapeutic benefit without undue adverse effects, or with medically acceptable physiological effects, which can be determined by those skilled in the medical arts. Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, direct delivery to the liver or lung, orally, intranasally, intratracheally, by inhalation, intravenously, intramuscularly, intraocularly, subcutaneously, intradermally, or by other routes of administration. Routes of administration may be combined, if desired.

Dosages of the viral vector will depend primarily on factors such as the condition being treated, the age, weight and health of the patient, and may thus vary among patients. For example, a therapeutically effective human dosage of the viral vector is generally in the range of from about 1 ml to about 100 ml of solution containing concentrations of from about 1×10^9 to 1×10^{16} genomes virus vector. A preferred human dosage may be about 1×10^{13} to 1×10^{16} AAV genomes. The dosage will be adjusted to balance the therapeutic benefit against any side effects and such dosages may vary depending upon the therapeutic application for which the recombinant vector is employed. The levels of expression of the transgene can be monitored to determine the frequency of dosage resulting in viral vectors, preferably AAV vectors containing the minigene. Optionally, dosage regimens similar to those described for therapeutic purposes may be utilized for immunization using the compositions of the invention.

Examples of therapeutic products and immunogenic products for delivery by the AAV9-containing vectors of the invention are provided below. These vectors may be used for a variety of therapeutic or vaccinal regimens, as described herein. Additionally, these vectors may be delivered in combination with one or more other vectors or active ingredients in a desired therapeutic and/or vaccinal regimen.

B. Therapeutic Transgenes

Useful therapeutic products encoded by the transgene include hormones and growth and differentiation factors including, without limitation, insulin, glucagon, growth hormone (GH), parathyroid hormone (PTH), growth hormone releasing factor (GRF), follicle stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotropin (hCG), vascular endothelial growth factor (VEGF), angiopoietins, angiostatin, granulocyte colony stimulating factor (GCSF), erythropoietin (EPO), connective tissue growth factor (CTGF), basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin growth factors I and II (IGF-I and IGF-II), any one of the transforming growth factor a superfamily, including TGFα, activins, inhibins, or any of the bone morphogenic proteins (BMP) BMPs 1–15, any one of the heregluin/neuregulin/ARIA/neu differentiation factor (NDF) family of growth factors, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophins NT-3 and NT-4/5, ciliary neurotrophic factor (CNTF), glial cell line derived neurotrophic factor (GDNF), neurturin, agrin, any one of the family of semaphorins/collapsins, netrin-1 and netrin-2, hepatocyte growth factor (HGF), ephrins, noggin, sonic hedgehog and tyrosine hydroxylase.

Other useful transgene products include proteins that regulate the immune system including, without limitation, cytokines and lymphokines such as thrombopoietin (TPO), interleukins (IL) IL-1 through IL-25 (including, e.g., IL-2, IL-4, IL-6, IL-12 and IL-18], monocyte chemoattractant protein, leukemia inhibitory factor, granulocyte-macrophage colony stimulating factor, Fas ligand, tumor necrosis factors α and β, interferons α, β, and γ, stem cell factor, flk-2/flt3 ligand. Gene products produced by the immune system are also useful in the invention. These include, without limitations, immunoglobulins IgG, IgM, IgA, IgD and IgE, chimeric immunoglobulins, humanized antibodies, single chain antibodies, T cell receptors, chimeric T cell receptors, single chain T cell receptors, class I and class II MHC molecules, as well as engineered immunoglobulins and MHC molecules. Useful gene products also include complement regulatory proteins such as complement regulatory proteins, membrane cofactor protein (MCP), decay accelerating factor (DAF), CR1, CF2 and CD59.

Still other useful gene products include any one of the receptors for the hormones, growth factors, cytokines, lymphokines, regulatory proteins and immune system proteins. The invention encompasses receptors for cholesterol regulation, including the low density lipoprotein (LDL) receptor, high density lipoprotein (HDL) receptor, the very low density lipoprotein (VLDL) receptor, and the scavenger receptor. The invention also encompasses gene products such as members of the steroid hormone receptor superfamily including glucocorticoid receptors and estrogen receptors, Vitamin D receptors and other nuclear receptors. In addition, useful gene products include transcription factors such as jun, fos, max, mad, serum response factor (SRF), AP-1, AP2, myb, MyoD and myogenin, ETS-bbx containing proteins, TFE3, E2F, ATF1, ATF2, ATF3, ATF4, ZFS, NFAT, CREB, HNF-4, C/EBP, SP1, CCAAT-box binding proteins, interferon regulation factor (IRF-1), Wilms tumor protein, ETS-binding protein, STAT, GATA-box binding proteins, e.g., GATA-3, and the forkhead family of winged helix proteins.

Other useful gene products include, carbamoyl synthetase I, ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, argilase, fumarylacetacetate hydrolase, phenylalanine hydroxylase, alpha-1 antitrypsin, glucose-6-phosphatase, porphobilinogen deaminase, factor VIII, factor IX, cystathione beta-synthase, branched chain ketoacid decarboxylase, albumin, isovaleryl-coA dehydrogenase, propionyl CoA carboxylase, methyl malonyl CoA mutase, glutaryl CoA dehydrogenase, insulin, beta-glucosidase, pyruvate carboxylate, hepatic phosphorylase, phosphorylase kinase, glycine decarboxylase, H-protein, T-protein, a cystic fibrosis transmembrane regulator (CFTR) sequence, and a dystrophin cDNA sequence. Still other useful gene products include enzymes such as may be useful in enzyme replacement therapy, which is useful in a variety of conditions resulting from deficient activity of enzyme. For example, enzymes that contain mannose-6-phosphate may be utilized in therapies for lysosomal storage diseases (e.g., a suitable gene includes that encoding β-glucuronidase (GUSB)).

Other useful gene products include non-naturally occurring polypeptides, such as chimeric or hybrid polypeptides having a non-naturally occurring amino acid sequence containing insertions, deletions or amino acid substitutions. For example, single-chain engineered immunoglobulins could be useful in certain immunocompromised patients. Other types of non-naturally occurring gene sequences include antisense molecules and catalytic nucleic acids, such as ribozymes, which could be used to reduce overexpression of a target.

Reduction and/or modulation of expression of a gene is particularly desirable for treatment of hyperproliferative conditions characterized by hyperproliferating cells, as are cancers and psoriasis. Target polypeptides include those

polypeptides which are produced exclusively or at higher levels in hyperproliferative cells as compared to normal cells. Target antigens include polypeptides encoded by oncogenes such as myb, myc, fyn, and the translocation gene bcr/abl, ras, src, P53, neu, trk and EGRF. In addition to oncogene products as target antigens, target polypeptides for anti-cancer treatments and protective regimens include variable regions of antibodies made by B cell lymphomas and variable regions of T cell receptors of T cell lymphomas which, in some embodiments, are also used as target antigens for autoimmune disease. Other tumor-associated polypeptides can be used as target polypeptides such as polypeptides which are found at higher levels in tumor cells including the polypeptide recognized by monoclonal antibody 17-IA and folate binding polypeptides.

Other suitable therapeutic polypeptides and proteins include those which may be useful for treating individuals suffering from autoimmune diseases and disorders by conferring a broad based protective immune response against targets that are associated with autoimmunity including cell receptors and cells which produce "self"-directed antibodies. T cell mediated autoimmune diseases include Rheumatoid arthritis (RA), multiple sclerosis (MS), Sjögren's syndrome, sarcoidosis, insulin dependent diabetes mellitus (IDDM), autoimmune thyroiditis, reactive arthritis, ankylosing spondylitis, scleroderma, polymyositis, dermatomyositis, psoriasis, vasculitis, Wegener's granulomatosis, Croln's disease and ulcerative colitis. Each of these diseases is characterized by T cell receptors (TCRs) that bind to endogenous antigens and initiate the inflammatory cascade associated with autoimmune diseases.

C. Immunogenic Transgenes

Alternatively, or in addition, the vectors of the invention may contain AAV9 sequences of the invention and a transgene encoding a peptide, polypeptide or protein which induces an immune response to a selected immunogen. For example, immunogens may be selected from a variety of viral families. Example of desirable viral families against which an immune response would be desirable include, the picornavirus family, which includes the genera *rhinoviruses*, which are responsible for about 50% of cases of the common cold; the genera *enteroviruses*, which include *polioviruses, coxsackieviruses, echoviruses*, and human *enteroviruses* such as hepatitis A virus; and the genera *apthoviruses*, which are responsible for foot and mouth diseases, primarily in non-human animals. Within the picornavirus family of viruses, target antigens include the VP1, VP2, VP3, VP4, and VPG. Another viral family includes the calcivirus family, which encompasses the Norwalk group of viruses, which are an important causative agent of epidemic gastroenteritis. Still another viral family desirable for use in targeting antigens for inducing immune responses in humans and non-human animals is the togavirus family, which includes the genera *alphavirus*, which include Sindbis viruses, Ross-River virus, and Venezuelan, Eastern & Western Equine encephalitis, and rubivirus, including Rubella virus. The flaviviridae family includes dengue, yellow fever, Japanese encephalitis, St. Louis encephalitis and tick borne encephalitis viruses. Other target antigens may be generated from the Hepatitis C or the coronavirus family, which includes a number of non-human viruses such as infectious bronchitis virus (poultry), porcine transmissible gastroenteric virus (pig), porcine hemagglutinating encephalomyelitis virus (pig), feline infectious peritonitis virus (cats), feline enteric coronavirus (cat), canine coronavirus (dog), and human respiratory coronaviruses, which may cause the common cold and/or non-A, B or C hepatitis. Within the coronavirus family, target antigens include the E1 (also called M or matrix protein), E2 (also called S or Spike protein), E3 (also called HE or hemagglutin-elterose) glycoprotein (not present in all coronaviruses), or N (nucleocapsid). Still other antigens may be targeted against the rhabdovirus family, which includes the genera *vesiculovirus* (e.g., Vesicular Stomatitis Virus), and the general lyssavirus (e.g., rabies). Within the rhabdovirus family, suitable antigens may be derived from the G protein or the N protein. The family filoviridae, which includes hemorrhagic fever viruses such as Marburg and Ebola virus may be a suitable source of antigens. The paramyxovirus family includes parainfluenza Virus Type 1, parainfluenza Virus Type 3, bovine parainfluenza Virus Type 3, rubulavirus (mumps virus, parainfluenza Virus Type 2, parainfluenza virus Type 4, Newcastle disease virus (chickens), rinderpest, morbillivirus, which includes measles and canine distemper, and pneumovirus, which includes respiratory syncytial virus. The influenza virus is classified within the family orthomyxovirus and is a suitable source of antigen (e.g., the HA protein, the N1 protein). The bunyavirus family includes the genera *bunyavirus* (California encephalitis, La Crosse), *phlebovirus* (Rift Valley Fever), *hantavirus* (puremala is a hemahagin fever virus), *nairovirus* (Nairobi sheep disease) and various unassigned bungaviruses. The arenavirus family provides a source of antigens against LCM and Lassa fever virus. The reovirus family includes the genera *reovirus, rotavirus* (which causes acute gastroenteritis in children), *orbiviruses*, and *cultivirus* (Colorado Tick fever, Lebombo (humans), equine encephalosis, blue tongue). The retrovirus family includes the subfamily oncorivirinal which encompasses such human and veterinary diseases as feline leukemia virus, HTLVI and HTLVII, lentivirinal (which includes HIV, simian immunodeficiency virus, feline immunodeficiency virus, equine infectious anemia virus, and spuinavirinal). The papovavirus family includes the sub-family polyomaviruses (BKU and JCU viruses) and the sub-family papillomavirus (associated with cancers or malignant progression of papilloma). The adenovirus family includes viruses (EX, AD7, ARD, O.B.) which cause respiratory disease and/or enteritis. The parvovirus family feline parvovirus (feline enteritis), feline panleucopeniavirus, canine parvovirus, and porcine parvovirus. The herpesvirus family includes the sub-family alphaherpesvirinae, which encompasses the genera *simplexvirus* (HSVI, HSVII), *varicellovirus* (pseudorabies, *varicella zoster*) and the sub-family betaherpesvirinae, which includes the genera *cytomegalovirus* (HCMV, *muromegalovirus*) and the sub-family gammaherpesvirinae, which includes the genera *lymphocryptovirus*, EBV (Burkitts lymphoma), infectious rhinotracheitis, Marek's disease virus, and rhadinovirus. The poxvirus family includes the sub-family chordopoxyirinae, which encompasses the genera *orthopoxvirus* (Variola major (Smallpox) and *Vaccinia* (Cowpox)), *parapoxvirus, avipoxvirus, leporipoxvirus, suipoxvirus*, and the sub-family entomopoxyirinae. The hepadnavirus family includes the Hepatitis B virus. One unclassified virus which may be suitable source of antigens is the Hepatitis delta virus. Another virus which is a source of antigens is Nipan Virus. Still other viral sources may include avian infectious bursal disease virus and porcine respiratory and reproductive syndrome virus. The alphavirus family includes equine arteritis virus and various Encephalitis viruses.

The present invention may also encompass immunogens which are useful to immunize a human or non-human animal against other pathogens including bacteria, fungi, parasitic microorganisms or multicellular parasites which infect human and non-human vertebrates, or from a cancer cell or

tumor cell. Examples of bacterial pathogens include pathogenic gram-positive cocci include *pneumococci; staphylococci* (and the toxins produced thereby, e.g., enterotoxin B); and *streptococci*. Pathogenic gram-negative cocci include meningococcus; gonococcus. Pathogenic enteric gram-negative *bacilli* include enterobacteriaceae; *pseudomonas, acinetobacteria* and *eikenella; melioidosis; salmonella; shigella; haemophilus; moraxella; H. ducreyi* (which causes chancroid); *brucella* species (brucellosis); *Francisella tularensis* (which causes tularemia); *Yersinia pestis* (plague) and other *yersinia* (*pasteurella*); *streptobacillus moniliformis* and *spirillum*; Gram-positive *bacilli* include *listeria monocytogenes; erysipelothrix rhusiopathiae; Corynebacterium diphtheria* (diphtheria); *cholera; B. anthracis* (anthrax); donovanosis (granuloma inguinale); and bartonellosis. Diseases caused by pathogenic anaerobic bacteria include tetanus; botulism (*Clostriduin botulimum* and its toxin); *Clostridium peifringens* and its epsilon toxin; other *clostridia*; tuberculosis; leprosy; and other mycobacteria. Pathogenic spirochetal diseases include syphilis; treponematoses: yaws, pinta and endemic syphilis; and leptospirosis. Other infections caused by higher pathogen bacteria and pathogenic fungi include glanders (*Burkholderia* mallet); actinomycosis; nocardiosis; cryptococcosis, blastomycosis, histoplasmosis and coccidioidomycosis; candidiasis, aspergillosis, and mucormycosis; sporotrichosis; paracoccidiodomycosis, petriellidiosis, torulopsosis, mycetoma and chromomycosis; and dermatophytosis. Rickettsial infections include Typhus fever, Rocky Mountain spotted fever, Q fever (*Coxiella burnetti*), and Rickettsialpox. Examples of mycoplasma and chlamydial infections include: mycoplasma pneumoniae; lymphogranuloma venereum; psittacosis; and perinatal chlamydial infections. Pathogenic eukaryotes encompass pathogenic protozoans and helminths and infections produced thereby include: amebiasis; malaria; leishmaniasis; trypanosomiasis; toxoplasmosis; *Pneumocystis carinii; Trichans; Toxoplasma gondii*; babesiosis; giardiasis; trichinosis; filariasis; schistosomiasis; nematodes; trematodes or flukes; and cestode (tapeworm) infections.

Many of these organisms and/or the toxins produced thereby have been identified by the Centers for Disease Control [(CDC), Department of Heath and Human Services, USA], as agents which have potential for use in biological attacks. For example, some of these biological agents, include, *Bacillus anthracis* (anthrax), *Clostridium botulinum* and its toxin (botulism), *Yersinia pestis* (plague), variola major (smallpox), *Francisella tularensis* (tularemia), and viral hemorrhagic fevers [filoviruses (e.g., Ebola, Marburg], and arenaviruses [e.g., Lassa, Machupo]), all of which are currently classified as Category A agents; *Coxiella burnetti* (Q fever); *Brucella* species (brucellosis), *Burkholderia mallei* (glanders), *Burkholderia pseudomallei* (meloidosis), *Ricinus communis* and its toxin (ricin toxin), *Clostridium perfringens* and its toxin (epsilon toxin), *Staphylococcus* species and their toxins (enterotoxin B), *Chlamydia psittaci* (psittacosis), water safety threats (e.g., *Vibrio cholerae, Crytosporidium parvum*), Typhus fever (*Richettsia powazekii*), and viral encephalitis (alphaviruses, e.g., Venezuelan equine encephalitis; eastern equine encephalitis; western equine encephalitis); all of which are currently classified as Category B agents; and Nipan virus and hantaviruses, which are currently classified as Category C agents. In addition, other organisms, which are so classified or differently classified, may be identified and/or used for such a purpose in the future. It will be readily understood that the viral vectors and other constructs described herein are useful to deliver antigens from these organisms, viruses, their toxins or other by-products, which will prevent and/or treat infection or other adverse reactions with these biological agents.

Administration of the vectors of the invention to deliver immunogens against the variable region of the T cells elicit an immune response including CTLs to eliminate those T cells. In rheumatoid arthritis (RA), several specific variable regions of T cell receptors (TCRs) which are involved in the disease have been characterized. These TCRs include V-3, V-14, V-17 and V-17. Thus, delivery of a nucleic acid sequence that encodes at least one of these polypeptides will elicit an immune response that will target T cells involved in RA. In multiple sclerosis (MS), several specific variable regions of TCRs which are involved in the disease have been characterized. These TCRs include V-7 and V-10. Thus, delivery of a nucleic acid sequence that encodes at least one of these polypeptides will elicit an immune response that will target T cells involved in MS. In scleroderma, several specific variable regions of TCRs which are involved in the disease have been characterized. These TCRs include V-6, V-8, V-14 and V-16, V-3C, V-7, V-14, V-15, V-16, V-28 and V-12. Thus, delivery of a nucleic acid molecule that encodes at least one of these polypeptides will elicit an immune response that will target T cells involved in scleroderma.

Thus, a rAAV9-derived recombinant viral vector of the invention provides an efficient gene transfer vehicle which can deliver a selected transgene to a selected host cell in vivo or ex vivo even where the organism has neutralizing antibodies to one or more AAV serotypes. In one embodiment, the rAAV and the cells are mixed ex vivo; the infected cells are cultured using conventional methodologies; and the transduced cells are re-infused into the patient.

The vectors of the invention are particularly well suited to gene delivery for therapeutic purposes and for immunization, including inducing protective immunity. Further, the vectors of the invention may also be used for production of a desired gene product in vitro. For in vitao production, a desired product (e.g., a protein) may be obtained from a desired culture following transfection of host cells with a rAAV containing the molecule encoding the desired product and culturing the cell culture under conditions which permit expression. The expressed product may then be purified and isolated, as desired. Suitable techniques for transfection, cell culturing, purification, and isolation are known to those of skill in the art.

The following examples illustrate several aspects and embodiments of the invention.

EXAMPLES

Example 1

Production of Recombinant AAV9 Viral Genomes Equipped with AAV2 ITRs

Chimeric packaging constructs are generated by fusing AAV2 rep with cap sequences of novel AAV serotypes. These chimeric packaging constructs are used, initially, for pseudotyping recombinant AAV genomes carrying AAV2 ITRs by triple transfection in 293 cell using Ad5 helper plasmid. These pseudotyped vectors are used to evaluate performance in transduction-based serological studies and evaluate gene transfer efficiency of novel AAV serotypes in different animal models including NHP and rodents, before intact and infectious viruses of these novel serotypes are isolated.

A. pAA V2GFP

The AAV2 plasmid which contains the AAV2 ITRs and green fluorescent protein expressed under the control of a constitutive promoter. This plasmid contains the following elements: the AAV2 ITRs, a CMV promoter, the GFP coding sequences,

B. Cloning of Trans Plasmid

To construct the chimeric trans-plasmid for production of recombinant pseudotyped AAV9 vectors, pSE18 plasmid (Xiao et al., 1999, *J. Virol* 73:3994–4003) was partially digested with Xho I to linearize the plasmid at the Xho I site at the position of 3169 bp only. The Xho I cut ends were then filled in and ligated back. This modified p5E18 plasmid was restricted with Xba I and Xho I in a complete digestion to remove the AAV2 cap gene sequence and replaced with a 2267 bp Spe I/Xho I fragment containing the AAV9 cap gene which was isolated from pCRAAV9 6-5+15-4 plasmid.

The resulting plasmid contains the AAV2 rep sequences for Rep78/68 under the control of the AAV2 P5 promoter, and the AAV2 rep sequences for Rep52/40 under the control of the AAV2 P19 promoter. The AAV9 capsid sequences are under the control of the AAV2 P40 promoter, which is located within the Rep sequences. This plasmid further contains a spacer 5' of the rep ORF.

Alternatively, a similar plasmid can be constructed which utilizes the AAV9 rep sequences and the native AAV9 promoter sequences. This plasmid is then used for production of rAAV9, as described herein.

C. Production of Pseudotyped rAAV

The rAAV particles (AAV2 vector in AAV9 capsid) are generated using an adenovirus-free method. Briefly, the cis plasmid (pAAV2.1 lacZ plasmid containing AAV2 ITRs), and the trans plasmid pCRAAV9 6-5+15-4 (containing the AAV2 rep and AAV9cap) and a helper plasmid, respectively, are simultaneously co-transfected into 293 cells in a ratio of 1:1:2 by calcium phosphate precipitation.

For the construction of the pAd helper plasmids, pBG10 plasmid was purchased from Microbix (Canada). A RsrII fragment containing L2 and L3 is deleted from pBHG10, resulting in the first helper plasmid, pAd F13. Plasmid Ad F1 was constructed by cloning Asp700/SalI fragment with a PmeI/SgfI deletion, isolating from pBHG10, into Bluescript. MLP, L2, L2 and L3 were deleted in the pAd F1. Further deletions of a 2.3 kb NruI fragment and, subsequently, a 0.5 kb RsrII/NruI fragment generated helper plasmids pAd F5 and pAd F6, respectively. The helper plasmid, termed p F6, provides the essential helper functions of E2a and E4 ORF6 not provided by the E1-expressing helper cell, but is deleted of adenoviral capsid proteins and functional E1 regions).

Typically, 50 μg of DNA (cis:trans:helper) is transfected onto a 150 mm tissue culture dish. The 293 cells are harvested 72 hours post-transfection, sonicated and treated with 0.5% sodium deoxycholate (37 C for 10 min.) Cell lysates are then subjected to two rounds of a CsCl gradient. Peak fractions containing rAAV vector are collected, pooled and dialyzed against PBS.

Example 2

Mouse Model of Familial Hypercholesterolemia

The following experiment will demonstrate that the AAV2/9 rAAV constructed as described herein delivers the LDL receptor and expresses LDL receptor in an amount sufficient to reduce the levels of plasma cholesterol and triglycerides in animal models of familial hypercholesterolemia.

The rAAV2/9 constructs used in the following experiment contain the cDNA for murine VLDL receptor (LDLR) driven from a β-actin promoter and enhanced from the cytomegalovirus enhancer (AAV-CB-VLDLR). LDL receptor-deficient mice (Jackson Laboratories) on a high cholesterol diet which exhibit the symptoms of familial hypercholesterolemia, (FH mice) are infused intravenously with 4×10^{12} AAV-CB-LDLR or a saline control and the plasma cholesterol level was monitored.

The plasma cholesterol and triglyceride profiles of FH mice infused with VLDLR virus are determined as follows. The mice treated as described above are fasted for six (6) hours, and blood samples collected by retro-orbital venous plexus puncture with heparinized capillary tubes. Plasma is separated by centrifugation. The concentration of total cholesterol and triglyceride is determined using kits purchased from Wako Chemicals.

Mice in the control group show gradually increased cholesterol levels until reaching a steady state in about 4 weeks post injection. In mice receiving a LDLR virus, plasma cholesterol levels steadily decrease starting 3 weeks post-virus administration.

A cholesterol profile of plasma from the FH mice infused with LDLR virus is obtained by FPLC analysis. Mouse plasma is pooled from 4 mice in each group. Plasma samples are isolated from control and AAV-VLDLR infused animals at pre-injection (PI) or 2 months (2M) post virus infusion and are analyzed by FPLC fractionation followed by cholesterol assay.

Example 3

In vivo Transduction with AAV9 Serotype Vectors

The performance of vector based on the new AAV9 serotype is evaluated in murine models of muscle and liver-directed gene transfer and compared to vectors based on the known serotypes AAV1, AAV2 and AAV5. Vectors expressing secreted proteins are used to quantitate relative transduction efficiencies between different serotypes through ELISA analysis of sera. The cellular distribution of transduction within the target organ is evaluated using lacZ expressing vectors and X-gal histochemistry.

For this experiment, recombinant AAV genomes, AAV2CBhA1AT, AAV2A1bhA1AT, AAV2CMVrhCG, AAV2TBGrhCG, AAV2TBGcFIX, AAV2CMVLacZ and AAV2TBGLacZ are packaged with AAV9, or AAV1, AAV2, or AAV5 capsid proteins. In all constructs, minigene cassettes are flanked with AAV2 ITRs. cDNAs of human α-antitrypsin (A1AT) [Xiao, W., et al., (1999) *J Virol* 73, 3994–4003] β-subunit of rhesus monkey choriogonadotropic hormone (CG) [Zoltick, P. W. & Wilson, J. M. (2000) *Mol Ther* 2, 657–9] canine factor IX [Wang, L., et al., (1997) *Proc Natl Acad Sci USA* 94, 11563–6] and bacterial β-galactosidase (i.e., Lac Z) genes are used as reporter genes. For liver-directed gene transfer, either mouse albumin gene promoter (Alb) [Xiao, W. (1999), cited above] or human thyroid hormone binding globulin gene promoter (TBG) [Wang (1997), cited above] is used to drive liver specific expression of reporter genes. In muscle-directed gene transfer experiments, either cytomegalovirus early promoter (CMV) or chicken β-actin promoter with CMV enhancer (CB) is employed to direct expression of reporters.

For muscle-directed gene transfer, vectors are injected into the right tibialis anterior of 4–6 week old NCR nude or C57BL/6 mice (Taconic, Germantown, N.Y.). In liver-directed gene transfer studies, vectors are infused intiaportally

into 7–9 week old NCR nude or C57BL/6 mice (Taconic, Gerinantown, N.Y.). Serum samples are collected intraorbitally at different time points after vector administration. Muscle and liver tissues are harvested at different time points for cryosectioning and Xgal histochemical staining from animals that received the lacZ vectors. For the re-administration experiment, C56BL/6 mice initially received AAV2/1, 2/2, 2/5, and 2/9 CBA1AT vectors intramuscularly and followed for A1AT gene expression for 7 weeks. Previous studies indicated that immune competent C57BL/6 mice elicit limited humoral responses to the human A1AT protein when expressed from AAV vectors [Xiao, W., et al., (1999) *J Virol* 73, 3994–4003]. Animals are then treated with AAV2/9 TBGcFIX intraportally and studied for cFIX gene expression.

ELISA based assays are performed to quantify serum levels of hA1AT, rhCG and cFIX proteins as described previously [Gao, G. P., et al., (1996) *J Virol* 70, 8934–43; Zoltick, P. W. & Wilson, J. M. (2000) *Mol Ther* 2, 657–9; Wang, L., et al., *Proc Natl Acad Sci USA* 94, 11563–6]. The experiments are completed when animals are sacrificed for harvest of muscle and liver tissues for DNA extraction and quantitative analysis of genome copies of vectors present in target tissues by TaqMan using the same set of primers and probe as in titration of vector preparations [Zhang, Y., et al., (2001) *Mol Ther* 3, 697–707].

All publications cited in this specification are incorporated herein by reference. While the invention has been described with reference to particularly preferred embodiments, it will be appreciated that modifications can be made without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the claims.

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gggtcagaga ctgctcagag tgtttccccg gcgtgtcaga atctcaaccg gtcgtcagaa   1980
agaggacgta tcggaaactc tgtgcgattc atcatctgct ggggcgggct cccgagattg   2040
cttgctcggc ctgcgatctg gtcaacgtgg acctggatga ctgtgtttct gagcaataaa   2100
tgacttaaac caggtatggc tgccgatggt tatcttccag attggctcga ggacaacctc   2160
tctgagggca ttcgcgagtg gtgggacctg aaacctggag ccccgaaacc caaagccaac   2220
cagcaaaagc aggacgacgg ccggggtctg gtgcttcctg gctacaagta cctcggaccc   2280
ttcaacggac tcgacaaggg ggagcccgtc aacgcggcgg acgcagcggc cctcgagcac   2340
gacaaggcct acgaccagca gctcaaagcg ggtgacaatc cgtacctgcg gtataaccac   2400
gccgacgccg agtttcagga gcgtctgcaa gaagatacgt cttttggggg caacctcggg   2460
cgagcagtct tccaggccaa gaagcgggtt ctcgaacctc tcggtctggt tgaggaaggc   2520
gctaagacgg ctcctggaaa gaagagaccg gtagagcagt caccccaaga accagactca   2580
tcctcgggca tcggcaaatc aggccagcag cccgctaaaa agagactcaa ttttggtcag   2640
actggcgact cagagtcagt ccccgaccca caacctctcg gagaacctcc agaagccccc   2700
tcaggtctgg gacctaatac aatggcttca ggcggtggcg ctccaatggc agacaataac   2760
gaaggcgccg acggagtggg taattcctcg ggaaattggc attgcgattc cacatggctg   2820
ggggacagag tcatcaccac cagcacccga acctgggcat tgcccaccta caacaaccac   2880
ctctacaagc aaatctccaa tggaacatcg ggaggaagca ccaacgacaa cacctacttt   2940
ggctacagca ccccctgggg gtattttgac ttcaacagat tccactgcca cttctcacca   3000
cgtgactggc agcgactcat caacaacaac tggggattcc ggccaaagag actcaacttc   3060
aagctgttca acatccaggt caaggaggtt acgacgaacg aaggcaccaa gaccatcgcc   3120
aataacctta ccagcaccgt ccaggtcttt acggactcgg agtaccagct accgtacgtc   3180
ctaggctctg cccaccaagg atgcctgcca ccgtttcctg cagacgtctt catggttcct   3240
cagtacggct acctgacgct caacaatgga agtcaagcgt taggacgttc ttctttctac   3300
tgtctggaat acttcccttc tcagatgctg agaaccggca acaactttca gttcagctac   3360
actttcgagg acgtgccttt ccacagcagc tacgcacaca gccagagtct agatcgactg   3420
atgaaccccc tcatcgacca gtacctatac tacctggtca gaacacagac aactggaact   3480
gggggaactc aaactttggc attcagccaa gcaggcccta gctcaatggc caatcaggct   3540
agaaactggg tacccgggcc ttgctaccgt cagcagcgcg tctccacaac caccaaccaa   3600
aataacaaca gcaactttgc gtggacggga gctgctaaat tcaagctgaa cgggagagac   3660
tcgctaatga atcctggcgt ggctatggca tcgcacaaag acgacgagga ccgcttcttt   3720
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ccatcaagtg gcgttctcat atttggcaag caaggagccg ggaacgatgg agtcgactac   3780

agccaggtgc tgattacaga tgaggaagaa attaaagcca ccaaccctgt agccacagag   3840

gaatacggag cagtggccat caacaaccag gccgctaaca cgcaggcgca aactggactt   3900

gtgcataacc agggagttat tcctggtatg gtctggcaga accgggacgt gtacctgcag   3960

ggccctattt gggctaaaat acctcacaca gatggcaact ttcacccgtc tcctctgatg   4020

ggtggatttg gactgaaaca cccacctcca cagattctaa ttaaaaatac accagtgccg   4080

gcagatcctc ctcttacctt caatcaagcc aagctgaact ctttcatcac gcagtacagc   4140

acgggacaag tcagcgtgga aatcgagtgg gagctgcaga aagaaaacag caagcgctgg   4200

aatccagaga tccagtatac ttcaaactac tacaaatcta caaatgtgga ctttgctgtc   4260

aataccgaag gtgtttactc tgagcctcgc cccattggta ctcgttacct cacccgtaat   4320

ttgtaattgc ctgttaatca ataaaccggt taattcgttt cagttgaact ttggtctctg   4380

cg                                                                  4382
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<210> SEQ ID NO 2
<211> LENGTH: 736
<212> TYPE: PRT
<213> ORGANISM: capsid protein of adeno-associated virus serotype 9

<400> SEQUENCE: 2

```
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1               5                   10                  15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
            20                  25                  30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
        35                  40                  45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
                85                  90                  95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
145                 150                 155                 160

Lys Ser Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
            180                 185                 190

Glu Ala Pro Ser Gly Leu Gly Pro Asn Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ser
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255
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Tyr Lys Gln Ile Ser Asn Gly Thr Ser Gly Gly Ser Thr Asn Asp Asn
            260                 265                 270

Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg
        275                 280                 285

Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn
    290                 295                 300

Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile
305                 310                 315                 320

Gln Val Lys Glu Val Thr Thr Asn Glu Gly Thr Lys Thr Ile Ala Asn
                325                 330                 335

Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu
            340                 345                 350

Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro
        355                 360                 365

Ala Asp Val Phe Met Val Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn
    370                 375                 380

Gly Ser Gln Ala Leu Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe
385                 390                 395                 400

Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Thr
                405                 410                 415

Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu
            420                 425                 430

Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Val
        435                 440                 445

Arg Thr Gln Thr Thr Gly Thr Gly Gly Thr Gln Thr Leu Ala Phe Ser
    450                 455                 460

Gln Ala Gly Pro Ser Ser Met Ala Asn Gln Ala Arg Asn Trp Val Pro
465                 470                 475                 480

Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Thr Thr Thr Asn Gln Asn
                485                 490                 495

Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Ala Lys Phe Lys Leu Asn
            500                 505                 510

Gly Arg Asp Ser Leu Met Asn Pro Gly Val Ala Met Ala Ser His Lys
        515                 520                 525

Asp Asp Glu Asp Arg Phe Phe Pro Ser Ser Gly Val Leu Ile Phe Gly
    530                 535                 540

Lys Gln Gly Ala Gly Asn Asp Gly Val Asp Tyr Ser Gln Val Leu Ile
545                 550                 555                 560

Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn Pro Val Ala Thr Glu Glu
                565                 570                 575

Tyr Gly Ala Val Ala Ile Asn Asn Gln Ala Ala Asn Thr Gln Ala Gln
            580                 585                 590

Thr Gly Leu Val His Asn Gln Gly Val Ile Pro Gly Met Val Trp Gln
        595                 600                 605

Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
    610                 615                 620

Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
625                 630                 635                 640

Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
                645                 650                 655

Asp Pro Pro Leu Thr Phe Asn Gln Ala Lys Leu Asn Ser Phe Ile Thr
            660                 665                 670
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Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
        675                 680                 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn
    690                 695                 700

Tyr Tyr Lys Ser Thr Asn Val Asp Phe Ala Val Asn Thr Glu Gly Val
705                 710                 715                 720

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


<210> SEQ ID NO 3
<211> LENGTH: 623
<212> TYPE: PRT
<213> ORGANISM: rep protein of adeno-associated virus serotype 9

<400> SEQUENCE: 3

Met Pro Gly Phe Tyr Glu Ile Val Ile Lys Val Pro Ser Asp Leu Asp
1               5                   10                  15

Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Asn Trp Val Ala Glu
            20                  25                  30

Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Arg Asn Leu Ile
        35                  40                  45

Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
    50                  55                  60

Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
65                  70                  75                  80

Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Leu His Val Leu Val Glu
                85                  90                  95

Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
            100                 105                 110

Arg Glu Lys Leu Val Gln Thr Ile Tyr Arg Gly Ile Glu Pro Thr Leu
        115                 120                 125

Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
    130                 135                 140

Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
145                 150                 155                 160

Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Glu Tyr Ile
                165                 170                 175

Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
            180                 185                 190

Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Leu Asn
        195                 200                 205

Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
    210                 215                 220

Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
225                 230                 235                 240

Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
                245                 250                 255

Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
            260                 265                 270

Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ser
        275                 280                 285

Leu Pro Val Asp Ile Thr Gln Asn Arg Ile Tyr Arg Ile Leu Gln Leu
    290                 295                 300

Asn Gly Tyr Asp Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
305                 310                 315                 320
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Gln Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
                325                 330                 335

Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
            340                 345                 350

Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
        355                 360                 365

Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
    370                 375                 380

Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
385                 390                 395                 400

Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
                405                 410                 415

Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
            420                 425                 430

Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
        435                 440                 445

Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
    450                 455                 460

Glu Val Lys Glu Phe Phe Arg Trp Ala Ser Asp His Val Thr Glu Val
465                 470                 475                 480

Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Ser Lys Arg Pro Ala
                485                 490                 495

Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
            500                 505                 510

Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
        515                 520                 525

Asp Arg Tyr Gln Asn Lys Cys Ser Arg His Ala Gly Met Leu Gln Met
    530                 535                 540

Leu Leu Pro Cys Lys Thr Cys Glu Arg Met Asn Gln Asn Phe Asn Ile
545                 550                 555                 560

Cys Phe Thr His Gly Val Arg Asp Cys Ser Glu Cys Phe Pro Gly Val
                565                 570                 575

Ser Glu Ser Gln Pro Val Val Arg Lys Arg Thr Tyr Arg Lys Leu Cys
            580                 585                 590

Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys Ser Ala
        595                 600                 605

Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu Gln
    610                 615                 620


<210> SEQ ID NO 4
<211> LENGTH: 735
<212> TYPE: PRT
<213> ORGANISM: adeno-associated virus serotype 2

<400> SEQUENCE: 4

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Thr Leu Ser
1               5                   10                  15

Glu Gly Ile Arg Gln Trp Trp Lys Leu Lys Pro Gly Pro Pro Pro Pro
            20                  25                  30

Lys Pro Ala Glu Arg His Lys Asp Asp Ser Arg Gly Leu Val Leu Pro
        35                  40                  45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60

Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
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65                  70                  75                  80

Arg Gln Leu Asp Ser Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
                85                  90                  95

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

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Glu Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

Pro Val Glu His Ser Pro Val Glu Pro Asp Ser Ser Ser Gly Thr Gly
145                 150                 155                 160

Lys Ala Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

Gly Asp Ala Asp Ser Val Pro Asp Pro Gln Pro Leu Gly Gln Pro Pro
            180                 185                 190

Ala Ala Pro Ser Gly Leu Gly Thr Asn Thr Met Ala Thr Gly Ser Gly
        195                 200                 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ser
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Thr Trp Met Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

Tyr Lys Gln Ile Ser Ser Gln Ser Gly Ala Ser Asn Asp Asn His Tyr
            260                 265                 270

Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe His
        275                 280                 285

Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn Trp
    290                 295                 300

Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln Val
305                 310                 315                 320

Lys Glu Val Thr Gln Asn Asp Gly Thr Thr Thr Ile Ala Asn Asn Leu
                325                 330                 335

Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu Pro Tyr
            340                 345                 350

Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala Asp
        355                 360                 365

Val Phe Met Val Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly Ser
    370                 375                 380

Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro Ser
385                 390                 395                 400

Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe Glu
                405                 410                 415

Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp Arg
            420                 425                 430

Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser Arg Thr
        435                 440                 445

Asn Thr Pro Ser Gly Thr Thr Thr Gln Ser Arg Leu Gln Phe Ser Gln
    450                 455                 460

Ala Gly Ala Ser Asp Ile Arg Asp Gln Ser Arg Asn Trp Leu Pro Gly
465                 470                 475                 480

Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Ser Ala Asp Asn Asn
                485                 490                 495
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Asn Ser Glu Tyr Ser Trp Thr Gly Ala Thr Lys Tyr His Leu Asn Gly
            500                 505                 510

Arg Asp Ser Leu Val Asn Pro Gly Pro Ala Met Ala Ser His Lys Asp
        515                 520                 525

Asp Glu Glu Lys Phe Phe Pro Gln Ser Gly Val Leu Ile Phe Gly Lys
    530                 535                 540

Gln Gly Ser Glu Lys Thr Asn Val Asp Ile Glu Lys Val Met Ile Thr
545                 550                 555                 560

Asp Glu Glu Glu Ile Arg Thr Thr Asn Pro Val Ala Thr Glu Gln Tyr
                565                 570                 575

Gly Ser Val Ser Thr Asn Leu Gln Arg Gly Asn Arg Gln Ala Ala Thr
            580                 585                 590

Ala Asp Val Asn Thr Gln Gly Val Leu Pro Gly Met Val Trp Gln Asp
        595                 600                 605

Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His Thr
    610                 615                 620

Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu Lys
625                 630                 635                 640

His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala Asn
                645                 650                 655

Pro Ser Thr Thr Phe Ser Ala Ala Lys Phe Ala Ser Phe Ile Thr Gln
            660                 665                 670

Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln Lys
        675                 680                 685

Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn Tyr
    690                 695                 700

Asn Lys Ser Val Asn Val Asp Phe Thr Val Asp Thr Asn Gly Val Tyr
705                 710                 715                 720

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


<210> SEQ ID NO 5
<211> LENGTH: 736
<212> TYPE: PRT
<213> ORGANISM: adeno-associated virus serotype 1

<400> SEQUENCE: 5

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1               5                   10                  15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
            20                  25                  30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
        35                  40                  45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
                85                  90                  95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
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    130                 135                 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
145                 150                 155                 160

Lys Thr Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
            180                 185                 190

Ala Thr Pro Ala Ala Val Gly Pro Thr Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ala
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

Tyr Lys Gln Ile Ser Ser Ala Ser Thr Gly Ala Ser Asn Asp Asn His
            260                 265                 270

Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe
        275                 280                 285

His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn
    290                 295                 300

Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln
305                 310                 315                 320

Val Lys Glu Val Thr Thr Asn Asp Gly Val Thr Thr Ile Ala Asn Asn
                325                 330                 335

Leu Thr Ser Thr Val Gln Val Phe Ser Asp Ser Glu Tyr Gln Leu Pro
            340                 345                 350

Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala
        355                 360                 365

Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly
    370                 375                 380

Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro
385                 390                 395                 400

Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe
                405                 410                 415

Glu Glu Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp
            420                 425                 430

Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Asn Arg
        435                 440                 445

Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn Lys Asp Leu Leu Phe Ser
    450                 455                 460

Arg Gly Ser Pro Ala Gly Met Ser Val Gln Pro Lys Asn Trp Leu Pro
465                 470                 475                 480

Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Lys Thr Asp Asn
                485                 490                 495

Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala Ser Lys Tyr Asn Leu Asn
            500                 505                 510

Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr Ala Met Ala Ser His Lys
        515                 520                 525

Asp Asp Glu Asp Lys Phe Phe Pro Met Ser Gly Val Met Ile Phe Gly
    530                 535                 540

Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala Leu Asp Asn Val Met Ile
545                 550                 555                 560
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Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn Pro Val Ala Thr Glu Arg
                565                 570                 575

Phe Gly Thr Val Ala Val Asn Phe Gln Ser Ser Ser Thr Asp Pro Ala
            580                 585                 590

Thr Gly Asp Val His Ala Met Gly Ala Leu Pro Gly Met Val Trp Gln
        595                 600                 605

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
    610                 615                 620

Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
625                 630                 635                 640

Lys Asn Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
                645                 650                 655

Asn Pro Pro Ala Glu Phe Ser Ala Thr Lys Phe Ala Ser Phe Ile Thr
            660                 665                 670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
        675                 680                 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Val Gln Tyr Thr Ser Asn
    690                 695                 700

Tyr Ala Lys Ser Ala Asn Val Asp Phe Thr Val Asp Asn Asn Gly Leu
705                 710                 715                 720

Tyr Thr Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Pro Leu
                725                 730                 735


<210> SEQ ID NO 6
<211> LENGTH: 736
<212> TYPE: PRT
<213> ORGANISM: adeno-associated virus serotype 3

<400> SEQUENCE: 6

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1               5                   10                  15

Glu Gly Ile Arg Glu Trp Trp Ala Leu Lys Pro Gly Val Pro Gln Pro
            20                  25                  30

Lys Ala Asn Gln Gln His Gln Asp Asn Arg Arg Gly Leu Val Leu Pro
        35                  40                  45

Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60

Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
                85                  90                  95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Ile Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Glu Ala Ala Lys Thr Ala Pro Gly Lys Lys Gly
    130                 135                 140

Ala Val Asp Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Val Gly
145                 150                 155                 160

Lys Ser Gly Lys Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
            180                 185                 190

Ala Ala Pro Thr Ser Leu Gly Ser Asn Thr Met Ala Ser Gly Gly Gly
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        195                 200                 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ser
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Gln Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

Tyr Lys Gln Ile Ser Ser Gln Ser Gly Ala Ser Asn Asp Asn His Tyr
            260                 265                 270

Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe His
        275                 280                 285

Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn Trp
    290                 295                 300

Gly Phe Arg Pro Lys Lys Leu Ser Phe Lys Leu Phe Asn Ile Gln Val
305                 310                 315                 320

Arg Gly Val Thr Gln Asn Asp Gly Thr Thr Thr Ile Ala Asn Asn Leu
                325                 330                 335

Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu Pro Tyr
            340                 345                 350

Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala Asp
        355                 360                 365

Val Phe Met Val Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly Ser
    370                 375                 380

Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro Ser
385                 390                 395                 400

Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Thr Phe Glu
                405                 410                 415

Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp Arg
            420                 425                 430

Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Asn Arg Thr
        435                 440                 445

Gln Gly Thr Thr Ser Gly Thr Thr Asn Gln Ser Arg Leu Leu Phe Ser
    450                 455                 460

Gln Ala Gly Pro Gln Ser Met Ser Leu Gln Ala Arg Asn Trp Leu Pro
465                 470                 475                 480

Gly Pro Cys Tyr Arg Gln Gln Arg Leu Ser Lys Thr Ala Asn Asp Asn
                485                 490                 495

Asn Asn Ser Asn Phe Pro Trp Thr Ala Ala Ser Lys Tyr His Leu Asn
            500                 505                 510

Gly Arg Asp Ser Leu Val Asn Pro Gly Pro Ala Met Ala Ser His Lys
        515                 520                 525

Asp Asp Glu Glu Lys Phe Phe Pro Met His Gly Asn Leu Ile Phe Gly
    530                 535                 540

Lys Glu Gly Thr Thr Ala Ser Asn Ala Glu Leu Asp Asn Val Met Ile
545                 550                 555                 560

Thr Asp Glu Glu Glu Ile Arg Thr Thr Asn Pro Val Ala Thr Glu Gln
                565                 570                 575

Tyr Gly Thr Val Ala Asn Asn Leu Gln Ser Ser Asn Thr Ala Pro Thr
            580                 585                 590

Thr Gly Thr Val Asn His Gln Gly Ala Leu Pro Gly Met Val Trp Gln
        595                 600                 605

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
    610                 615                 620
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Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
625                 630                 635                 640

Lys His Pro Pro Pro Gln Ile Met Ile Lys Asn Thr Pro Val Pro Ala
                645                 650                 655

Asn Pro Pro Thr Thr Phe Ser Pro Ala Lys Phe Ala Ser Phe Ile Thr
            660                 665                 670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
        675                 680                 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn
    690                 695                 700

Tyr Asn Lys Ser Val Asn Val Asp Phe Thr Val Asp Thr Asn Gly Val
705                 710                 715                 720

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


<210> SEQ ID NO 7
<211> LENGTH: 738
<212> TYPE: PRT
<213> ORGANISM: adeno-associated virus serotype 8

<400> SEQUENCE: 7

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1               5                   10                  15

Glu Gly Ile Arg Glu Trp Trp Ala Leu Lys Pro Gly Ala Pro Lys Pro
            20                  25                  30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
        35                  40                  45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

Gln Gln Leu Gln Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
                85                  90                  95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

Pro Val Glu Pro Ser Pro Gln Arg Ser Pro Asp Ser Ser Thr Gly Ile
145                 150                 155                 160

Gly Lys Lys Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln
                165                 170                 175

Thr Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro
            180                 185                 190

Pro Ala Ala Pro Ser Gly Val Gly Pro Asn Thr Met Ala Ala Gly Gly
        195                 200                 205

Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser
    210                 215                 220

Ser Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val
225                 230                 235                 240

Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His
                245                 250                 255

Leu Tyr Lys Gln Ile Ser Asn Gly Thr Ser Gly Gly Ala Thr Asn Asp
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            260                 265                 270

Asn Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn
        275                 280                 285

Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn
    290                 295                 300

Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Ser Phe Lys Leu Phe Asn
305                 310                 315                 320

Ile Gln Val Lys Glu Val Thr Gln Asn Glu Gly Thr Lys Thr Ile Ala
                325                 330                 335

Asn Asn Leu Thr Ser Thr Ile Gln Val Phe Thr Asp Ser Glu Tyr Gln
            340                 345                 350

Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe
        355                 360                 365

Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn
    370                 375                 380

Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr
385                 390                 395                 400

Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Thr Tyr
                405                 410                 415

Thr Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser
            420                 425                 430

Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu
        435                 440                 445

Ser Arg Thr Gln Thr Thr Gly Gly Thr Ala Asn Thr Gln Thr Leu Gly
    450                 455                 460

Phe Ser Gln Gly Gly Pro Asn Thr Met Ala Asn Gln Ala Lys Asn Trp
465                 470                 475                 480

Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Thr Thr Thr Gly
                485                 490                 495

Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr Ala Gly Thr Lys Tyr His
            500                 505                 510

Leu Asn Gly Arg Asn Ser Leu Ala Asn Pro Gly Ile Ala Met Ala Thr
        515                 520                 525

His Lys Asp Asp Glu Glu Arg Phe Phe Pro Ser Asn Gly Ile Leu Ile
    530                 535                 540

Phe Gly Lys Gln Asn Ala Ala Arg Asp Asn Ala Asp Tyr Ser Asp Val
545                 550                 555                 560

Met Leu Thr Ser Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr
                565                 570                 575

Glu Glu Tyr Gly Ile Val Ala Asp Asn Leu Gln Gln Gln Asn Thr Ala
            580                 585                 590

Pro Gln Ile Gly Thr Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val
        595                 600                 605

Trp Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile
    610                 615                 620

Pro His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe
625                 630                 635                 640

Gly Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val
                645                 650                 655

Pro Ala Asp Pro Pro Thr Thr Phe Asn Gln Ser Lys Leu Asn Ser Phe
            660                 665                 670

Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu
        675                 680                 685
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Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr
    690                 695                 700

Ser Asn Tyr Tyr Lys Ser Thr Ser Val Asp Phe Ala Val Asn Thr Glu
705                 710                 715                 720

Gly Val Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg
                725                 730                 735

Asn Leu
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What is claimed is:

1. An isolated adeno-associated virus (AAV) comprising an AAV capsid protein having the amino acid sequence of SEQ ID NO:2.

2. The isolated AAV according to claim **1**, wherein the AAV capsid protein was encoded by nt 2116 to 4323 of SEQ ID NO:1.

3. The isolated AAV according to claim **1**, wherein said AAV further comprises a minigene having AAV inverted terminal repeats and a heterologous gene operably linked to regulatory sequences which direct its expression in a host cell.

4. An isolated host cell comprising an adeno-associated virus according to claim **1**.

5. A composition comprising said AAV according to claim **1** and a physiologically compatible carrier.

6. A method of delivering a transgene to a cell, said method comprising the step of contacting the cell with said AAV according to claim **1**, wherein said AAV comprises a heterologous gene.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,198,951 B2
APPLICATION NO. : 10/496799
DATED : April 3, 2007
INVENTOR(S) : Gao et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

(1) Col. 4, line 17, replace "invented" with -- inverted --;

(2) Col. 4, line 56, replace "alt" with -- art --;

(3) Col. 5, line 45, after aa 133 to 165; add line "aa 134 to 165; aa 137 to 143; aa 154 to 156; aa 194 to 208; aa 261 to 274; aa 262 to 274;"

(4) Col. 5, line 46, delete "aa 5"

(5) Col. 5, line 46, after aa 534 to 571; add line "aa 581 to 601;"

(6) Col. 6, line 5, replace "(Sieberetal.," with -- (Sieber et al., --;

(7) Col. 9, line 49, replace "(May 2001This" with -- (May 2001). This --;

(8) Col. 11, line 9, replace "αchain)," with -- α chain), --;

(9) Col. 12, line 19, replace "rep78168" with -- rep 78/68 --;

(10) Col. 12, line 54, delete "is"

(11) Col. 13, line 2, replace "PS" with -- P5 --;

(12) Col. 17, line 52, replace "a superfamily" with -- α superfamily --;

(13) Col. 18, line 28, "bbx" with -- box --;

(14) Col. 18, line 29, "ZFS" with -- ZF5 --;

(15) Col. 18, line 36, replace "argilase" with -- arginase --;

(16) Col. 19, line 28, replace "Croln's" with -- Crohn's --;

(17) Col. 20, line 35, replace "spuinavirinal" with -- spumavirinal --;

(18) Col. 20, line 52, replace "dopoxyirinae" with -- dopoxvirinae --;

(19) Col. 20, line 54, insert "capripoxvirus" between avipoxvirus and leporipoxvirus;

(20) Col. 20, line 55, replace "entomapoxyinnae" with -- Intomopoxvirinae --;

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,198,951 B2
APPLICATION NO. : 10/496799
DATED : April 3, 2007
INVENTOR(S) : Gao et al.

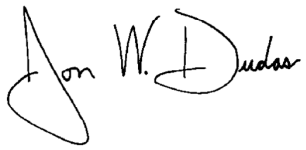
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

(21) Col. 21, line 18, replace "peifringens" with -- perfringens --;

(22) Col. 21, line 23, replace "mallet)" with -- mallei --;

Signed and Sealed this

Twenty-ninth Day of April, 2008



JON W. DUDAS
Director of the United States Patent and Trademark Office