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(54) **INFLUENZA VIRUS REPLICATION BY INHIBITING MICRORNA LEC7C BINDING TO INFLUENZA VIRAL CRNA AND MRNA**

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(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

A vector, composition and method to improve influenza virus replication by inhibiting miRNA lec-7C binding to influenza virus mRNA and/or cRNA.

19 Claims, 13 Drawing Sheets

Specification includes a Sequence Listing.

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Figure 1

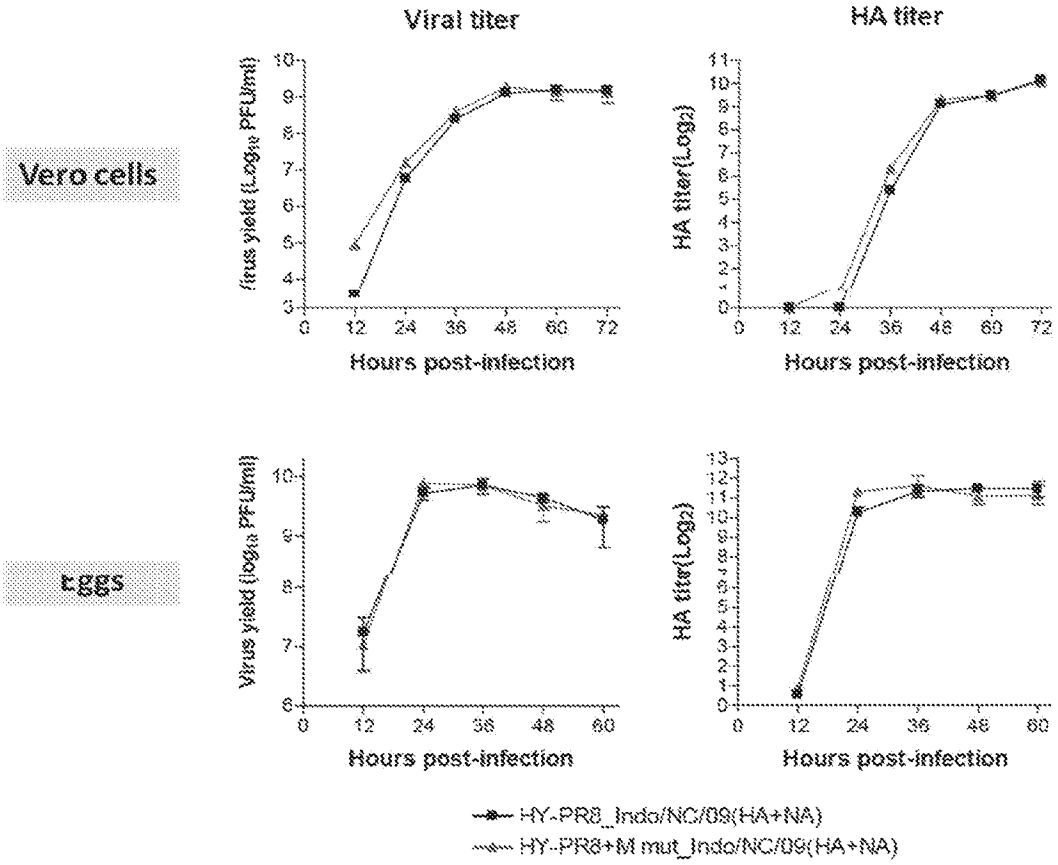
Viral titer
HA titer
Vero cells
eggs
Virus yield (Log10 PFU/ml)
HA titer(Log2)
Hours post-infection
HY-PR8_Indo/NC/09(HA+NA)
HY-PR8+M mut_Indo/NC/09(HA+NA)

Figure 2.

M (PR8UW)

AGCAAAAGCA GGTAGATATT GAAAGATGAG TCTTCTAACC GAGGTCGAAA
CGTACGTACT CTCTATCATC CCGTCAGGCC CCCTCAAAGC CGAGATCGCA
CAGAGACTTG AAGATGTCTT TGCAGGGAAG AACACCGATC TTGAGGTTCT
CATGGAATGG CTAAAGACAA GACCAATCCT GTCACCTCTG ACTAAGGGGA
TTTTAGGATT TGTGTTCACG CTCACCGTGC CCAGTGAGCG AGGACTGCAG
CGTAGACGCT TTGTCCAAAA TGCCCTTAAT GGGAACGGGG ATCCAAATAA
CATGGACAAA GCAGTTAAAC TGTATAGGAA GCTCAAGAGG GAGATAACAT
TCCATGGGGC CAAAGAAATC TCACTCAGTT ATTCTGCTGG TGCACTTGCC
AGTTGTATGG GCCTCATATA CAACAGGATG GGGGCTGTGA CCACTGAAGT
GGCATTTGGC CTGGTATGTG CAACCTGTGA ACAGATTGCT GACTCCCAGC
ATCGGTCTCA TAGGCAAATG GTGACAACAA CCAATCCACT AATCAGACAT
GAGAACAGAA TGGTTTTAGC CAGCACTACA GCTAAGGCTA TGGAGCAAAT
GGCTGGATCG AGTGAGCAAG CAGCAGAGGC CATGGAGGTT GCTAGTCAGG
CTAGACAAAT GGTGCAAGCG ATGAGAACCA TTGGGACTCA TCCTAGCTCC
AGTGCTGGTC TGAAAAATGA TCTTCTTGAA AATTTGCAGG CCTATCAGAA
ACGAATGGGG GTGCAGATGC AACGGTTCAA GTGATCCTCT CACTATTGCC
GCAAATATCA TTGGGATCTT GCACTTGACA TTGTGGATTC TTGATCGTCT
TTTTTTCAAA TGCATTTACC GTCGCTTTAA ATACGGACTG AAAGGAGGGC
CTTCTACGGA AGGAGTGCCA AAGTCTATGA GGGAAGAATA TCGAAAGGAA
CAGCAGAGTG CTGTGGATGC TGACGATGGT CATTTTGTCA GCATAGAGCT
GGAGTAAAAA ACTACCTTGT TTCTACT

M (Cambridge)

agcaaaagca ggtagatatt gaaagatgag tcttctaacc gaggtcgaaa cgtacgttct
ctctatcatc ccgtcaggcc ccctcaaagc cgagatcgca cagagacttg aagatgtctt
tgcagggaag aacaccgatc ttgaggttct catggaatgg ctaaagacaa gaccaatcct
gtcacctctg actaagggga ttttaggatt tgtgttcacg ctcaccgtgc ccagtgagcg
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aatcagacat gagaacagaa tggttttagc cagcactaca gctaaggcta tggagcaaat
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ggtgcaagcg atgagaacca ttgggactca tcctagctcc agtgctggtc tgaaaaatga
tcttcttgaa aatttgcagg cctatcagaa acgaatgggg gtgcagatgc aacggttcaa
gtgatcctct cgctattgcc gcaaatatca ttgggatctt gcacttgata ttgtggattc
ttgatcgtct ttttttcaaa tgcatttacc gtcgctttaa atacggactg aaaggagggc
cttctacgga aggagtgcca aagtctatga gggaagaata tcgaaaggaa cagcagagtg
ctgtggatgc tgacgatggt cattttgtca gcatagagct ggagtaaaaa actaccttgt
ttctact

Figure 3.

M1 target site:

M1	5'	...GCTGGAGTAAAAAACTACCTTG...3'
let-7c	3'	UUGGUAUGUUGGAUGAUGGAGU 5'

PR8(Cambridge)

PB2

AGCGAAAGCAGGTCAATTTATATTCAATATGGAAAGAATAAAAGAACTAAGAAATCTAATGTCGCAGTCTCGCACCCGCGAGATA
CTCACAAAAACCACCGTGGACCATATGGCCATAATCAAGAAGTACACATCAGGAAGACAGGAGAAGAACCCAGCACTTAGGATG
AAATGGATGATGGCAATGAAATATCCAATTACAGCAGACAAGAGGATAACGGAAATGATTCCTGAGAGAAATGAGCAAGGACAA
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CCAATGACAAATACAGTTCATTATCCAAAAATCTACAAAACTTATTTTGAAAGAGTCGAAAGGCTAAAGCATGGAACCTTTGGC
CCTGTCCATTTTAGAAACCAAGTCAAAATACGTCGGAGAGTTGACATAAATCCTGGTCATGCAGATCTCAGTGCCAAGGAGGCA
CAGGATGTAATCATGGAAGTTGTTTTCCCTAACGAAGTGGGAGCCAGGATACTAACATCGGAATCGCAACTAACGATAACCAAA
GAGAAGAAAGAAGAACTCCAGGATTGCAAAATTTCTCCTTTGATGGTTGCATACATGTTGGAGAGAGAACTGGTCCGCAAAACG
AGATTCCTCCCAGTGGCTGGTGGAACAAGCAGTGTGTACATTGAAGTGTTGCATTTGACTCAAGGAACATGCTGGGAACAGATG
TATACTCCAGGAGGGGAAGTGAAGAATGATGATGTTGATCAAAGCTTGATTATTGCTGCTAGGAACATAGTGAGAAGAGCTGCA
GTATCAGCAGACCCACTAGCATCTTTATTGGAGATGTGCCACAGCACACAGATTGGTGGAATTAGGATGGTAGACATCCTTAAG
CAGAACCCAACAGAAGAGCAAGCCGTGGATATATGCAAGGCTGCAATGGGACTGAGAATTAGCTCATCCTTCAGTTTTGGTGGA
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TTTCAGTCTTTAGTACCTAAGGCCATTAGAGGCCAATACAGTGGGTTTGTAAGAACTCTGTTCCAACAAATGAGGGATGTGCTT
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TTTACTGTGAATGTGAGGGGATCAGGAATGAGAATACTTGTAAGGGGCAATTCTCCTGTATTCAACTACAACAAGGCCACGAAG
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GAGAAGGCTAATGTGCTAATTGGGCAAGGAGACGTGGTGTTGGTAATGAAACGAAAACGGGACTCTAGCATACTTACTGACAGC
CAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAGTGTCGAATAGTTTAAAAACGACCTTGTTTCTACT

(SEQ ID NO:11)

PB1

AGCGAAAGCAGGCAAACCATTTGAATGGATGTCAATCCGACCTTACTTTTCTTAAAAGTGCCAGCACAAAATGCTATAAGCACA
ACTTTCCCTTATACCGGAGACCCTCCTTACAGCCATGGGACAGGAACAGGATACACCATGGATACTGTCAACAGGACACATCAG
TACTCAGAAAAGGGAAGATGGACAACAAACACCGAAACTGGAGCACCGCAACTCAACCCGATTGATGGGCCACTGCCAGAAGAC
AATGAACCAAGTGGTTATGCCCAAACAGATTGTGTATTGGAAGCAATGGCTTTCCTTGAGGAATCCCATCCTGGTATTTTTGAA
AACTCGTGTATTGAAACGATGGAGGTTGTTCAGCAAACACGAGTAGACAAGCTGACACAAGGCCGACAGACCTATGACTGGACT
TTAAATAGAAACCAGCCTGCTGCAACAGCATTGGCCAACACAATAGAAGTGTTCAGATCAAATGGCCTCACGGCCAATGAGTCA
GGAAGGCTCATAGACTTCCTTAAGGATGTAATGGAGTCAATGAAAAAAGAAGAAATGGGGATCACAACTCATTTTCAGAGAAAG
AGACGGGTGAGAGACAATATGACTAAGAAAATGATAACACAGAGAACAATAGGTAAAAGGAAACAGAGATTGAACAAAAGGGGT
TATCTAATTAGAGCATTGACCCTGAACACAATGACCAAAGATGCTGAGAGAGGGAAGCTAAAACGGAGAGCAATTGCAACCCCA
GGGATGCAAATAAGGGGGTTTGTATACTTTGTTGAGACACTGGCAAGGAGTATATGTGAGAAACTTGAACAATCAGGGTTGCCA
GTTGGAGGCAATGAGAAGAAAGCAAAGTTGGCAAATGTTGTAAGGAAGATGATGACCAATTCTCAGGACACCGAACTTTCTTTC
ACCATCACTGGAGATAACACCAAATGGAACGAAAATCAGAATCCTCGGATGTTTTTGGCCATGATCACATATATGACCAGAAAT
CAGCCCGAATGGTTCAGAAATGTTCTAAGTATTGCTCCAATAATGTTCTCAAACAAAATGGCGAGACTGGGAAAAGGGTATATG
TTTGAGAGCAAGAGTATGAAACTTAGAACTCAAATACCTGCAGAAATGCTAGCAAGCATTGATTTGAAATATTTCAATGATTCA
ACAAGAAAGAAGATTGAAAAAATCCGACCGCTCTTAATAGAGGGGACTGCATCATTGAGCCCTGGAATGATGATGGGCATGTTC
AATATGTTAAGCACTGTATTAGGCGTCTCCATCCTGAATCTTGGACAAAAGAGATACACCAAGACTACTTACTGGTGGGATGGT
CTTCAATCCTCTGACGATTTTGCTCTGATTGTGAATGCACCCAATCATGAAGGGATTCAAGCCGGAGTCGACAGGTTTTATCGA
ACCTGTAAGCTACTTGGAATCAATATGAGCAAGAAAAAGTCTTACATAAACAGAACAGGTACATTTGAATTCACAAGTTTTTTC
TATCGTTATGGGTTTGTTGCCAATTTCAGCATGGAGCTTCCCAGTTTTGGGGTGTCTGGGATCAACGAGTCAGCGGACATGAGT
ATTGGAGTTACTGTCATCAAAAACAATATGATAAACAATGATCTTGGTCCAGCAACAGCTCAAATGGCCCTTCAGTTGTTCATC
AAAGATTACAGGTACACGTACCGATGCCATAGAGGTGACACACAAATACAAACCCGAAGATCATTTGAAATAAAGAAACTGTGG
GAGCAAACCCGTTCCAAAGCTGGACTGCTGGTCTCCGACGGAGGCCCAAATTTATACAACATTAGAAATCTCCACATTCCTGAA
GTCTGCCTAAAATGGGAATTGATGGATGAGGATTACCAGGGGCGTTTATGCAACCCACTGAACCCATTTGTCAGCCATAAAGAA
ATTGAATCAATGAACAATGCAGTGATGATGCCAGCACATGGTCCAGCCAAAAACATGGAGTATGATGCTGTTGCAACAACACAC
TCCTGGATCCCCAAAAGAAATCGATCCATCTTGAATACAAGTCAAAGAGGAGTACTTGAAGATGAACAAATGTACCAAAGGTGC
TGCAATTTATTTGAAAAATTCTTCCCCAGCAGTTCATACAGAAGACCAGTCGGGATATCCAGTATGGTGGAGGCTATGGTTTCC
AGAGCCCGAATTGATGCACGGATTGATTTCGAATCTGGAAGGATAAAGAAAGAAGAGTTCACTGAGATCATGAAGATCTGTTCC
ACCATTGAAGAGCTCAGACGGCAAAAATAGTGAATTTAGCTTGTCCTTCATGAAAAAATGCCTTGTTTCTACT

(SEQ ID NO:10)

FIG. 4

PR8(Cambridge)

PA

AGCGAAAGCAGGTACTGATTCAAAATGGAAGATTTTGTGCGACAATGCTTCAATCCGATGATTGTCGAGCTTGCGGAAAAAACA
ATGAAAGAGTATGGGGAGGACCTGAAAATCGAAACAAACAAATTTGCAGCAATATGCACTCACTTGGAAGTATGCTTCATGTAT
TCAGATTTCCACTTCATCAATGAGCAAGGCGAGTCAATAATCGTAGAACTTGGTGATCCTAATGCACTTTTGAAGCACAGATTT
GAAATAATCGAGGGAAGAGATCGCACAATGGCCTGGACAGTAGTAAACAGTATTTGCAACACTACAGGGGCTGAGAAACCAAAG
TTTCTACCAGATTTGTATGATTACAAGGAAAATAGATTCATCGAAATTGGAGTAACAAGGAGAGAAGTTCACATATACTATCTG
GAAAAGGCCAATAAAATTAAATCTGAGAAAACACACATCCACATTTTCTCGTTCACTGGGGAAGAAATGGCCACAAGGGCCGAC
TACACTCTCGATGAAGAAAGCAGGGCTAGGATCAAAACCAGGCTATTCACCATAAGACAAGAAATGGCCAGCAGAGGCCTCTGG
GATTCCTTTCGTCAGTCCGAGAGAGGAGAAGAGACAATTGAAGAAAGGTTTGAAATCACAGGAACAATGCGCAAGCTTGCCGAC
CAAAGTCTCCCGCCGAACTTCTCCAGCCTTGAAAATTTTAGAGCCTATGTGGATGGATTCGAACCGAACGGCTACATTGAGGGC
AAGCTGTCTCAAATGTCCAAAGAAGTAAATGCTAGAATTGAACCTTTTTTGAAAACAACACCACGACCACTTAGACTTCCGAAT
GGGCCTCCCTGTTCTCAGCGGTCCAAATTCCTGCTGATGGATGCCTTAAAATTAAGCATTGAGGACCCAAGTCATGAAGGAGAG
GGAATACCGCTATATGATGCAATCAAATGCATGAGAACATTCTTTGGATGGAAGGAACCCAATGTTGTTAAACCACACGAAAAG
GGAATAAATCCAAATTATCTTCTGTCATGGAAGCAAGTACTGGCAGAACTGCAGGACATTGAGAATGAGGAGAAAATTCCAAAG
ACTAAAAATATGAAAAAAACAAGTCAGCTAAAGTGGGCACTTGGTGAGAACATGGCACCAGAAAAGGTAGACTTTGACGACTGT
AAAGATGTAGGTGATTTGAAGCAATATGATAGTGATGAACCAGAATTGAGGTCGCTTGCAAGTTGGATTCAGAATGAGTTCAAC
AAGGCATGCGAACTGACAGATTCAAGCTGGATAGAGCTTGATGAGATTGGAGAAGATGTGGCTCCAATTGAACACATTGCAAGC
ATGAGAAGGAATTATTTCACATCAGAGGTGTCTCACTGCAGAGCCACAGAATACATAATGAAGGGGGTGTACATCAATACTGCC
TTACTTAATGCATCTTGTGCAGCAATGGATGATTTCCAATTAATTCCAATGATAAGCAAGTGTAGAACTAAGGAGGGAAGGCGA
AAGACCAACTTGTATGGTTTCATCATAAAAGGAAGATCCCACTTAAGGAATGACACCGACGTGGTAAACTTTGTGAGCATGGAG
TTTTCTCTCACTGACCCAAGACTTGAACCACACAAATGGGAGAAGTACTGTGTTCTTGAGATAGGAGATATGCTTCTAAGAAGT
GCCATAGGCCAGGTTTCAAGGCCCATGTTCTTGTATGTGAGGACAAATGGAACCTCAAAAATTAAAATGAAATGGGGAATGGAG
ATGAGGCGTTGTCTCCTCCAGTCACTTCAACAAATTGAGAGTATGATTGAAGCTGAGTCCTCTGTCAAAGAGAAAGACATGACC
AAAGAGTTCTTTGAGAACAAATCAGAAACATGGCCCATTGGAGAGTCTCCCAAAGGAGTGGAGGAAAGTTCCATTGGGAAGGTC
TGCAGGACTTTATTAGCAAAGTCGGTATTTAACAGCTTGTATGCATCTCCACAACTAGAAGGATTTTCAGCTGAATCAAGAAAA
CTGCTTCTTATCGTTCAGGCTCTTAGGGACAATCTGGAACCTGGGACCTTTGATCTTGGGGGGCTATATGAAGCAATTGAGGAG
TGCCTAATTAATGATCCCTGGGTTTTGCTTAATGCTTCTTGGTTCAACTCCTTCCTTACACATGCATTGAGTTAGTTGTGGCAG
TGCTACTATTTGCTATCCATACTGTCCAAAAAAGTACCTTGTTTCTACT

(SEQ ID NO: 12)

NP

AGCAAAAGCAGGGTAGATAATCACTCACTGAGTGACATCAAAATCATGGCGTCCCAAGGCACCAAACGGTCTTACGAACAGATG
GAGACTGATGGAGAACGCCAGAATGCCACTGAAATCAGAGCATCCGTCGGAAAAATGATTGGTGGAATTGGACGATTCTACATC
CAAATGTGCACAGAACTTAAACTCAGTGATTATGAGGGACGGTTGATCCAAAACAGCTTAACAATAGAGAGAATGGTGCTCTCT
GCTTTTGACGAAAGGAGAAATAAATACCTGGAAGAACATCCCAGTGCGGGGAAAGATCCTAAGAAAACTGGAGGACCTATATAC
AGAAGAGTAAACGGAAAGTGGATGAGAGAACTCATCCTTTATGACAAAGAAGAAATAAGGCGAATCTGGCGCCAAGCTAATAAT
GGTGACGATGCAACGGCTGGTCTGACTCACATGATGATCTGGCATTCCAATTTGAATGATGCAACTTATCAGAGGACAAGGGCT
CTTGTTCGCACCGGAATGGATCCCAGGATGTGCTCTCTGATGCAAGGTTCAACTCTCCCTAGGAGGTCTGGAGCCGCAGGTGCT
GCAGTCAAAGGAGTTGGAACAATGGTGATGGAATTGGTCAGGATGATCAAACGTGGGATCAATGATCGGAACTTCTGGAGGGGT
GAGAATGGACGAAAAACAAGAATTGCTTATGAAAGAATGTGCAACATTCTCAAAGGGAAATTTCAAACTGCTGCACAAAAAGCA
ATGATGGATCAAGTGAGAGAGAGCCGGAACCCAGGGAATGCTGAGTTCGAAGATCTCACTTTTCTAGCACGGTCTGCACTCATA
TTGAGAGGGTCGGTTGCTCACAAGTCCTGCCTGCCTGCCTGTGTGTATGGACCTGCCGTAGCCAGTGGGTACGACTTTGAAAGA
GAGGGATACTCTCTAGTCGGAATAGACCCTTTCAGACTGCTTCAAAACAGCCAAGTGTACAGCCTAATCAGACCAAATGAGAAT
CCAGCACACAAGAGTCAACTGGTGTGGATGGCATGCCATTCTGCCGCATTTGAAGATCTAAGAGTATTGAGCTTCATCAAAGGG
ACGAAGGTGGTCCCAAGAGGGAAGCTTTCCACTAGAGGAGTTCAAATTGCTTCCAATGAAAATATGGAGACTATGGAATCAAGT
ACACTTGAACTGAGAAGCAGGTACTGGGCCATAAGGACCAGAAGTGGAGGAAACACCAATCAACAGAGGGCATCTGCGGGCCAA
ATCAGCATACAACCTACGTTCTCAGTACAGAGAAATCTCCCTTTTGACAGAACAACCGTTATGGCAGCATTCACTGGGAATACA
GAGGGGAGAACATCTGACATGAGGACCGAAATCATAAGGATGATGGAAAGTGCAAGACCAGAAGATGTGTCTTTCCAGGGGCGG
GGAGTCTTCGAGCTCTCGGACGAAAAGGCAGCGAGCCCGATCGTGCCTTCCTTTGACATGAGTAATGAAGGATCTTATTTCTTC
GGAGACAATGCAGAGGAGTACGACAATTAAAGAAAAATACCCTTGTTTCTACT

(SEQ ID NO: 13)

M

AGCAAAAGCAGGTAGATATTGAAAGATGAGTCTTCTAACCGAGGTCGAAACGTACGTTCTCTCTATCATCCCGTCAGGCCCCCT
CAAAGCCGAGATCGCACAGAGACTTGAAGATGTCTTTGCAGGGAAGAACACCGATCTTGAGGTTCTCATGGAATGGCTAAAGAC
AAGACCAATCCTGTCACCTCTGACTAAGGGGATTTTAGGATTTGTGTTCACGCTCACCGTGCCCAGTGAGCGAGGACTGCAGCG
TAGACGCTTTGTCCAAAATGCCCTTAATGGGAACGGGGATCCAAATAACATGGACAAAGCAGTTAAACTGTATAGGAAGCTCAA
GAGGGAGATAACATTCCATGGGGCCAAAGAAATCTCACTCAGTTATTCTGCTGGTGCACTTGCCAGTTGTATGGGCCTCATATA
CAACAGGATGGGGGCTGTGACCACTGAAGTGGCATTTGGCCTGGTATGTGCAACCTGTGAACAGATTGCTGACTCCCAGCATCG

FIG. 4 (CONT.)

PR8(Cambridge)

GTCTCATAGGCAAATGGTGACAACAACCAACCCACTAATCAGACATGAGAACAGAATGGTTTTAGCCAGCACTACAGCTAAGGC
TATGGAGCAAATGGCTGGATCGAGTGAGCAAGCAGCAGAGGCCATGGAGGTTGCTAGTCAGGCTAGGCAAATGGTGCAAGCGAT
GAGAACCATTGGGACTCATCCTAGCTCCAGTGCTGGTCTGAAAAATGATCTTCTTGAAAATTTGCAGGCCTATCAGAAACGAAT
GGGGGTGCAGATGCAACGGTTCAAGTGATCCTCTCGCTATTGCCGCAAATATCATTGGGATCTTGCACTTGATATTGTGGATTC
TTGATCGTCTTTTTTTTCAAATGCATTTACCGTCGCTTTAAATACGGACTGAAAGGAGGGCCTTCTACGGAAGGAGTGCCAAAGT
CTATGAGGGAAGAATATCGAAAGGAACAGCAGAGTGCTGTGGATGCTGACGATGGTCATTTTGTCAGCATAGAGCTGGAGTAAA
AAACTACCTTGTTTCTACT

SEQ ID NO: 14)

NS

AGCAAAAGCAGGGTGACAAAGACATAATGGATCCAAACACTGTGTCAAGCTTTCAGGTAGATTGCTTTCTTTGGCATGTCCGCA
AACGAGTTGCAGACCAAGAACTAGGTGATGCCCCATTCCTTGATCGGCTTCGCCGAGATCAGAAATCCCTAAGAGGAAGGGGCA
GCACTCTTGGTCTGGACATCGAGACAGCCACACGTGCTGGAAAGCAGATAGTGGAGCGGATTCTGAAAGAAGAATCCGATGAGG
CACTTAAAATGACCATGGCCTCTGTACCTGCGTCGCGTTACCTAACCGACATGACTCTTGAGGAAATGTCAAGGGAATGGTCCA
TGCTCATACCCAAGCAGAAAGTGGCAGGCCCTCTTTGTATCAGAATGGACCAGGCGATCATGGATAAAAACATCATACTGAAAG
CGAACTTCAGTGTGATTTTTGACCGGCTGGAGACTCTAATATTGCTAAGGGCTTTCACCGAAGAGGGAGCAATTGTTGGCGAAA
TTTCACCATTGCCTTCTCTTCCAGGACATACTGCTGAGGATGTCAAAAATGCAGTTGGAGTCCTCATCGGAGGACTTGAATGGA
ATGATAACACAGTTCGAGTCTCTGAAACTCTACAGAGATTCGCTTGGAGAAGCAGTAATGAGAATGGGAGACCTCCACTCACTC
CAAAACAGAAACGAGAAATGGCGGGAACAATTAGGTCAGAAGTTTGAAGAAATAAGATGGTTGATTGAAGAAGTGAGACACAAA
CTGAAGGTAACAGAGAATAGTTTTGAGCAAATAACATTTATGCAAGCCTTACATCTATTGCTTGAAGTGGAGCAAGAGATAAGA
ACTTTCTCATTTCAGCTTATTTAATAATAAAAAACACCCTTGTTTCTACT

SEQ ID NO: 15

FIG. 4 (CONT.)

PA target site:

PA 5' ...ATACTGCCAAAAAAGTACCTT...3'

PB2 target site:

PB2 5' ...CGAATAGTTTAAAAACGACCTTGT...3'

PA

AGCGAAAGCA GGTACTGATC CAAAATGGAA GATTTTGTGC GACAATGCTT CAATCCGATG
ATTGTCGAGC TTGCGGAAAA AACAATGAAA GAGTATGGGG AGGACCTGAA AATCGAAACA
AACAAATTTG CAGCAATATG CACTCACTTG GAAGTATGCT TCATGTATTC AGATTTTCAC
TTCATCAATG AGCAAGGCGA GTCAATAATC GTAGAACTTG GTGATCCAAA TGCACTTTTG
AAGCACAGAT TTGAAATAAT CGAGGGAAGA GATCGCACAA TGGCCTGGAC AGTAGTAAAC
AGTATTTGCA ACACTACAGG GGCTGAGAAA CCAAAGTTTC TACCAGATTT GTATGATTAC
AAGGAGAATA GATTCATCGA AATTGGAGTA ACAAGGAGAG AAGTTCACAT ATACTATCTG
GAAAAGGCCA ATAAAATTAA ATCTGAGAAA ACACACATCC ACATTTTCTC GTTCACTGGG
GAAGAAATGG CCACAAAGGC AGACTACACT CTCGATGAAG AAAGCAGGGC TAGGATCAAA
ACCAGACTAT TCACCATAAG ACAAGAAATG GCCAGCAGAG GCCTCTGGGA TTCCTTTCGT
CAGTCCGAGA GAGGAGAAGA GACAATTGAA GAAAGGTTTG AAATCACAGG AACAATGCGC
AAGCTTGCCG ACCAAAGTCT CCCGCCGAAC TTCTCCAGCC TTGAAAATTT TAGAGCCTAT
GTGGATGGAT TCGAACCGAA CGGCTACATT GAGGGCAAGC TGTCTCAAAT GTCCAAAGAA
GTAAATGCTA GAATTGAACC TTTTTTGAAA ACAACACCAC GACCACTTAG ACTTCCGAAT
GGGCCTCCCT GTTCTCAGCG GTCCAAATTC CTGCTGATGG ATGCCTTAAA ATTAAGCATT
GAGGACCCAA GTCATGAAGG AGAGGGAATA CCGCTATATG ATGCAATCAA ATGCATGAGA
ACATTCTTTG GATGGAAGGA ACCCAATGTT GTTAAACCAC ACGAAAAGGG AATAAATCCA
AATTATCTTC TGTCATGGAA GCAAGTACTG GCAGAACTGC AGGACATTGA GAATGAGGAG
AAAATTCCAA AGACTAAAAA TATGAAGAAA ACAAGTCAGC TAAAGTGGGC ACTTGGTGAG
AACATGGCAC CAGAAAAGGT AGACTTTGAC GACTGTAAAG ATGTAGGTGA TTTGAAGCAA
TATGATAGTG ATGAACCAGA ATTGAGGTCG CTTGCAAGTT GGATTCAGAA TGAGTTTAAC
AAGGCATGCG AACTGACAGA TTCAAGCTGG ATAGAGCTCG ATGAGATTGG AGAAGATGTG
GCTCCAATTG AACACATTGC AAGCATGAGA AGGAATTATT TCACATCAGA GGTGTCTCAC
TGCAGAGCCA CAGAATACAT AATGAAGGGA GTGTACATCA ATACTGCCTT GCTTAATGCA
TCTTGTGCAG CAATGGATGA TTTCCAATTA ATTCCAATGA TAAGCAAGTG TAGAACTAAG
GAGGGAAGGC GAAAGACCAA CTTGTATGGT TTCATCATAA AAGGAAGATC CCACTTAAGG
AATGACACCG ACGTGGTAAA CTTTGTGAGC ATGGAGTTTT CTCTCACTGA CCCAAGACTT
GAACCACATA AATGGGAGAA GTACTGTGTT CTTGAGATAG GAGATATGCT TATAAGAAGT
GCCATAGGCC AGGTTTCAAG GCCCATGTTC TTGTATGTGA GAACAAATGG AACCTCAAAA
ATTAAAATGA AATGGGGAAT GGAGATGAGG CGTTGCCTCC TCCAGTCACT TCAACAAATT

FIG. 5A

GAGAGTATGA TTGAAGCTGA GTCCTCTGTC AAAGAGAAAG ACATGACCAA AGAGTTCTTT
GAGAACAAAT CAGAAACATG GCCCATTGGA GAGTCCCCCA AAGGAGTGGA GGAAAGTTCC
ATTGGGAAGG TCTGCAGGAC TTTATTAGCA AAGTCGGTAT TCAACAGCTT GTATGCATCT
CCACAACTAG AAGGATTTTC AGCTGAATCA AGAAAACTGC TTCTTATCGT TCAGGCTCTT
AGGGACAACC TGGAACCTGG GACCTTTGAT CTTGGGGGGC TATATGAAGC AATTGAGGAG
TGCCTGATTA ATGATCCCTG GGTTTTGCTT AATGCTTCTT GGTTCAACTC CTTCCTTACA
CATGCATTGA GTTAGTTGTG GCAGTGCTAC TATTTGCTAT CCATACTGTC CAAAAAAGTA
CCTTGTTTCT ACT

PB2

AGCGAAAGCA GGTCAATTAT ATTCAATATG GAAAGAATAA AAGAACTACG AAATCTAATG
TCGCAGTCTC GCACCCGCGA GATACTCACA AAAACCACCG TGGACCATAT GGCCATAATC
AAGAAGTACA CATCAGGAAG ACAGGAGAAG AACCCAGCAC TTAGGATGAA ATGGATGATG
GCAATGAAAT ATCCAATTAC AGCAGACAAG AGGATAACGG AAATGATTCC TGAGAGAAAT
GAGCAAGGAC AAACTTTATG GAGTAAAATG AATGATGCCG GATCAGACCG AGTGATGGTA
TCACCTCTGG CTGTGACATG GTGGAATAGG AATGGACCAA TAACAAATAC AGTTCATTAT
CCAAAAATCT ACAAAACTTA TTTTGAAAGA GTCGAAAGGC TAAAGCATGG AACCTTTGGC
CCTGTCCATT TTAGAAACCA AGTCAAAATA CGTCGGAGAG TTGACATAAA TCCTGGTCAT
GCAGATCTCA GTGCCAAGGA GGCACAGGAT GTAATCATGG AAGTTGTTTT CCCTAACGAA
GTGGGAGCCA GGATACTAAC ATCGGAATCG CAACTAACGA TAACCAAAGA GAAGAAAGAA
GAACTCCAGG ATTGCAAAAT TTCTCCTTTG ATGGTTGCAT ACATGTTGGA GAGAGAACTG
GTCCGCAAAA CGAGATTCCT CCCAGTGGCT GGTGGAACAA GCAGTGTGTA CATTGAAGTG
TTGCATTTGA CTCAAGGAAC ATGCTGGGAA CAGATGTATA CTCCAGGAGG GGAAGTGAGG
AATGATGATG TTGATCAAAG CTTGATTATT GCTGCTAGGA ACATAGTGAG AAGAGCTGCA
GTATCAGCAG ATCCACTAGC ATCTTTATTG GAGATGTGCC ACAGCACACA GATTGGTGGA
ATTAGGATGG TAGACATCCT TAGGCAGAAC CCAACAGAAG AGCAAGCCGT GGATATATGC
AAGGCTGCAA TGGGACTGAG AATTAGCTCA TCCTTCAGTT TTGGTGGATT CACATTTAAG
AGAACAAGCG GATCATCAGT CAAGAGAGAG GAAGAGGTGC TTACGGGCAA TCTTCAAACA
TTGAAGATAA GAGTGCATGA GGGATATGAA GAGTTCACAA TGGTTGGGAG AAGAGCAACA
GCCATACTCA GAAAAGCAAC CAGGAGATTG ATTCAGCTGA TAGTGAGTGG GAGAGACGAA
CAGTCGATTG CCGAAGCAAT AATTGTGGCC ATGGTATTTT CACAAGAGGA TTGTATGATA
AAAGCAGTCA GAGGTGATCT GAATTTCGTC AATAGGGCGA ATCAACGATT GAATCCTATG
CATCAACTTT TAAGACATTT TCAGAAGGAT GCGAAAGTGC TTTTTCAAAA TTGGGGAGTT
GAACCTATCG ACAATGTGAT GGGAATGATT GGGATATTGC CCGACATGAC TCCAAGCATC
GAGATGTCAA TGAGAGGAGT GAGAATCAGC AAAATGGGTG TAGATGAGTA CTCCAGCACG
GAGAGGGTAG TGGTGAGCAT TGACCGTTTT TTGAGAATCC GGGACCAACG AGGAAATGTA
CTACTGTCTC CCGAGGAGGT CAGTGAAACA CAGGGAACAG AGAAACTGAC AATAACTTAC
TCATCGTCAA TGATGTGGGA GATTAATGGT CCTGAATCAG TGTTGGTCAA TACCTATCAA
TGGATCATCA GAAACTGGGA AACTGTTAAA ATTCAGTGGT CCCAGAACCC TACAATGCTA
TACAATAAAA TGGAATTTGA ACCATTTCAG TCTTTAGTAC CTAAGGCCAT TAGAGGCCAA
TACAGTGGGT TTGTAAGAAC TCTGTTCCAA CAAATGAGGG ATGTGCTTGG GACATTTGAT
ACCGCACAGA TAATAAAACT TCTTCCCTTC GCAGCCGCTC CACCAAAGCA AAGTAGAATG
CAGTTCTCCT CATTTACTGT GAATGTGAGG GGATCAGGAA TGAGAATACT TGTAAGGGGC
AATTCTCCTG TATTCAACTA TAACAAGGCC ACGAAGAGAC TCACAGTTCT CGGAAAGGAT
GCTGGCACTT TAACTGAAGA CCCAGATGAA GGCACAGCTG GAGTGGAGTC CGCTGTTCTG
AGGGGATTCC TCATTCTGGG CAAAGAAGAC AAGAGATATG GGCCAGCACT AAGCATCAAT

FIG. 5B

GAACTGAGCA ACCTTGCGAA AGGAGAGAAG GCTAATGTGC TAATTGGGCA AGGAGACGTG
GTGTTGGTAA TGAAACGGAA ACGGGACTCT AGCATACTTA CTGACAGCCA GACAGCGACC
AAAAGAATTC GGATGGCCAT CAATTAGTGT CGAATAGTTT AAAAACGACC TTGTTTCTAC T

FIG. 5C

Influenza B virus M gene

```
1 atgtcgctgt ttggagacac aattgcctac ctgctttcat tgacagaaga tggagaaggc
       61 aaagcagaac tagcagaaaa attacactgc tggttcggtg ggaaagaatt tgacctagac
      121 tctgccttgg aatggataaa aaacaaaaga tgcttaactg atatacaaaa agcactaatt
      181 ggtgcctcta tctgcttttt aaaacccaaa gaccaggaaa gaaaaagaag attcatcaca
      241 gagcccctat caggaatggg aacaacagca acaaaaaaga aaggcctgat tctagctgag
      301 agaaaagatga gaagatgtgt gagctttcat gaagcatttg aaatagcaga aggccatgaa
      361 agctcagcgc tactatattg tctcatggtc atgtacctga atcctggaaa ttattcaatg
      421 caagtaaaac taggaacgct ctgtgctttg tgcgagaaac aagcatcaca ttcacacagg
      481 gctcacagca gagcagcgag atcttcagtg cccggagtga gacgagaaat gcagatggtc
      541 tcagctatga acacagcaaa aacaatgaat ggaatgggaa aaggagaaga cgtccaaaaa
      601 ctggcagaag agctgcaaag caacattgga gtcttgagat ctcttggggc aagtcaaaag
      661 aatggggaag gaattgcaaa ggatgtaatg gaagtgctaa agcagagctc tatgggaaat
      721 tcagctcttg tgaagaaata cctataatgc tcgaaccatt tcagattctt tcaatttgtt
      781 cttttatctt atcagctctc catttcatgg cttggacaat agggcatttg aatcaaataa
      841 aaagaggtgt aaacatgaag atacgaataa aaggtccaaa taaagagaca ataaacagag
      901 aggtatcaat tttgagacac agttaccaaa aagaaatcca ggccaaagaa acaatgaagg
      961 aagtactctc tgacaacatg gaagtattga gtgaccacat agtaattgag gggctttctg
     1021 ccgaagagat aataaaaatg ggtgaaacag ttttggaagt agaagaattg cattaa
```

Influenza B virus PB2 gene

```
1 atgacattgg ccaaaattga attgttaaaa caactgctaa gggacaatga agccaaaaca
       61 gttttgaagc aaacaacggt agaccaatat aacataataa gaaaattcaa tacatcaagg
      121 attgaaaaga atccttcact aaggatgaag tgggccatgt gttctaattt tcccttggct
      181 ctaaccaagg gcgatatggc aaatagaatc cccttggaat acaaaggaat acaacttaaa
      241 acaaatgctg aagacatagg aaccaaaggc caaatgtgct caatagcagc agttacttgg
      301 tggaatacat atggaccaat aggagatact gaaggtttcg aaagggtcta cgaaagcttt
      361 tttctcagaa aaatgagact tgacaacgcc acttggggcc gaataacttt tggcccagtt
      421 gaaagagtga gaaaaagggt actgctaaac cctctcacca aggaaatgcc tccggatgag
      481 gcgagcaatg tgataatgga aatattgttc cctaaagaag caggaatacc aagagaatcc
      541 acttggatac atagggaact gataaaagaa aaaagagaaa aattgaaagg aacaatgata
      601 actccaatcg tactggcata catgcttgaa agagaactgg ttgctcgaag aagattcttg
      661 ccagtggcag gagcaacatc agctgagttc atagaaatgc tacactgctt acaaggtgaa
      721 aattggagac aaatatatca cccaggaggg aataaattaa ctgagtctag gtctcaatca
      781 atgatagtag cttgtagaaa aataatcaga agatcaatag tcgcttcaaa cccactggag
      841 ctagctgtag aaattgcaaa caagactgtg atagatactg aaccttttaaa gtcatgtctg
```

FIG. 6A

```
 901 gcagccatag acggaggtga tgtagcttgt gacataataa gagctgcatt aggactaaag
 961 atcagacaaa gacaaagatt tggacggctt gagctaaaaa gaatatcagg aagaggattc
1021 aaaaatgatg aagaaatatt aatagggaac ggaacaatac agaagattgg aatatgggac
1081 ggggaagagg agttccatgt aagatgtggt gaatgcaggg gaatattaaa aaagagtaaa
1141 atgaaactgg aaaaactact gataaattca gccaaaaagg aggatatgag agatttaata
1201 atcttatgca tggtattttc tcaagacact aggatgttcc aaggggtgag aggagaaata
1261 aattttctta atcgagcagg ccaactttta tctccaatgt accaactcca acgatatttt
1321 ttgaatagaa gcaacgacct ttttgatcaa tggggtatg aggaatcacc caaagcaagt
1381 gaactacatg ggataaatga atcaatgaat gcatctgact atacattgaa aggggttgta
1441 gtgacaagaa atgtaattga cgactttagc tctactgaaa cagaaaaagt atccataaca
1501 aaaaatctta gtttaataaa aaggactggg gaagtcataa tgggagctaa tgacgtgagt
1561 gaattagaat cacaagcaca gctgatgata acatatgata cacctaaaat gtgggaaatg
1621 ggaacaacca aagaactggt gcaaaacact tatcaatggg tgctaaaaaa cttggtaaca
1681 ctgaaggctc agtttcttct aggaaaagag gacatgtttc aatgggatgc atttgaagca
1741 tttgagagca taattcctca gaagatggct ggtcagtaca gtggatttgc aagagcagtg
1801 ctcaaacaaa tgagagacca ggaggttatg aaaactgacc agttcataaa gttgttgcct
1861 ttttgtttct caccaccaaa attaaggagc aatggggagc cttatcaatt cttaaaactt
1921 gtgttgaaag gaggagggga aaatttcatc gaagtaagga aagggtcccc tctattttcc
1981 tataatccac aaacagaagt cctaactata tgcggcagaa tgatgtcatt aaaagggaaa
2041 attgaagatg aagaaaggaa tagatcaatg gggaatgcag tattagcagg ctttctcgtt
2101 agtggcaagt atgacccaga tcttggagat ttcaaaacta ttgaagaact tgaaaagctg
2161 aaaccggggg aaaaggcaaa catcttactt tatcaaggaa agccagttaa agtagttaaa
2221 aggaaaaggt atagtgcttt gtccaatgac atttcacaag gaataaagag acaaagaatg
2281 acagttgagt ccatggggtg ggccttgagc taa
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Influenza B virus PA gene

```
1 atgacattgg ccaaaattga attgttaaaa caactgctaa gggacaatga agccaaaaca
  61 gttttgaagc aaacaacggt agaccaatat aacataataa gaaaattcaa tacatcaagg
 121 attgaaaaga atccttcact aaggatgaag tgggccatgt gttctaattt tcccttggct
 181 ctaaccaagg gcgatatggc aaatagaatc ccctttggaat acaaaggaat acaacttaaa
 241 acaaatgctg aagacatagg aaccaaaggc caaatgtgct caatagcagc agttacttgg
 301 tggaatacat atggaccaat aggagatact gaaggtttcg aaagggtcta cgaaagcttt
 361 tttctcagaa aaatgagact tgacaacgcc acttggggcc gaataacttt tggcccagtt
 421 gaaagagtga gaaaaagggt actgctaaac cctctcacca aggaaatgcc tccggatgag
 481 gcgagcaatg tgataatgga aatattgttc cctaaagaag caggaatacc aagagaatcc
 541 acttggatac atagggaact gataaaagaa aaaagagaaa aattgaaagg aacaatgata
 601 actccaatcg tactggcata catgcttgaa agagaactgg ttgctcgaag aagattcttg
 661 ccagtggcag gagcaacatc agctgagttc atagaaatgc tacactgctt acaaggtgaa
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FIG. 6B

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 721 aattggagac aaatatatca cccaggaggg aataaattaa ctgagtctag gtctcaatca
 781 atgatagtag cttgtagaaa aataatcaga agatcaatag tcgcttcaaa cccactggag
 841 ctagctgtag aaattgcaaa caagactgtg atagatactg aacctttaaa gtcatgtctg
 901 gcagccatag acggaggtga tgtagcttgt gacataataa gagctgcatt aggactaaag
 961 atcagacaaa gacaaagatt tggacggctt gagctaaaaa gaatatcagg aagaggattc
1021 aaaaatgatg aagaaatatt aatagggaac ggaacaatac agaagattgg aatatgggac
1081 ggggaagagg agttccatgt aagatgtggt gaatgcaggg gaatattaaa aaagagtaaa
1141 atgaaactgg aaaaactact gataaattca gccaaaaagg aggatatgag agatttaata
1201 atcttatgca tggtattttc tcaagacact aggatgttcc aaggggtgag aggagaaata
1261 aatttcctta atcgagcagg ccaactttta tctccaatgt accaactcca acgatatttt
1321 ttgaatagaa gcaacgacct ttttgatcaa tgggggtatg aggaatcacc caaagcaagt
1381 gaactacatg ggataaatga atcaatgaat gcatctgact atacattgaa aggggttgta
1441 gtgacaagaa atgtaattga cgactttagc tctactgaaa cagaaaaagt atccataaca
1501 aaaaatctta gtttaataaa aaggactggg gaagtcataa tgggagctaa tgacgtgagt
1561 gaattagaat cacaagcaca gctgatgata acatatgata cacctaaaat gtgggaaatg
1621 ggaacaacca aagaactggt gcaaaacact tatcaatggg tgctaaaaaa cttggtaaca
1681 ctgaaggctc agtttcttct aggaaaagag gacatgtttc aatgggatgc atttgaagca
1741 tttgagagca taattcctca gaagatggct ggtcagtaca gtggatttgc aagagcagtg
1801 ctcaaacaaa tgagagacca ggaggttatg aaaactgacc agttcataaa gttgttgcct
1861 ttttgtttct caccaccaaa attaaggagc aatggggagc cttatcaatt cttaaaactt
1921 gtgttgaaag gaggagggga aaatttcatc gaagtaagga aagggtcccc tctattttcc
1981 tataatccac aaacagaagt cctaactata tgcggcagaa tgatgtcatt aaaagggaaa
2041 attgaagatg aagaaaggaa tagatcaatg gggaatgcag tattagcagg ctttctcgtt
2101 agtggcaagt atgacccaga tcttggagat ttcaaaacta ttgaagaact tgaaaagctg
2161 aaaccggggg aaaaggcaaa catcttactt tatcaaggaa agccagttaa agtagttaaa
2221 aggaaaaggt atagtgcttt gtccaatgac atttcacaag gaataaagag acaaagaatg
2281 acagttgagt ccatggggtg ggccttgagc taa
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Influenza C virus M1

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1 caatggcaca tgaaatactg attgctgaaa cagaggcatt tctaaaaaat gttgctcctg
  61 agaccaggac agcaataatt tcagcaataa caggtggaaa atcagcctgc aaatcagcag
 121 ctaaactgat taagaatgaa catcttccct taatgtctgg agaagctacc acaatgcaca
 181 ttgttatgag gtgcttatat cctgaaataa aaccatggaa gaaggcaagc gacatgctga
 241 ataaagcaac ttctagtttg aaaaaatcag aaggaagaga catcagaaag caaatgaaag
 301 cagctggaga cttcttggga gtggagtcaa tgatgaaaat gagggccttc agagatgacc
 361 aaataatgga aatggttgaa gaagtatatg atcacccaga cgactacaca ccagacatcc
 421 gaataggaac aatcacagct tggttgagat gcaaaaacaa gaaaagtgaa agatacagga
 481 gtaatgtctc agaaagtgga cgaacagctt taaaaattca tgaagtaaga aaagccagca
 541 cagcaatgaa cgaaattgct ggtattactg gccttggaga agaagcacta tctctccaaa
 601 gacaaacaga aagtttggcc atattatgca atcacacttt tggaagtaat ataatgagac
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FIG. 6C

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 661 cccacttgga aaaagcaata aaaggagttg aaggcagagt tggagagatg ggacgaatgg
 721 caatgaaatg gttagttgtt ataatatatt tctctataac aagtcaacct gcttctgctt
 781 gcaatctaaa aacctgtcta aacctattta acaatactga tgcagtaact gttcattgtt
 841 ttaatgaaaa ccaaggatac atgctaacat tagcctcttt gggattagga ataattacta
 901 tgttgtattt attagtaaaa atcataattg aacttgtcaa tggttttgtg ctcggcagat
 961 gggagagatg gtgtggagat ataaagacca caattatgcc tgaaattgac tcgatggaaa
1021 aagatattgc cctctctagg gagagacttg acctgggaga ggatgctcct gacgaaaccg
1081 acaactcacc aattcctttt tccaatgatg gtatttttga aatt
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FIG. 6D

INFLUENZA VIRUS REPLICATION BY INHIBITING MICRORNA LEC7C BINDING TO INFLUENZA VIRAL CRNA AND MRNA

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing date of U.S. application Ser. No. 62/169,346, filed on Jun. 1, 2015, the disclosure of which is incorporated by reference herein.

STATEMENT OF GOVERNMENT RIGHTS

The invention was made with Government support under HHSN272201400008C awarded by the National Institutes of Health. The Government has certain rights in the invention.

BACKGROUND

MicroRNAs are single-stranded RNA molecules that are 21-23 nucleotides in length (Wienholds, 2005). Several hundred miRNAs have been identified in plants, animals and viral RNA genomes (Bartel, 2004; Liu et al., 2005). In animals, miRNAs regulate many cellular processes by binding to 3'-UTRs of their target mRNAs, causing translational repression of the target mRNA (Bartel, 2004). Recent studies showed that viral miRNAs play an important role in regulating viral infection in host cells by targeting the cellular or viral genes. Viral miRNAs are capable of controlling expression of viral or cellular genes.

Cellular miRNAs can regulate viral infections. Host-derived miR-24 and miR-93 have been found to target the VSV large protein (L protein) and phosphoprotein (P protein) genes, respectively. A deficiency in miR-24 and miR-93 was responsible for increased vesicular stomatitis virus (VSV) propagation in Dicer1 knockout cells (Otsuka et al., 2007). Furthermore, Lecellier et al. reported that host miR-32 effectively inhibits the accumulation of the retrovirus primate foamy virus type 1 (PFV-1) in human cells by targeting a sequence in the genome of the PFV-1 (Lecellier et al., 2005). Huang et al. reported that a cluster of cellular miRNAs, including miR-28, miR-125b, miR-150, miR-223 and miR-382, targets the 3' ends of various human immunodeficiency virus 1 (HIV-1) mRNAs. These host miRNAs are enriched in resting $CD4^+$ T cells as compared to activated $CD4^+$ T cells, indicating that these cellular miRNAs are important to maintain HIV-1 latency (Huange et al., 2007). Inhibition of miR-122, a cellular miRNA highly and specifically expressed in the human liver, resulted in a marked loss in autonomous replication of hepatitis C viral RNAs, suggesting that miR-122 likely enhances propagation of the virus (Chang et al., 2008; Jopling et al., 2005).

SUMMARY

The invention provides for one or more M gene, PA gene or PB2 gene mutations that enhance the growth of influenza in cells/eggs, and could be incorporated into high-growth influenza vaccines produced through reverse genetics. As disclosed herein, a set of three nucleotide changes (G1012C, A1013U, U1014A) in the let-7c-binding sequence within the 3' UTR of the influenza M segment increased virus replication of a high growth influenza backbone (HY-PR8) in Vero cells, and had an effect in embryonated chicken eggs. The let-7c-binding sequence is also found in the 3' UTR of the PA gene and the PB2 gene, and so mutations in those regions may likewise enhance viral titers. The mutations can therefore be used to increase the yield of influenza vaccine viruses, which can provide for more cost-effective vaccine production, and may be used in combination with other mutations for enhancing growth. A single mutation in the let-7c-binding sequence may provide for increased viral titers, however, by incorporating two or more mutations, e.g., in consecutive (contiguous, adjacent) nucleotides, the reversion rate would likely be less. A single mutation in the let-7c-binding sequence in the 3' UTR of at least two of the M gene, the PA gene and the PB2 aerie, for instance, a different nucleotide is mutated in each binding sequence, or 2 or more mutations in the 3' UTR of at least two of the M gene, the PA gene and the PB2 gene, may also decrease reversion.

The invention provides a recombinant vector and recombinant influenza virus comprising an influenza M gene segment having a nucleotide other than U/T at position 1011, other than G at position 1012, other than A at position 1013, other than U/T at position 1014, other than G at position 1015, other than G at position 1016, other than A at position 1017, or any combination thereof, wherein the numbering refers to $cRNA^+$. In one embodiment, the mutant M gene segment has at least 2, 3 or 4 of the specified nucleotide alterations, e.g., 2, 3 or 4 consecutive nucleotide alterations. In one embodiment, the mutant M gene segment has at least 2, 3 or 4 non-consecutive nucleotide alterations. In one embodiment, the recombinant virus has HA and NA sequences from the same isolate. In one embodiment, the recombinant virus has HA and NA sequences from the same isolate and are from an isolate that is genetically distinct from the vaccine strain providing the PB1, PB2, PA, NP, NS, and M sequences (a 6+2 virus). In one embodiment the HA is H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H16, H17 or H18. In one embodiment, the NA is N1, N2, N3, N4, N5, N6, N7, N8, N9, N10, or N11.

In one embodiment, the invention provides a composition comprising a plurality of influenza virus vectors for a recombinant influenza virus, comprising a vector comprising a promoter operably linked to an influenza virus PA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB1 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB2 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus HA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an Influenza virus NP cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus mutant M cDNA linked to a transcription termination sequence, and a vector comprising a promoter operably linked to an influenza virus NS cDNA linked to a transcription termination sequence, wherein the cDNAs for PB1, PB2, PA, NP, and NS may have sequences for PB1, PB2, PA, NP, and NS that are from one or more influenza viruses that replicate to high titers in embryonated eggs. Vero cells or MDCK cells, wherein the mutant M cDNA has a nucleotide other than U/T at position 1011, a nucleotide other than G at position 1012, a nucleotide other than A at position 1013, a nucleotide other than U/T at position 1014, a nucleotide other than G at position 1015, a nucleotide other than G at position 1016, a nucleotide other than A at position 1017, or any combination thereof, wherein the numbering

refers to cRNA+ and optionally wherein the cDNA for HA and/or NA has sequences for a heterologous HA or NA; and optionally the composition comprises a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB1, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB2, and a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NP, and optionally a vector comprising a promoter operably linked to a DNA segment encoding influenza virus HA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus M1, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus M2, or a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NS2. In one embodiment, the cDNAs for PB1, PB2, PA, NP, NS, and M encode a polypeptide having substantially the same amino acid sequence as a corresponding polypeptide encoded by SEQ ID NOs:1-6. In one embodiment, the cDNA for M has two or more of a nucleotide other than G at position 1012, a nucleotide other than A at position 1013, or a nucleotide other than U at position 1014. In one embodiment, the cDNA for NS encodes a polypeptide having substantially the same amino acid sequence as a corresponding polypeptide encoded by SEQ ID NO:6 and which optionally encodes a Glu at residue 55 of NS1. In one embodiment, the promoter is a RNA polymerase I promoter, a RNA polymerase II promoter, a RNA polymerase III promoter, a T3 promoter or a T7 promoter. In one embodiment, the HA is a type A HA. In one embodiment, the HA is a type B HA. In one embodiment, the NA is N1 or N2. In one embodiment, the HA is R1, H3, H5 or H7. In one embodiment, a plurality of the vectors comprise a RNA polymerase I promoter or a RNA polymerase II promoter. In one embodiment, the RNA polymerase I promoter is a human RNA polymerase I promoter. In one embodiment, ail of the mRNA vectors comprise a RNA polymerase II promoter. In one embodiment, each vector encoding a viral protein is on a separate plasmid. In one embodiment, each vector that expresses vRNA cRNA is on a separate plasmid. In one embodiment, each of the vectors that express viral protein further comprise a RNA transcription termination sequence. In one embodiment, the NA or HA is a chimeric NA or HA. In one embodiment, the cDNA for HA does not encode a polypeptide corresponding to the polypeptide encoded by SEQ ID NO:7 or wherein the cDNA for NA does not encode a polypeptide corresponding to the polypeptide encoded by SEQ ID NO:8. In one embodiment, the HA is an avirulent H5 HA.

Also provided is a method to prepare influenza virus. The method includes: contacting a cell with one or more of: a vector comprising a promoter operably linked to an influenza virus PA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB1 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB2 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus HA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NP cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus M cDNA linked to a transcription termination sequence, and a vector comprising a promoter operably linked to an influenza virus NS cDNA linked to a transcription termination sequence, wherein the cDNAs for PB1, PB2, PA, NP, and NS may have sequences for PB1, PB2, PA, NP, and NS that are from one or more influenza viruses that replicate to high titers in embryonated eggs, Vero cells or MDCK cells, wherein the mutant M cDNA has a nucleotide other than U/T at position 1011, a nucleotide other than G at position 1012, a nucleotide other than A at position 1013, a nucleotide other than U/T at position 1014, a nucleotide other than G at position 1015, a nucleotide other than G at position 1016, a nucleotide other than A at position 1017, or any combination thereof, wherein the numbering refers to cRNA+ and optionally wherein the cDNA for HA and/or NA has sequences for a heterologous HA or NA; and optionally a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB1, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB2, and a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NP, and optionally a vector comprising a promoter operably linked to a DNA segment encoding influenza virus HA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus M1, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus M2, or a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NS2.

In addition, the invention provides an isolated recombinant influenza virus comprising a viral segment for PB1, PB2, PA, and NP that is from an influenza virus that replicates to high titers in embryonated eggs, Vero cells or MDCK cells, a viral segment for M having a nucleotide other than U/T at position 1011, a nucleotide other than G at position 1012, a nucleotide other than A at position 1013, a nucleotide other than U/T at position 1014, a nucleotide other than G at position 1015, a nucleotide other than G at position 1016, a nucleotide other than A at position 1017, or any combination thereof, wherein the numbering refers to cRNA+, a viral segment for NS with a Glu residue at position 55, a viral segment for a heterologous NA, and a viral segment for a heterologous HA.

The invention further provides a recombinant vector and recombinant influenza virus comprising an influenza PA gene segment having a nucleotide other than (3 at position 2210, a nucleotide other than U/T at position 2211, a nucleotide other than T/U at position 2212, a nucleotide other than U/T at position 2213, a nucleotide other than U/T at position 2217, a nucleotide other than A at position 2219, a nucleotide other than U/T at position 2220, a nucleotide other than G at position 2221, a nucleotide other than G at position 2222, a nucleotide other than A at position 2223, or any combination thereof, wherein the numbering refers to cRNA+, In one embodiment, the mutant PA gene segment has at least 2, 3 or 4 of the specified nucleotide alterations, e.g., 2, 3 or 4 consecutive nucleotide alterations. In one embodiment, the mutant PA gene segment has at least 2, 3 or 4 non-consecutive nucleotide alterations. In one embodiment, the recombinant virus has HA and NA sequences from the same isolate. In one embodiment, the recombinant virus has HA and NA sequences from the same isolate and which are from an isolate that is genetically distinct from the

vaccine strain providing the PB1, PB2, PA, NP, NS, and M sequences. In one embodiment, the recombinant virus has HA and NA sequences from the same isolate. In one embodiment, the recombinant virus has HA and NA sequences from the same isolate and which are from an isolate that is genetically distinct from the vaccine strain providing the PB1, PB2, PA, NP, NS, and M sequences. In one embodiment the HA is H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H16, H17 or H18. In one embodiment, the NA is N1, N2, N3, N4, N5, N6, N7, N8, N9, N10, or N11. In one embodiment, the isolated recombinant influenza virus has a heterologous HA gene segment, a heterologous NA gene segment, a chimeric HA gene segment, a chimeric NA gene. Methods of using the vector and virus are also provided.

The invention also provides a recombinant vector and recombinant influenza virus comprising an influenza PB2 gene segment having a nucleotide other than G at position 2326, a nucleotide other than U/T at position 2328, a nucleotide other than G at position 2329, a nucleotide other than G at position 2330, a nucleotide other than A at position 2331, or any combination thereof, wherein the numbering refers to $cRNA^{+}$. In one embodiment, the mutant PB2 gene segment has at least 2, 3 or 4 of the specified nucleotide alterations, e.g., 2, 3 or 4 consecutive nucleotide alterations. In one embodiment, the mutant PB2 gene segment has at least 2, 3 or 4 non-consecutive nucleotide alterations. In one embodiment, the recombinant virus has HA and NA sequences from the same isolate. In one embodiment, the recombinant virus has HA and NA sequences from the same isolate and which are from an isolate that is genetically distinct from the vaccine strain providing the PB1, PB2, PA, NP, NS, and M sequences. In one embodiment, the recombinant virus has HA and NA sequences from the same isolate. In one embodiment, the recombinant virus has HA and NA sequences from the same isolate and which are from an isolate that is genetically distinct from the vaccine strain providing the PB1, PB2, PA, NP, NS, and M sequences. In one embodiment the HA is H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H16, H17 or H18. In one embodiment, the NA is N1, N2, N3, N4, N5, N6, N7, N8, N9, N10, or N11. Methods of using the vector and virus are also provided.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1**. Growth curve of M mutant recombinant virus in Vero cells and eggs.

FIG. **2**. Exemplary influenza M gene segment sequences (let-7c binding sites are underlined) (SEQ ID NOs: 16-17).

FIG. **3**, Let-7c target site prediction in the vRNA of influenza A virus M gene. Let-7c was predicted to pair with residues in the 3' region of cRNA of M1 (SEQ ID NOs: 18-19).

FIG. **4**. Nucleotide sequence for PR8 (Cambridge) internal genes (SEQ ID Nos. 10-15).

FIGS. **5**A-C. Exemplary 3' UTR influenza PA and PB2 gene segment e and full-length sequences (let-7c binding sites are underlined) (SEQ ID NOs: 20-23).

FIGS. **6**A-**6**D. Exemplary influenza B gene M, PA and PB2 sequences and influenza. C virus M1 sequence.

DETAILED DESCRIPTION

Definitions

As used herein, the term "isolated" refers to in vitro preparation and/or isolation of a nucleic acid molecule, e.g., vector or plasmid, peptide or polypeptide (protein), or virus of the invention, so that it is not associated with in vivo substances, or is substantially purified from in vitro substances. An isolated virus preparation is generally obtained by in vitro culture and propagation, and/or via passage in eggs, and is substantially free from other infectious agents.

As used herein, "substantially purified" means the object species is the predominant species, e.g., on a molar basis it is more abundant than any other individual species in a composition, and preferably is at least about 80% of the species present, and optionally 90% or greater, e.g., 95%, 98%, 99% or more, of the species present in the composition.

As used herein, "substantially free" means below the level of detection for a particular infectious agent using standard detection methods for that agent.

A "recombinant" virus is one which has been manipulated in vitro, e.g., using recombinant DNA techniques, to introduce changes to the viral genome. Reassortant viruses can be prepared by recombinant or nonrecombinant techniques.

As used herein, the term "recombinant nucleic acid" or "recombinant DNA sequence or segment" refers to a nucleic acid, e.g., to DNA, that has been derived or isolated from a source, that may be subsequently chemically altered in vitro, so that its sequence is not naturally occurring, or corresponds to naturally occurring sequences that are not positioned as they would be positioned in the native genome. An example of DNA "derived" from a source, would be a DNA sequence that is identified as a useful fragment, and which is then chemically synthesized in essentially pure form. An example of such DNA "isolated" from a source would be a useful DNA sequence that is excised or removed from said source by chemical means, e.g., by the use of restriction endonucleases, so that it can be further manipulated, e.g., amplified, for use in the invention, by the methodology of genetic engineering.

As used herein, a "heterologous" influenza virus gene or gene segment is from an influenza virus source that is different than a majority of the other influenza viral genes or gene segments in a recombinant, e.g., reassortant, influenza virus.

The terms "isolated polypeptide", "isolated peptide" or "isolated protein" include a polypeptide, peptide or protein encoded by cDNA or recombinant RNA including one of synthetic origin, or some combination thereof.

The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule expressed from a recombinant DNA molecule. In contrast, the term "native protein" is used herein to indicate a protein isolated from a naturally occurring (i.e., a nonrecombinant) source. Molecular biological techniques may be used to produce a recombinant form of a protein with identical properties as compared to the native form of the protein.

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent identity between any two sequences can be accomplished using a mathematical algorithm.

Computer implementations f these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Alignments using these programs can be performed using the default parameters. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The algorithm may involve first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold

score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold. These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always>0) and N (penalty score for mismatching residues; always<0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when the cumulative alignment score falls off by the quantity X from its maximum achieved value, the cumulative score goes to zero or below due to the accumulation of one or more negative-scoring residue alignments, or the end of either sequence is reached.

In addition to calculating percent sequence identity, the BLAST algorithm may also perform a statistical analysis of the similarity between two sequences. One measure of similarity provided by the BLAST algorithm may be the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino add sequences would occur by chance. For example, a test nucleic acid sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid sequence to the reference nucleic acid sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

The BLASTN program (for nucleotide sequences) may use as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=−4, and a comparison of both strands. For amino acid sequences, the BLASTP program may use as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix. See http://www.ncbi.n1m.nih.gov. Alignment may also be performed manually by inspection.

For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Influenza Virus Structure and Propagation

Influenza A viruses possess a genome of eight single-stranded negative-sense viral RNAs (vRNAs) that encode at least ten proteins. The influenza virus life cycle begins with binding of the hemagglutinin (HA) to sialic acid-containing receptors on the surface of the host cell, followed by receptor-mediated endocytosis. The low pH in late endosomes triggers a conformational shift in the HA, thereby exposing the N-terminus of the HA2 subunit (the so-called fusion peptide). The fusion peptide initiates the fusion of the viral and endosomal membrane, and the matrix protein (M1) and RNP complexes are released into the cytoplasm. RNPs consist of the nucleoprotein (NP), which encapsidates vRNA, and the viral polymerase complex, which is formed by the PA. PB1, and PB2 proteins, RNPs are transported into the nucleus, where transcription and replication take place. The RNA polymerase complex catalyzes three different reactions: synthesis of an mRNA with a 5' cap and 3' polyA structure, of a full-length complementary RNA (cRNA), and of genomic vRNA using the cRNA as a template. Newly synthesized vRNAs, NP, and polymerase proteins are then assembled into RNPs, exported from the nucleus, and transported to the plasma membrane, where budding of progeny virus particles occurs. The neuraminidase (NA) protein plays a crucial role late in infection by removing sialic acid from sialyloligosaccharides, thus releasing newly assembled virions from the cell surface and preventing the self aggregation of virus particles. Although virus assembly involves protein-protein and protein-vRNA interactions, the nature of these interactions is largely unknown.

Although influenza B and C viruses are structurally and functionally similar to influenza A virus, there are some differences. For example, influenza B virus does not have a M2 protein with ion channel activity but has BM2 and has a gene segment with both NA and NB sequences. Influenza C virus has only seven gene segments.

Cell Lines that can be Used in the Present Invention

Any cell, e.g., any avian or mammalian cell, such as a human, e.g., 293T or PER.C6® cells, or canine, e.g., MDCK, bovine, equine, feline, swine, ovine, rodent, for instance mink, e.g., MvLu1 cells, or hamster, e.g., CHO cells, or non-human primate, e.g., Vero cells, including mutant cells, which supports efficient replication of influenza virus can be employed to isolate and/or propagate influenza viruses. Isolated viruses can be used to prepare a reassortant virus. In one embodiment, host cells for vaccine production are continuous mammalian or avian cell lines or cell strains. A complete characterization of the cells to be used, may be conducted so that appropriate tests for purity of the final product can be included. Data that can be used for the characterization of a cell includes (a) information on its origin, derivation, and passage history; (b) information on its growth and morphological characteristics; (c) results of tests of adventitious agents; (d) distinguishing features, such as biochemical, immunological, and cytogenetic patterns which allow the cells to be clearly recognized among other cell lines; and (e) results of tests for tumorigenicity. In one embodiment, the passage level, or population doubling, of the host cell used is as low as possible.

In one embodiment, the cells are WHO certified, or certifiable, continuous cell lines. The requirements for certifying such cell lines include characterization with respect to at least one of genealogy, growth characteristics, immunological markers, virus susceptibility tumorigenicity and storage conditions, as well as by testing in animals, eggs, and cell culture. Such characterization is used to confirm that the cells are free from detectable adventitious agents. In some countries, karyology may also be required. In addition, tumorigenicity may be tested in cells that are at the same passage level as those used for vaccine production, The virus may be purified by a process that has been shown to give consistent results, before vaccine production (see, e.g., World Health Organization, 1982).

Virus produced by the host cell may be highly purified prior to vaccine or gene therapy formulation, Generally, the purification procedures result in extensive removal of cellular DNA and other cellular components, and adventitious agents. Procedures that extensively degrade or denature DNA may also be used.

Influenza Vaccines

A vaccine of the invention includes an isolated recombinant influenza virus of the invention, and optionally one or more other isolated viruses including other isolated influenza viruses, one or more immunogenic proteins or glycoproteins of one or more isolated influenza viruses or one or more other pathogens, e.g., an immunogenic protein from one or more bacteria, non-influenza viruses, yeast or fungi,

or isolated nucleic acid encoding one or more viral proteins (e.g., DNA vaccines) including one or more immunogenic proteins of the isolated influenza virus of the invention. In one embodiment, the influenza viruses of the invention may be vaccine vectors for influenza virus or other pathogens.

A complete virion vaccine may be concentrated by ultrafiltration and then purified by zonal centrifugation or by chromatography. Viruses other than the virus of the invention, such as those included in a multivalent vaccine, may be inactivated before or after purification using formalin beta-propiolactone, for instance.

A subunit vaccine comprises purified glycoproteins. Such a vaccine may be prepared as follows: using viral suspensions fragmented by treatment with detergent, the surface antigens are purified, by ultracentrifugation for example. The subunit vaccines thus contain mainly HA protein, and also NA. The detergent used may be cationic detergent for example, such as hexadecyl trimethyl ammonium bromide (Bachmeyer, 1975), an anionic detergent such as ammonium deoxycholate (layer & Webster, 1976); or a nonionic detergent such as that commercialized under the name TRITON X100. The hemagglutinin may also be isolated after treatment of the virions with a protease such as bromelin, and then purified. The subunit vaccine may be combined with an attenuated virus of the invention in a multivalent vaccine.

A split vaccine comprises virions which have been subjected to treatment with agents that dissolve lipids. A split vaccine can be prepared as follows: an aqueous suspension of the purified virus obtained as above, inactivated or not, is treated, under stirring, by lipid solvents such as ethyl ether or chloroform, associated with detergents. The dissolution of the viral envelope lipids results in fragmentation of the viral particles. The aqueous phase is recuperated containing the split vaccine, constituted mainly of hemagglutinin and neuraminidase with their original lipid environment removed, and the core or its degradation products. Then the residual infectious particles are inactivated if this has not already been done. The split vaccine may be combined with an attenuated virus of the invention in a multivalent vaccine.

Inactivated Vaccines.

Inactivated influenza virus vaccines are provided by inactivating replicated virus using known methods, such as, but not limited to, formalin or β-propiolactone treatment. Inactivated vaccine types that can be used in the invention can include whole-virus (WV) vaccines or subvirion (SV) (split) vaccines. The WV vaccine contains intact, inactivated virus, while the SV vaccine contains purified virus disrupted with detergents that solubilize the lipid-containing viral envelope, followed by chemical inactivation of residual virus.

In addition, vaccines that can be used include those containing the isolated HA and NA surface proteins, which are referred to as surface antigen or subunit vaccines,

Live Attenuated Virus Vaccines.

Live, attenuated influenza virus vaccines, such as those including a recombinant virus of the invention can be used for preventing or treating influenza virus infection. Attenuation may be achieved in a single step by transfer of attenuated genes from an attenuated donor virus to a replicated isolate or reassorted virus according to known methods. Since resistance to influenza A virus is mediated primarily by the development of an immune response to the HA and/or NA glycoproteins, the genes coding for these surface antigens come from the reassorted viruses or clinical isolates. The attenuated genes are derived from an attenuated parent. In this approach, genes that confer attenuation generally do not code for the HA and NA glycoproteins.

Viruses (donor influenza viruses) are available that are capable of reproducibly attenuating influenza viruses, e.g., a cold adapted (ca) donor virus can be used for attenuated vaccine production. Live, attenuated reassortant virus vaccines can be generated by mating the ca donor virus with a virulent replicated virus. Reassortant progeny are then selected at 25° C. (restrictive for replication of virulent virus), in the presence of an appropriate antiserum, which inhibits replication of the viruses bearing the surface antigens of the attenuated ca donor virus. Useful reassortants are: (a) infectious, (b) attenuated for seronegative non-adult mammals and immunologically primed adult mammals, (c) immunogenic and (d) genetically stable. The immunogenicity of the ca reassortants parallels their level of replication. Thus, the acquisition of the six transferable genes of the ca donor virus by new wild-type viruses has reproducibly attenuated these viruses for use in vaccinating susceptible mammals both adults and non-adult.

Other attenuating mutations can be introduced into influenza virus genes by site-directed mutagenesis to rescue infectious viruses bearing these mutant genes. Attenuating mutations can be introduced into non-coding regions of the genome, as well as into coding regions. Such attenuating mutations can also be introduced into genes other than the HA or NA, e.g., the PB2 polymerase gene. Thus, new donor viruses can also be generated bearing attenuating mutations introduced by site-directed mutagenesis, and such new donor viruses can be used in the production of live attenuated reassortants vaccine candidates in a manner analogous to that described above for the ca donor virus. Similarly, other known and suitable attenuated donor strains can be reassorted with influenza virus to obtain attenuated vaccines suitable for use in the vaccination of mammals.

In one embodiment, such attenuated viruses maintain the genes from the virus that encode antigenic determinants substantially similar to those of the original clinical isolates. This is because the purpose of the attenuated vaccine is to provide substantially the same antigenicity as the original clinical isolate of the virus, while at the same time lacking pathogenicity to the degree that the vaccine causes minimal chance of inducing a serious disease condition in the vaccinated mammal.

The viruses in a multivalent vaccine can thus be attenuated or inactivated, formulated and administered, according to known methods, as a vaccine to induce an immune response in an animal, e.g., a mammal. Methods are well-known in the art for determining whether such attenuated or inactivated vaccines have maintained similar antigenicity to that of the clinical isolate or high growth strain derived therefrom, Such known methods include the use of antisera or antibodies to eliminate viruses expressing antigenic determinants of the donor virus; chemical selection (e.g., amantadine or rimantidine); HA and NA activity and inhibition; and nucleic acid screening (such as probe hybridization or PCR) to confirm that donor genes encoding the antigenic determinants (e.g., HA or NA genes) are not present in the attenuated viruses,

Pharmaceutical Compositions

Pharmaceutical compositions of the present invention, suitable for inoculation, e.g., nasal, parenteral or oral administration, comprise one or more influenza virus isolates, e.g., one or more attenuated or inactivated influenza viruses, a subunit thereof, isolated protein(s) thereof, and/or isolated nucleic acid encoding one or more proteins thereof, optionally further comprising sterile aqueous or non-aqueous solutions, suspensions, and emulsions. The compositions can further comprise auxiliary agents or excipients, as known in

the art. The composition of the invention is generally presented in the form of individual doses (unit doses).

Conventional vaccines generally contain about 0.1 to 200 μg, e.g., 30 to 100 μg, of HA from each of the strains entering into their composition. The vaccine forming the main constituent of the vaccine composition of the invention may comprise a single influenza virus, or a combination of influenza viruses, for example, at least two or three influenza viruses, including one or more reassortant(s).

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and/or emulsions, which may contain auxiliary agents or excipients known in the art. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Carriers or occlusive dressings can be used to increase skin permeability and enhance antigen absorption. Liquid dosage forms for oral administration may generally comprise a liposome solution containing the liquid dosage form. Suitable forms for suspending liposomes include emulsions, suspensions, solutions, syrups, and elixirs containing inert diluents commonly used in the art, such as purified water. Besides the inert diluents, such compositions can also include adjuvants, wetting agents, emulsifying and suspending agents, or sweetening, flavoring, or perfuming agents.

When a composition of the present invention is used for administration to an individual, it can further comprise salts, buffers, adjuvants, or other substances which are desirable for improving the efficacy of the composition. For vaccines, adjuvants, substances which can augment a specific immune response, can be used. Normally, the adjuvant and the composition are mixed prior to presentation to the immune system, or presented separately, but into the same site of the organism being immunized.

Heterogeneity in a vaccine may be provided by mixing replicated influenza viruses for at least two influenza virus strains, such as 2-20 strains or any range or value therein. Vaccines can be provided for variations in a single strain of an influenza virus, using techniques known in the art.

A pharmaceutical composition according to the present invention may further or additionally comprise at least one chemotherapeutic compound, for example, for gene therapy, immunosuppressants, anti-inflammatory agents or immune enhancers, and for vaccines, chemotherapeutics including, but not limited to, gamma globulin, amantadine, guanidine, hydroxybenzimidazole, interferon-α, interferon-β, interferon-γ, tumor necrosis factor-alpha, thiosemicarbarzones, methisazone, rifampin, ribavirin, a pyrimidine analog, a purine analog, foscarnet, phosphonoacetic add, acyclovir, dideoxynucleosides, a protease inhibitor, or ganciclovir.

The composition can also contain variable but small quantities of endotoxin-free formaldehyde, and preservatives, which have been found safe and not contributing to undesirable effects in the organism to which the composition is administered.

Pharmaceutical Purposes

The administration of the composition (or the antisera that it elicits) may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the compositions of the invention which are vaccines are provided before any symptom or clinical sign of a pathogen infection becomes manifest. The prophylactic administration of the composition serves to prevent or attenuate any subsequent infection. When provided prophylactically, the gene therapy compositions of the invention, are provided before any symptom or clinical sign of a disease becomes manifest. The prophylactic administration of the composition serves to prevent or attenuate one or more symptoms or clinical signs associated with the disease.

When provided therapeutically, a viral vaccine is provided upon the detection of a symptom or clinical sign of actual infection. The therapeutic administration of the compound(s) serves to attenuate any actual infection. When provided therapeutically, a gene therapy composition is provided upon the detection of a symptom or clinical sign of the disease. The therapeutic administration of the compound(s) serves to attenuate a symptom or clinical sign of that disease.

Thus, a vaccine composition of the present invention may be provided either before the onset of infection (so as to prevent or attenuate an anticipated infection) or after the initiation of an actual infection. Similarly, for gene therapy, the composition may be provided before any symptom or clinical sign of a disorder or disease is manifested or after one or more symptoms are detected.

A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient mammal. Such an agent is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. A composition of the present invention is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient, e.g., enhances at least one primary or secondary humoral or cellular immune response against at least one strain of an infectious influenza virus.

The "protection" provided need not be absolute, i.e., the influenza infection need not be totally prevented or eradicated, if there is a statistically significant improvement compared with a control population or set of mammals. Protection may be limited to mitigating the severity or rapidity of onset of symptoms or clinical signs of the influenza virus infection.

Pharmaceutical Administration

A composition of the present invention may confer resistance to one or more pathogens, e.g., one or more influenza virus strains, by either passive immunization or active immunization. In active immunization, an attenuated live vaccine composition is administered prophylactically to a host (e.g., a mammal), and the host's immune response to the administration protects against infection and/or disease. For passive immunization, the elicited antisera can be recovered and administered to a recipient suspected of having an infection caused by at least one influenza virus strain. A gene therapy composition of the present invention may yield prophylactic or therapeutic levels of the desired gene product by active immunization.

In one embodiment, the vaccine is provided to a mammalian female (at or prior to pregnancy or parturition), under conditions of time and amount sufficient to cause the production of an immune response which serves to protect both the female and the fetus or newborn (via passive incorporation of the antibodies across the placenta or in the mother's milk).

The present invention thus includes methods for preventing or attenuating a disorder or disease, e.g., an infection by at least one strain of pathogen. As used herein, a vaccine is said to prevent or attenuate a disease if its administration results either in the total or partial attenuation (i.e., suppression) of a clinical sign or condition of the disease, or in the total or partial immunity of the individual to the disease. As used herein, a gene therapy composition is said to prevent or attenuate a disease if its administration results either in the total or partial attenuation (i.e., suppression) of a clinical

sign or condition of the disease, or in the total or partial immunity of the individual to the disease.

A composition having at least one influenza virus of the present invention, including one which is attenuated and one or more other isolated viruses, one or more isolated viral proteins thereof, one or more isolated nucleic acid molecules encoding one or more viral proteins thereof, or a combination thereof, may be administered by any means that achieve the intended purposes.

For example, administration of such a composition may be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, oral or transdermal routes. Parenteral administration can be accomplished by bolus injection or by gradual perfusion over time.

A typical regimen for preventing, suppressing, or treating an influenza virus related pathology, comprises administration of an effective amount of a vaccine composition as described herein, administered as a single treatment, or repeated as enhancing or booster dosages, over a period up to and including between one week and about 24 months, or any range or value therein.

According to the present invention, an "effective amount" of a composition is one that is sufficient to achieve a desired effect. It is understood that the effective dosage may be dependent upon the species, age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect wanted. The ranges of effective doses provided below are not intended to limit the invention and represent dose ranges.

The dosage of a live, attenuated or killed virus vaccine for an animal such as a mammalian adult organism may be from about 10^2-10^{15}, e.g., 10^3-10^{12}, plaque forming units (PFU)/kg, or any range or value therein. The dose of inactivated vaccine may range from about 0.1 to 1000, e.g., 30 to 100 μg, of HA protein. However, the dosage should be a safe and effective amount as determined by conventional methods, using existing vaccines as a starting point.

The dosage of immunoreactive HA in each dose of replicated virus vaccine may be standardized to contain a suitable amount, e.g., 30 to 100 μg or any range or value therein, or the amount recommended by government agencies or recognized professional organizations. The quantity of NA can also be standardized, however, this glycoprotein may be labile during purification and storage.

The dosage of immunoreactive HA in each dose of replicated virus vaccine can be standardized to contain a suitable amount, e.g., 1-50 μg or any range or value therein, or the amount recommended by the U.S. Public Health Service (PHS), which is usually 15 μg per component for older children (greater than or equal to 3 years of age), and 7.5 μg per component for children less than 3 years of age. The quantity of NA can also be standardized, however, this glycoprotein can be labile during the processor purification and storage (Kendal et al., 1980; Kerr et al., 1975). Each 0.5-ml dose of vaccine may contains approximately 1-50 billion virus particles, and preferably 10 billion particles.

Mutations that Enhance Replication

Mutations that increase the replicative ability of viruses in cell culture and/or embryonated chicken eggs, including mutations in non-coding sequences, are useful to amplify influenza viruses and to establish robust influenza vaccine platforms. Currently, most influenza vaccines are generated in embryonated chicken eggs. Influenza vaccines generated in MDCK cells are now approved for human use in the U.S. and in Europe, and influenza vaccines derived from Vero cells are approved for human use in Europe.

The mutations may be generated by specifically altering one or more nucleotides or by random mutagenesis. For instance, virus libraries possessing random mutations in the 'internal' viral genes (i.e., all viral genes except those encoding the viral surface glycoproteins HA and NA) were generated using a vaccine virus isolate, e.g., UW-PR8, were generated and passaged in MDCK cells. The identified mutations result in higher virus titers in MDCK cells (and may also increase virus titers in Vero cells and/or embryonated chicken eggs), allowing more efficient influenza virus growth and more cost-effective vaccine production. In addition to mutations in the coding regions of the six internal gene segments, mutations in non-coding regions were observed to increase viral titers, including promoter mutations, for instance, C-to-U mutations at position 4 from the 3' end of the PB2. PB1, and/or PA vRNA segments. The resulting sequences may be also codon-usage optimized, e.g., optimized for expression in mammalian cells such as canine cells or primate cells, or avian cells, e.g., chicken embryos. As disclosed herein, mutations in the 3' UTR of the M gene segment corresponding to the cellular microRNA let-7c binding site in cRNA/mRNA can increase viral titers. For example, three nucleotide changes were introduced into the 3' UTR of the influenza M segment to alter the sequence targeted by let-7c. A virus possessing the respective mutations grew to higher titers in Vero cells and embryonated chicken egos when compared to wild-type virus. The respective mutation(s) in the M segment can therefore be used to increase the yield of influenza vaccine viruses and optionally be employed with other mutations that enhance vaccine virus properties, e.g., the substitutions discussed below and promoter mutation mentioned above. Thus, the mutations can be used in various combinations, with results influenced by the cell line (or egg) in use and the desired level of improvement in the replication of the virus.

In one embodiment, the invention provides isolated recombinant, e.g., reassortant, influenza viruses with one or more mutations in the 3' UTR of M, PA and/or PB2 and selected amino acid residues at one or more specified positions in one or more gene segments for PA, PB1, PB2, NP, M (encoding M1 and M2 proteins), and/or NS (encoding NS1 and NS2 proteins), e.g., in selected amino acid residues at specified positions of PB1, PB2 and NS1; PA, PB1, PB2, NP and NS1; PB1, PB2, NP, M, and NS1; PA, PB2, NP and NS1; or PA, PB1, PB2, NP, M, and NS1, and including HA and NA genes/proteins of interest, e.g., from annual and pandemic strains, which viruses are produced more efficiently and cost-effectively via cell culture (in MDCK or Vero cells) or in embryonated chicken eggs. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 142 in PA that results in enhanced growth in cells including MDCK cells, Vero cells or eggs relative to a corresponding virus with, for instance, a lysine at position 142 in PA, i.e., the residue at position 142 in PA in the PA gene segment in the recombinant influenza virus is not lysine but is a residue that is correlated with enhanced replication in MDCK cells, Vero cells or eggs, as well as optionally selected amino acid residues at one or more specified positions in PB1, PB2, NP, M1 and/or NS1. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 142 in PA that results in enhanced interaction with one or more host proteins in MDCK cells, Vero cells or eggs relative to a corresponding virus with, for instance, a lysine at position 142 in PA. In one embodiment, the recombinant reassortant influenza virus has an asparagine or glutamine at position 142 in PA as well as optionally selected amino acid residues

at one or more specified positions in PB1, PB2, NP, M1 and/or NS1. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 247 in PB1 that results in enhanced growth in cells including MDCK cells, Vero cells or eggs, relative to a corresponding virus with, for instance, a glutamine at position 247 in PB1, i.e., the residue at position 247 in PB1 in the PB1 gene segment in the recombinant influenza virus is not glutamine but is a residue that is correlated with enhanced replication in MDCK cells, Vero cells or eggs, as well as optionally selected amino acid residues at one or more specified positions PA, PB2, NP, M1 and/or NS1 which have are described herein. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 247 in PB1 that results in enhanced interaction with one or more host proteins in MDCK cells. Vero cells or eggs relative to a corresponding virus with, for instance, a glutamine at position 247 in PB1. In one embodiment, the recombinant reassortant influenza virus has a histidine, arginine lysine at position 247 in PB1 as well as optionally selected amino acid residues at one or more specified positions PA, PB2, NP, M1 and/or NS1 which are described herein. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 202 and/or position 323 in PB2 that results in enhanced growth in cells including MDCK cells, Vero cells or eggs, relative to a corresponding virus with, for instance, a methionine at position 202 or a phenylalanine at position 323 in PB2, i.e., the residue at position 202 and/or 323 in PB2 in the PB2 gene segment in the recombinant influenza virus is not methionine phenylalanine but is a residue that is correlated with enhanced replication in MDCK cells, Vero cells or eggs, as well as optionally selected amino acid residues at one or more specified positions PA, PB1, NP, M1 and/or NS which are described herein. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 323 in PB2 that results in an altered cap binding interaction relative to a corresponding virus with, for instance, a phenylalanine at position 323 in PB2. In one embodiment, the recombinant reassortant influenza virus has a leucine, alanine, threonine, valine, isoleucine, or glycine, at position 202 and/or position 323 in PB2 as well as optionally selected amino acid residues at one or more specified positions PA, PB1, NP, M1 and/or NS which are described herein. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 74 in NP that results in enhanced growth in cells including MDCK cells, Vero cells or eggs relative to a corresponding virus with, for instance, an arginine at position 74 in NP, i.e., the residue at position 74 in NP in the NP gene segment in the recombinant influenza virus is not arginine but is a residue that is correlated with enhanced replication in MDCK cells, Vero cells or eggs, as well as optionally selected amino acid residues at one or more specified positions PA, PB1, PB2, M1 and/or NS which are described herein. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 74 in NP that may alter folding, stability and/or interaction with other viral or host proteins relative to a corresponding virus with, for instance, an arginine at position 74 in NP. In one embodiment, the recombinant reassortant influenza virus has a lysine histidine at position 74 in NP as well as optionally selected amino acid residues at one or more specified positions PA, PB1, PB2, M1 and/or NS which are described herein. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 97 and/or position 100 in M1 that results in enhanced growth in cells including MDCK cells, Vero cells or eggs, relative to a corresponding virus with, for instance, a valine at position 97 or a tyrosine at position 100 in M1, i.e., the residue at position 97 and/or 100 in M1 in the M gene segment in the recombinant influenza virus is not valine or tyrosine, respectively, hut is a residue that is correlated with enhanced replication in MDCK cells, Vero cells or eggs, as well as selected amino acid residues at one or more specified positions PA, PB1, PB2, NP and/or NS1 which are described herein. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 97 in M1 that may alter dimerization relative to a corresponding virus with, for instance, a valine at position 97 in M1. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 100 in M1 that may alter virus assembly relative to a corresponding virus with, for instance, a tyrosine at position 100 in M1, In one embodiment, the recombinant reassortant influenza virus has a leucine, threonine, isoleucine, alanine, or glycine, at position 97 and/or a lysine, arginine, or histidine at position 100 in M1 as well as selected amino acid residues at one or more specified positions PA, PB1, PB2, NP and/or NS1 which are described herein. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 55 in NS1 that results in enhanced growth in cells including MDCK cells, Vero cells or eggs relative to a corresponding virus with, for instance, a lysine at position 55 in NS1, as well as selected amino acid residues at one or more specified positions PA, PB1, PB2, NP and/or M1 which are described herein. In one embodiment, the recombinant reassortant influenza virus has an asparagine, aspartic acid, glutamic acid or glutamine at position 55 in NS1 as well as selected amino acid residues at one or more specified positions PA, PB1, PB2, NP and/or M1 which are described herein. In one embodiment, the invention provides an isolated recombinant reassortant influenza virus having six "internal" gene segments from a vaccine influenza virus with two or more of the selected amino acid residues at specified positions described herein, and a NA gene segment selected from a first influenza virus isolate, and a HA gene segment from the same isolate or a different isolate.

In one embodiment, the influenza virus of the invention is a recombinant influenza virus with one or more mutations in the 3' UTR of M, PA and/or PB2 and having two or more of selected amino acid residues at specified positions in one or more gene segments for PA, PB1, PB2, NP, M1, and/or NS1, which can be employed with HA and NA genes of interest. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 142 in PA that results in enhanced growth in MDCK cells, Vero cells or eggs relative to a corresponding virus with, for instance, a lysine at position 142 in PA; an amino acid residue at position 247 in PB1 that results in enhanced growth in MDCK cells, Vero cells or eggs relative to a corresponding virus with, for instance, a glutamine at position 247 in PB1; an amino acid residue at position 202 and/or position 323 in PB2 that results in enhanced growth in MDCK cells, Vero cells or eggs relative to a corresponding virus with, for instance, a methionine at position 202 or a phenylalanine at position 323 in PB2; an amino acid residue at position 74 in NP that results in enhanced growth in MDCK cells, Vero cells or eggs relative to a corresponding virus with, for instance, a arginine at position 74 in NP; an amino acid residue at position 97 and/or position 100 in M1 that results in enhanced growth in MDCK cells, Vero cells or eggs relative to a corresponding virus with, for instance, a valine at position 97 or a tyrosine at position 100 in M1; or an

amino acid residue at position 55 in NS1 that results in enhanced growth in MDCK cells, Vero cells or eggs relative to a corresponding virus with, for instance, a lysine at position 55 in NS1, or combinations thereof.

In one embodiment, the influenza virus of the invention is a recombinant influenza virus with one or more mutations in the 3' UTR of M, PA and/or PB2 and having two or more of selected amino acid residues at specified positions in one or more gene segments for PA, PB1, PB2, NP, M1, and/or NS1, which can be employed with HA and NA genes of interest. In one embodiment, the recombinant reassortant influenza virus has two or more of a lysine at position 142 in PA; a glutamine at position 247 in PB1; a leucine at position 202 and/or at position 323 in PB2; a lysine at position 74 in NP; an alanine at position 97 and an histidine at position 100 in M1; or a glutamic acid at position 55 in NS1.

The invention provides isolated recombinant, e.g., reassortant, influenza viruses with one or more mutations in the 3' UTR of M, PA and/or PB2 and with one or more mutations in the 3' UTR of M, PA and/or PB2 and with selected amino acid residues at one or more specified positions in one or more gene segments for PA, PB1, PB2, NP, M1, and/or NS1, e.g., in selected amino acid residues at specified positions PB1, PB2 and NS; PB1, PB2, NP and NS; PA, PB1, PB2, NP and NS; PB1, PB2, NP, M and NS; or PA, PB1, PB2, NP, M, and NS, that include one or more of the characteristic residues described herein. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 105 and/or 401 in PA that results in enhanced growth in cells, e.g., MDCK cells, relative to a corresponding virus with, for instance, a phenylalanine or arginine at position 105 or 401, respectively, in PA. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 40, 54, 59, 62, 63, 66 (F2), 73 (F2), 75, 76, 78, 79, 80, 112, 180, 327, 507, 624, 644, 667, 694, 695, 697, 699, 700, 701, 702, 705, 713, and/or 714 in PB1 that results in enhanced growth in cells, e.g., MDCK cells, relative to a corresponding virus with, for instance, a methionine, arginine, threonine, glycine, alanine, asparagine, lysine, glutamic acid, aspartic acid, glutamic acid, proline, serine, glutamic acid, glycine, isoleucine, methionine, leucine, valine, isoleucine, asparagine, leucine, glutamic acid, phenyalanine, phenylalanine, proline, serine, tyrosine, serine or methionine, at position 40, 54, 59, 62, 63, 66 (F2), 73 (F2), 75, 76, 78, 79, 80, 112, 180, 504, 507, 624, 644, 667, 694, 695, 697, 699, 700, 701, 702, 705, 713, or 714, respectively, in PB1. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 57, 58, 59, 61, 66, 202, 323, 368, 391, 504, 591, 677, 678, or 679 in PB2 that results in enhanced growth in cells, e.g., MDCK cells, relative to a corresponding virus with, for instance, an isoleucine, threonine, alanine, lysine, methionine, methionine, phenylalanine, arginine, glutamic acid, isoleucine, glutamine, glutamic acid, aspartic acid or phenylalanine, at position 57, 58, 59, 61, 66, 202, 323, 368, 391, 504, 591, 677, 678 or 679, respectively, in PB2. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 116, 224, 293, 371, 417, 422 or 442 in NP that results in enhanced growth in cells, e.g., MDCK cells, relative to a corresponding virus with, for instance, a leucine, asparagine, arginine, methionine, aspartic acid, arginine threonine, at position 116, 224, 293, 371, 417, 422, or 442, respectively, in NP. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 90 in M1 that results in enhanced growth in cells relative to a corresponding virus with, for instance, a serine at position 90 in M1. In one embodiment, the recombinant reassortant influenza virus has an amino acid residue at position 30, 49, 140, 161 or 223 in NS1 that results in enhanced growth in MDCK cells relative to a corresponding virus with, for instance, a proline, alanine, glutamine, threonine or glutamic acid, respectively, at position 30, 49, 140, 161 or 223, respectively, in NS1. IN one embodiment, the recombinant reassortant influenza virus does not have a valine at residue 504 in PB2 and a leucine at residue 550 in PA.

In one embodiment, the influenza virus of the invention is a recombinant influenza virus with one or more mutations in the 3' UTR of M, PA and/or PB2 and having a particular amino acid residue at specified positions in one, two, three or more of PA, PB1, PB2, NP, M1 and/or NS1 and having an amino acid sequence with at least 80%, e.g., 90%, 92%, 95%, 97%, 98%, or 99%, including any integer between 80 and 99, contiguous amino acid sequence identity to a corresponding polypeptide encoded by one of SEQ ID Nos. 1-6 or 10-15, such as a polypeptide with a residue other than K142, S225, K356 or I550 in PA; other than E112, Q247, M507 or V644 in PB1; other than M202, F323 or I504 in PB2; other than R74, I112, I116, T442, or N417 in NP; other than V97 and/or Y100 in M1; and/or other than R140 or K55 in NS. The residue other than the specified residue may be conservative substitution. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine and tryptophan; a group of amino acids having basic side chains is lysine, arginine and histidine; and a group of amino acids having sulfur-containing side chain is cysteine and methionine. In one embodiment, conservative amino acid substitution groups are: threonine-valine-leucine-isoleucine-alanine; phenylalanine-tyrosine; lysine-arginine; alanine-valine; glutamic-aspartic; and asparagine-glutamine.

In one embodiment, the influenza virus of the invention is a recombinant influenza virus with one or more mutations in the 3' UTR of M, PA and/or PB2 and having a particular amino acid residue at specified positions in one or more of PA, PB1, PB2, NP, M1 and/or NS1 and an amino acid sequence with at least 80%, e.g., 90%, 92%, 95%, 97%, 98%, or 99%, including any integer between 80 and 99, contiguous amino acid sequence identity to a corresponding polypeptide encoded by one of SEQ ID Nos. 1-6 or 10-15, such as a polypeptide with a residue that is a conservative substitution relative to M202 in PB2, R74 in NP, and/or V97 in M1.

In one embodiment, the influenza virus of the invention is a recombinant influenza virus with one or more mutations in the 3' UTR of M, PA and/or PB2 and having a particular amino acid residue at specified positions in PA, PB1, PB2, NP, M1 and/or NS1 and an amino acid sequence with at least 80%, e.g., 90%, 92%, 95%, 97%, 98%, or 99%, including any integer between 80 and 99, contiguous amino acid sequence identity to a corresponding polypeptide encoded by one of SEQ ID Nos. 1-6 or 10-15, e.g., a polypeptide with a residue that is a non-conservative substitution relative to K142 in PA, Q247 in PB1, M202, F323 or I504 in PB2, R74I112, I116, J442 or N417 in NP, V97 and/or Y100 in M1, and/or K55 or R140 in NS1.

In one embodiment, the influenza virus of the invention is a recombinant influenza virus with one or more mutations in

the 3' UTR of M, PA and/or PB2 and having a particular amino acid residue at specified positions in PA, PB1, PB2, NP, M1 and/or NS1 and an amino acid sequence with at least 80%, e.g., 90%, 92%, 95%, 97%, 98%, or 99%, including any integer between 80 and 99, contiguous amino acid sequence identity to a corresponding polypeptide encoded by one of SEQ ID Nos. 1-6 or 10-15, e.g., a PB2 gene segment with a residue other than isoleucine and that is a conservative substitution for isoleucine at residue 504; a PB1 gene segment with a non-conservative substitution for E112; a PA gene segment with a substitution for S225; a NP gene segment with a conservative substitution for R74 and N417; a M gene segment with a conservative substitution for V97 and a non-conservative substitution for Y100; and a NS gene segment with a non-conservative substitution for K55.

In one embodiment, the influenza virus of the invention is a recombinant influenza virus with one or more mutations in the 3' UTR of M, PA and/or PB2 and having a particular amino acid residue at specified positions in PA, PB1, PB2, NP, M1 and/or NS1 and an amino acid sequence with at least 80%, e.g., 90%, 92%, 95%, 97%, 98%, or 99%, including any integer between 80 and 99, contiguous amino acid sequence identity to a corresponding polypeptide encoded by one of SEQ ID Nos. 1-6 or 10-15, e.g., a PB2 gene segment with a non-conservative substitution for M202 and F323; a PB1 gene segment with a non-conservative substitution for Q247; a PA gene segment with a non-conservative substitution for K142; a NP gene segment with a conservative substitution for R74; a M gene segment with a conservative substitution for V97 and a non-conservative substitution for Y100; and a NS gene segment with a conservative substitution for K55E.

In one embodiment, the influenza virus of the invention is a recombinant influenza virus with one or more mutations in the 3' UTR of M, PA and/or PB2 and having a particular amino acid residue at specified positions in PA, PB1, PB2, NP, M1 and/or NS1 and an amino acid sequence with at least 80%, e.g., 90%, 92%, 95%, 97%, 98%, or 99%, including any integer between 80 and 99, contiguous amino acid sequence identity to a corresponding polypeptide encoded by one of SEQ ID Nos. 1-6 or 10-15, e.g., a PB2 segment with a conservative substitution for I504; a PB1 segment with a conservative substitution for M40L and a non-conservative substitution for G180; a PA segment with a conservative substitution for R401; a NP segment with a conservative substitution for I116; a NS gene segment with a conservative substitution for A30 or R118.

In one embodiment, the influenza virus of the invention is a recombinant influenza virus with one or more mutations in the 3' UTR of M, PA and/or PB2 and having a particular amino acid residue at specified positions in one or more of PA, PB1, PB2, NP, M1 and/or NS1 and an amino acid sequence with at least 80%, e.g., 90%, 92%, 95%, 97% or 99%, including any integer between 80 and 99, contiguous amino add sequence identity to a corresponding polypeptide encoded by one of SEQ ID Nos. 1-6 or 10-15, such as a polypeptide with a residue that is a non-conservative substitution relative to K142 in PA, 0247 in PB1. F323 in PB2, Y100 in M1, and/or K55 in NS1. In one embodiment, the amino acid residue that is replaced has an aliphatic side chain, amide-containing side chain, basic side chain, or sulfur containing side chain and the replacement of an aromatic side chain or acidic side chain (a nonconservative substitution). In one embodiment, the recombinant influenza virus has a residue that is a neutral or positively charged residue that is replaced with a polar or negatively charged residue.

Also included are any combination of the selected amino acid residues at specified positions described herein.

Gene segments for M, PB2 and/or PA with one or more mutations in the 3' UTR of M, PA and/or PB2, and where the M, PA and PB2 and the PB1. NP, and/or NS gene segments optionally have the residues at the specified positions, may be combined with a gene segment for HA, e.g., H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, or H17 and a gene segment for NA, e.g., N1, N2, N3, N4, N5, N6, N7, N8, N9, N10, or N11, and any combination of HA and NA, to provide the reassortant vaccine viruses of the invention. In one embodiment, the HA is H1, H5 or H7. In one embodiment the NA is N1 or N9. In one embodiment, the HA gene segment in the reassortant virus is heterologous to the gene segments for PA, PB1, PB2, NP, M and NS. In one embodiment, the NA gene segment in the reassortant virus is heterologous to the gene segments for PA, PB1, PB2, NP, M and NS. In one embodiment, the HA gene segment in the reassortant virus has gene segments for PA, PB1, PB2, NP, M and NS from one influenza virus isolate or strain ("parent"), or a variant thereof, e.g., one with gene segments encoding influenza virus proteins with at least 95%, 96%, 97%, 98%, 99%, or 99.5% amino acid sequence identity, or having 1, 2, 5, 10, or 20 substitutions relative, to sequences in a parent influenza virus isolate or strain. In one embodiment, the parent strain has gene segments with sequences corresponding to SEQ ID Nos. 1-6 or 10-15. In one embodiment, the HA gene segment in the reassortant virus is a chimeric HA gene segment, e.g., a chimera of heterologous HA ectodomain sequences linked to HA signal peptide sequences and/or HA transmembrane domain sequences from the HA gene segment of the parent isolate or strain, or variant thereof. In one embodiment, the NA gene segment in the isolated recombinant virus is a chimeric NA gene segment e.g., a chimera of heterologous NA ectodomain sequences linked to NA transmembrane domain sequences from the NA gene segment of the parent isolate or strain, or variant thereof, and/or stalk sequences from the parent isolate or strain, or variant thereof. In one embodiment, the NA gene segment in the isolated recombinant virus is a chimeric NA gene segment e.g., a chimera of heterologous NA ectodomain sequences linked to NA transmembrane domain sequences from the NA gene segment of the parent isolate or strain, or variant thereof, and/or stalk sequences from a second isolate or strain, or variant thereof. In one embodiment, the isolated recombinant virus has a heterologous HA gene segment, a heterologous NA gene segment, a chimeric HA gene segment, a chimeric NA gene segment, or any combination thereof. The nucleic acid sequences employed to prepare vRNA may be ones that introduce the residues at the specified positions via recombinant methodology or may be selected as having the residues at the specified positions.

A/Puerto Rico/8/34 (H1N1), "PR8," virus serves as the genetic backbone for generation of inactivated influenza vaccines. Occasionally, vaccine strains based on PR8 backbone replicate to relatively low titers in eggs and cell culture resulting in delayed vaccine production and vaccine shortage. To determine if high yield vaccine strain backbones for propagation in MDCK cells, chicken eggs and Vero cells can be prepared to supply the demand of seasonal flu and highly pathogenic pandemic viruses, various mutagenesis strategies were employed. For example, PR8 backbone random mutant libraries were screened for high replicative mutants, e.g., by introducing random mutations to internal PR8 genes by error prone PCR, introducing mutations that confer high replication and high polymerase activity, and optimizing

PR8 internal gene via codon bias. In another approach, the HA gene was optimized to increase virus replication and HA content, e.g., by optimizing the HA promoter to generate a strong promoter, optimizing the HA noncoding region, and/or optimizing the HA signal peptide.

As described herein, an influenza virus isolate useful as a vaccine virus (e.g., A/Puerto Rico/8/34, "PR8," including a specific isolate such as UW-PR8) to carry heterologous gene segments for NA and/or HA, was serially passaged in MDCK cells, e.g., about 10-12-times although fewer passages may be employed, to obtain virus with enhanced replication in those cells. In one embodiment, viruses obtained after serial passage which have enhanced replication, have titers that are at least 1 or 2 logs higher than viruses that were not serially passaged. In one embodiment, viruses obtained after serial passage had substitutions in two or more internal gene segments relative to the parent virus.

Thus, for vaccine viruses that are to be grown or passaged in cells in culture, e.g., MDCK or Vero cells or eggs, selection of sequences with, or replacement of, the disclosed residues at the specified positions in one or more of PA, PB1, PB2, NP, M1 and/or NS1, that confer enhanced growth of the virus in cultured cells when employed with HA and NA sequences of interest, can result in significantly higher viral titers. Thus, the invention provides a method to select for influenza viruses with enhanced replication in cell culture. The method includes providing cells suitable for influenza vaccine production; serially culturing one or more influenza virus isolates in the cells; and isolating serially cultured virus with enhanced growth relative to the one or more isolates prior to serial culture. In one embodiment, the cells are canine or primate, e.g., human or monkey, cells.

In one embodiment, the influenza virus of the invention is a recombinant influenza virus having two or more of selected amino acid residues at specified positions in one or more of PA, PB1, PB2, NP, M1, and/or NS1, which can be employed with HA and NA genes of interest. In one embodiment, the recombinant reassortant influenza virus has an asparagine or glutamine at position 142 in PA, a cysteine at position 225, an arginine or histidine at position 356 in PA, or a leucine, valine, threonine, or glycine at position 550 in PA; a histidine, arginine or lysine at position 247 in PB1, a valine, leucine, isoleucine, threonine, alanine or glycine at position 507 in PB1 and/or an alanine, glycine, leucine or isoleucine at position 644 in PB1; a leucine, alanine, valine, isoleucine, glycine, or threonine at position 202 and/or position 323 in PB2, or a valine, leucine, glycine, threonine, or alanine at position 504 in PB2; a lysine or a histidine at position 74 in NP or a leucine, valine, glycine or alanine at position 112, 116 or 442 in NP; a leucine, isoleucine, alanine, glycine, or threonine, at position 97 and/or a lysine, arginine or histidine position 100 in M1; or an asparagine, aspartic acid, glutamic acid or glutamine at position 55 or glutamine or asparagine at position 140 in NS1.

The invention provides a plurality of influenza virus vectors of the invention, e.g., those useful to prepare reassortant viruses including 6:1:1 reassortants, 6:2 reassortants and 7:1 reassortants. A 6:1:1 reassortant within the scope of the present invention is an influenza virus with 6 internal gene segments from a vaccine virus, a NA gene segment from a different (second) viral isolate, and a HA gene segment from a third isolate; a 6:2 reassortant within the scope of the present invention is an influenza virus with 6 internal gene segments from a vaccine virus, and a NA gene segment and a HA gene segment from a different (second) viral isolate; and a 7:1 reassortant within the scope of the present invention is an influenza virus with 6 internal gene segments and a NA gene segment from a vaccine virus, and a HA gene segment from a different viral source than the vaccine virus, or an influenza virus with 6 internal gene segments and a HA gene segment from the vaccine virus, and a NA gene segment is from a different viral source than the vaccine virus.

In one embodiment of the invention, the plurality includes vectors for vRNA production selected from a vector comprising a promoter operably linked to an influenza virus PA DNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB1 DNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB2 DNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus HA DNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NP DNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NA DNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus M DNA linked to a transcription termination sequence, and a vector comprising a operably linked to an influenza virus NS DNA linked to a transcription termination sequence. In one embodiment, the DNAs for vRNA production of PB1, PB2, PA, NP, M, and NS, have sequences from an influenza virus that replicates to high titers in cultured mammalian cells such as MDCK cells, Vero cells or PER.C6® cells and also optionally embryonated eggs, and/or from a vaccine virus, e.g., one that does not cause significant disease in humans. The DNA for vRNA production of NA may be from any NA, e.g., any of N1-N10, and the DNA for vRNA production of HA may be from any HA, e.g., H1-H17. In one embodiment, the DNAs for vRNA production may be for an influenza B or C virus. The DNAs for vRNA production of NA and HA may be from different strains or isolates (6:1:1 reassortants) or from the same strain or isolate (6:2 reassortants), or the NA may be from the same strain or isolate as that for the internal genes (7:1 reassortant). The plurality also includes vectors for mRNA production selected from a vector encoding influenza virus PA, a vector encoding influenza virus PB1, a vector encoding influenza virus PB2, and a vector encoding influenza virus NP, and optionally one or more vectors encoding NP, NS, M, e.g., M1 and M2, HA or NA. The vectors encoding viral proteins may further include a transcription termination sequence.

Viruses that may provide the internal genes for reassortants within the scope of the invention include viruses that have high titers in MDCK cells, e.g., titers of at least about 10^5 PFU/mL, e.g., at least 10^6 PFU/mL, 10^7 PFU/mL or 10^8 PFU/mL; high titers in embryonated eggs, e.g., titers of at least about 10^7 EID_{50}/mL, e.g., at least 10^8 EID_{50}/mL, 10^9 EID_{50}/mL or 10^{10} EID_{50}/mL; high titers in cells such as MDCK cells, e.g., titers of at least about 10^7 PFU/mL, e.g., at least 10^8 PFU/mL, or high titers in two of more of those host cells.

In one embodiment, the titers of the reassortant viruses of the invention in cells such as MDCK cells or Vero cells may be over 1 log, 2 logs, 3 logs, or greater, than titers of the corresponding virus without particular residues at the specified positions.

Other reassortants with internal genes from other PR8 isolates or vaccine viruses may be employed in recombinant reassortant viruses of the invention. In particular, 5:1:2 reassortants having UW-PR8 PB1, PB2, PA, NP, and M ("5") and PR8(Cam) NS ("1"); 6:1:1 reassortants having

UW-PR8 NA, PB1, PB2, PA, NP, and M ("6") and PR8 (Cam) NS ("1"); and 7:1 reassortants having UW-PR8 PB1, PB2, PA, NP, M, NA, and NS ("7") may be employed.

In one embodiment, the DNAs for the internal genes for PB1, PB2, PA, NP, M, and NS encode proteins with substantially the same activity as a corresponding polypeptide encoded by one of SEQ ID NOs:1-6 or 10-15. As used herein, "substantially the same activity" includes an activity that is about 0.1%, 1%, 10%, 30%, 50%, 90%, e.g., up to 100% or more, or detectable protein level that is about 80%, 90% or more, the activity or protein level, respectively, of the corresponding full-length polypeptide. In one embodiment, the nucleic acid a sequence encoding a polypeptide which is substantially the same as, e.g., having at least 80%, e.g., 90%, 92%, 95%, 97%, 98%, or 99%, including any integer between 80 and 99, contiguous amino acid sequence identity to, a polypeptide encoded by one of SEQ ID NOs:1-6 or 10-15. In one embodiment, the isolated and/or purified nucleic acid molecule comprises a nucleotide sequence which is substantially the same as, e.g., having at least 50%, e.g., 60%, 70%, 80% or 90%, including any integer between 50 and 100, or more contiguous nucleic acid sequence identity to one of SEQ ID NOs:1-6 or 10-15 and, in one embodiment, also encodes a polypeptide having at least 80%, e.g., 90%, 92%, 95%, 97%, 98%, or 99%, including any integer between 80 and 99, contiguous amino acid sequence identity to a polypeptide encoded by one of SEQ ID NOs:1-6 or 10-15. In one embodiment, the influenza virus polypeptide has one or more, for instance, 2, 5, 10, 15, 20 or more, conservative amino acids substitutions, e.g., conservative substitutions of up to 10% or 20% of 2, 5, 10, 15, 20 or more, of a combination of conservative and non-conservative amino acids substitutions, e.g., conservative substitutions of up to 10% or 20% of the residues, or relative to a polypeptide encoded by one of SEQ IS NOs:1-6 or 10-15, and has a characteristic residue in two or more of PA, PB1, PB2, NP, M1, and/or NS1 the residues, relative to a polypeptide encoded by one of SEQ ID NOs: 1-6 or 10-15, and has a characteristic residue in two or more of the gene segments for PA. PB1, PB2, NP, M1, and/or NS1, e.g., there is an asparagine or glutamine at position 142 in PA; a histidine, arginine or lysine at position 247 in PB1; a leucine, alanine, valine, isoleucine, glycine, or serine at position 202 and/or position 323 in PB2; a lysine or a histidine at position 74 in NP; a leucine, isoleucine, alanine, glycine, or serine at position 202 and/or a lysine, arginine, or histidine position 100 in M1; or an asparagine, aspartic acid, glutamic acid or glutamine at position 44 in NS1. In one embodiment, the influenza virus polypeptide has one or more, for instance, 2, 3, 4, 5, 6, 7 or 8 conservative and/or nonconservative amino acid substitutions, relative to a polypeptide encoded by one of SEQ ID NOs:1-6 or 10-15, e.g., those in virus isolates 1, 4, 36, 38, P17, P25 or P61 in Table 4.

The invention thus includes the use of isolated and purified vectors or plasmids, which express or encode influenza virus proteins, or express or encode influenza vRNA, both native and recombinant vRNA. The vectors comprise influenza cDNA, e.g., influenza A (e.g., any influenza A gene including any of the 16 HA or 9 NA subtypes), B or C DNA (see Fields *Virology* (Fields et al. (eds.), Lippincott, Williams and Wickens (2006), which is specifically incorporated by reference herein). Any suitable promoter or transcription termination sequence may be employed to express a protein or peptide, e.g., a viral protein or peptide, a protein or peptide of a nonviral pathogen, or a therapeutic protein or peptide.

A composition or plurality of vectors of the invention may also comprise a heterologous gene or open reading frame of interest, e.g., a foreign gene encoding an immunogenic peptide or protein useful as a vaccine or in gene replacement, for instance, may encode an epitope useful in a cancer therapy or vaccine, or a peptide or polypeptide useful in gene therapy. When preparing virus, the vector or plasmid comprising the gene or cDNA of interest may substitute for a vector or plasmid for an influenza viral gene or may be in addition to vectors or plasmids for all influenza viral genes. Thus, another embodiment of the invention comprises a composition or plurality of vectors as described above in which one of the vectors is replaced with, or further comprises, 5' influenza virus sequences optionally including 5' influenza virus coding sequences or a portion thereof, linked to a desired nucleic acid sequence, e.g., a desired cDNA, linked to 3' influenza virus sequences optionally including 3' influenza virus coding sequences or a portion thereof. In one embodiment, the desired nucleic acid sequence such as a cDNA is in an antisense (antigenomic) orientation. The introduction of such a vector in conjunction with the other vectors described above to a host cell permissive for influenza virus replication results in recombinant virus comprising vRNA corresponding to the heterologous sequences of the vector.

The promoter in a vector for vRNA production may be a RNA polymerase I promoter, a RNA polymerase II promoter, a RNA polymerase III promoter, a T7 promoter, or a T3 promoter, and optionally the vector comprises a transcription termination sequence such as a RNA polymerase I transcription termination sequence, a RNA polymerase II transcription termination sequence, a RNA polymerase III transcription termination sequence, or a ribozyme. Ribozymes within the scope of the invention include, but are not limited to, tetrahyrnena ribozymes, RNase P, hammerhead ribozymes, hairpin ribozymes, hepatitis ribozyme, as well as synthetic ribozymes. In one embodiment, the RNA polymerase I promoter is a human RNA polymerase I promoter.

The promoter or transcription termination sequence in a vRNA or virus protein expression vector may be the same or different relative to the promoter or any other vector. In one embodiment, the vector or plasmid which expresses influenza vRNA comprises a promoter suitable for expression in at least one particular host cell, e.g., avian or mammalian host cells such as canine, feline, equine, bovine, ovine, or primate cells including human cells, or for expression in more than one host.

In one embodiment, at least one vector for vRNA comprises a RNA polymerase II promoter linked to a ribozyme sequence linked to viral coding sequences linked to another ribozyme sequences, optionally linked to a RNA polymerase II transcription termination sequence. In one embodiment, at least 2, e.g., 3, 4, 5, 6, 7 or 8, vectors for vRNA production comprise a RNA polymerase II promoter, a first ribozyme sequence, which is 5' to a sequence corresponding to viral sequences including viral coding sequences, which is 5' to a second ribozyme sequence, which is 5' to a transcription termination sequence. Each RNA polymerase II promoter in each vRNA vector may be the same or different as the RNA polymerase II promoter in any other vRNA vector. Similarly, each ribozyme sequence in each vRNA vector may be the same or different as the ribozyme sequences in any other vRNA vector. In one embodiment, the ribozyme sequences in a single vector are not the same.

In one embodiment, the invention provides a plurality of influenza virus vectors for a reassortant, comprising a vector

for vRNA production comprising a promoter operably linked to an influenza virus PA DNA linked to a transcription termination sequence, a vector for vRNA production comprising a promoter operably linked to an influenza virus PB1 DNA linked to a transcription termination sequence, a vector for vRNA production comprising a promoter operably linked to an influenza virus PB2 DNA linked to a transcription termination sequence, a vector for vRNA production comprising a promoter operably linked to an influenza virus HA DNA linked to a transcription termination sequence, a vector for vRNA production comprising a promoter operably linked to an influenza virus NP DNA linked to a transcription termination sequence, a vector for vRNA production comprising a promoter operably linked to an influenza virus NA DNA linked to a transcription termination sequence, a vector for vRNA production comprising a promoter operably linked to an influenza virus M DNA linked to a transcription termination sequence, and a vector for vRNA production comprising a promoter operably linked to an influenza virus NS cDNA linked to a transcription termination sequence, wherein the DNAs for PB1, PB2, PA, NP, NS, and M are from one or more influenza vaccine seed viruses and contain two or more of the characteristic residues at the specified position(s); and a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding influenza virus PA, a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding influenza virus PB1, a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding influenza virus PB2, and a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding influenza virus NP, and optionally a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding influenza virus HA, a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding influenza virus NA, a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding influenza virus M1, a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding influenza virus M2, or a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding influenza virus NS2. In one embodiment, at least one vector comprises sequences corresponding to those encoding PB1, PB2, PA, NP, M, or NS, or a portion thereof, having substantially the same activity as a corresponding polypeptide encoded by one of SEQ ID NOs:1-6 or 10-15, e.g., a sequence encoding a polypeptide with at least 80%, e.g., 85%, 90%, 92%, 95%, 98%, 99% or 100%, including any integer between 80 and 100, amino acid identity to a polypeptide encoded by one of SEQ ID NOs:1-6 or 10-15. Optionally, two vectors may be employed in place of the vector comprising a promoter operably linked to an influenza virus M cDNA linked to a transcription termination sequence, e.g., a vector comprising a promoter operably linked to an influenza virus M1 cDNA linked to a transcription termination sequence and a vector comprising a promoter operably linked to an influenza virus M2 cDNA linked to a transcription termination sequence.

A plurality of the vectors of the invention may be physically linked or each vector may be present on an individual plasmid or other, e.g., linear, nucleic acid delivery vehicle. In one embodiment, each vRNA production vector is on a separate plasmid. In one embodiment, each mRNA production vector is on a separate plasmid.

The invention also provides a method to prepare influenza virus. The method comprises contacting a cell with a plurality of the vectors of the invention, e.g., sequentially or simultaneously, in an amount effective to yield infectious influenza virus. The invention also includes isolating virus from a cell contacted with the plurality of vectors. Thus, the invention further provides isolated virus, as well as a host cell contacted with the plurality of vectors or virus of the invention. In another embodiment, the invention includes contacting the cell with one or more vectors, either vRNA or protein production vectors, prior to other vectors, either vRNA or protein production vectors. In one embodiment, the promoter for vRNA vectors employed in the method is a RNA polymerase I promoter, a RNA polymerase II promoter, a RNA polymerase III promoter, a T3 promoter or a T7 promoter. In one embodiment, the RNA polymerase I promoter is a human RNA polymerase I promoter. In one embodiment, each vRNA vector employed in the method is on a separate plasmid. In one embodiment, the vRNA vectors employed in the method are on one plasmid or on two or three different plasmids. In one embodiment, each mRNA vector employed in the method is on a separate plasmid. In one embodiment, the mRNA vectors for PA, PB1, PB2 and NP employed in the method are on one plasmid or on two or three different plasmids.

In one embodiment, the invention provides a method to select for influenza viruses with enhanced replication in cell culture. The method includes providing cells suitable for influenza vaccine production; serially culturing one or more influenza virus isolates in the cells; and isolating serially cultured virus with enhanced growth relative to the one or more isolates prior to serial culture. In one embodiment, the cells are rodent or primate cells.

The methods of producing virus described herein, which do not require helper virus infection, are useful in viral mutagenesis studies, and in the production of vaccines (e.g., for AIDS, influenza, hepatitis B, hepatitis C, rhinovirus, filoviruses, malaria, herpes, and foot and mouth disease) and gene therapy vectors (e.g., for cancer, AIDS, adenosine deaminase, muscular dystrophy, ornithine transcarbamylase deficiency and central nervous system tumors). Thus, a virus for use in medical therapy (e.g., for a vaccine or gene therapy) is provided.

The invention also provides isolated viral polypeptides, and methods of preparing and using recombinant virus of the invention. The methods include administering to a host organism, e.g., a mammal, an effective amount of the influenza virus of the invention, e.g., an inactivated virus preparation, optionally in combination with an adjuvant and/or a carrier, e.g., in an amount effective to prevent or ameliorate infection of an animal such as a mammal by that virus or an antigenically closely related virus. In one embodiment, the virus is administered intramuscularly while in another embodiment, the virus is administered intranasally. In some dosing protocols, all doses may be administered intramuscularly or intranasally, while in others a combination of intramuscular and intranasal administration is employed. The vaccine may further contain other isolates of influenza virus including recombinant influenza virus, other pathogen(s), additional biological agents or microbial components, e.g., to form a multivalent vaccine. In one embodiment, intranasal vaccination, for instance containing with inactivated influenza virus, and a mucosal adjuvant may induce virus-specific IgA and neutralizing antibody in the nasopharynx as well as serum IgG.

The influenza virus of the invention may employed with other anti-virals, e.g., amantadine, rimantadine, and/or neuraminidase inhibitors, e.g., may be administered separately in conjunction with those anti-virals, for instance, administered before, during and/or after.

The invention will be described by the following non-limiting examples.

Example

Improvement of Virus Replication by Inhibiting MicroRNA Let-7c Binding to Influenza A Virus NV1 mRNA

MicroRNAs (miRNAs) represent a class of small non-coding RNAs that target mRNAs, triggering either translation repression or RNA degradation. MicroRNA let-7c inhibits M1 protein expression of an H1N1 influenza A virus in infected A549 cells (Ma et al., 2012). Database screening indicated that the let-7c seed sequence is a perfect complementary sequence match to the 3' untranslated region (UTR) of viral gene M cRNA.

MiRNA let-7c has been confirmed in many species, including canine, chicken and monkey, thus inhibiting miRNA Let-7c binding to M RNA might increase the virus replication in cell culture and eggs.

Experimental:

The noncoding region of M gene is mutated to attenuate Let-7c binding to M cRNA. For example, one or more mutations are introduced into the let-7c binding site in cRNA or viral mRNA. In one embodiment, the mutations include one or more of: G1012C. A1013U, U1014A (numbers refer to positive-sense orientation). The M gene mutant virus is rescued, e.g., with other sequences for high growth PR8 variant. The mutant M gene is tested for an increase in virus yield.

As shown in FIG. **1**, the mutations in the 3' noncoding region of M increase virus replication of a particular high growth backbone (HY-PR8) in Vero cells, and have a small effect in eggs.

Therefore, these mutations are useful alone or in conjunction with one or more other mutations that have a desirable phenotype, e.g., enhanced yield, into a vaccine backbone such as HY-PR8. Moreover, because let-7c may also bind the 3' untranslated region (UTR) of viral gene PA cRNA or PB2 cRNA, alterations in the 3' untranslated region (UTR) of viral gene PA cRNA or PB2 cRNA may also enhance viral titers (see FIG. **5**). Further, the 3'UTR in the M, PA and PB2 gene segments in influenza B and C viruses (see FIG. **6**) may likewise be modified.

Sequences of PR8 (UW) Genes:

PA

(SEQ ID NO: 1)

AGCGAAAGCA GGTACTGATC CAAAATGGAA GATTTTGTGC GACAATGCTT CAATCCGATG

ATTGTCGAGC TTGCGGAAAA AACAATGAAA GAGTATGGGG AGGACCTGAA AATCGAAACA

AACAAATTTG CAGCAATATG CACTCACTTG GAAGTATGCT TCATGTATTC AGATTTTCAC

TTCATCAATG AGCAAGGCGA GTCAATAATC GTAGAACTTG GTGATCCAAA TGCACTTTTG

AAGCACAGAT TTGAAATAAT CGAGGGAAGA GATCGCACAA TGGCCTGGAC AGTAGTAAAC

AGTATTTGCA ACACTACAGG GGCTGAGAAA CCAAAGTTTC TACCAGATTT GTATGATTAC

AAGGAGAATA GATTCATCGA AATTGGAGTA ACAAGGAGAG AAGTTCACAT ATACTATCTG

GAAAAGGCCA ATAAAATTAA ATCTGAGAAA ACACACATCC ACATTTTCTC GTTCACTGGG

GAAGAAATGG CCACAAAGGC AGACTACACT CTCGATGAAG AAAGCAGGGC TAGGATCAAA

ACCAGACTAT TCACCATAAG ACAAGAAATG GCCAGCAGAG GCCTCTGGGA TTCCTTTCGT

CAGTCCGAGA GAGGAGAAGA GACAATTGAA GAAAGGTTTG AAATCACAGG AACAATGCGC

AAGCTTGCCG ACCAAAGTCT CCCGCCGAAC TTCTCCAGCC TTGAAAATTT TAGAGCCTAT

GTGGATGGAT TCGAACCGAA CGGCTACATT GAGGGCAAGC TGTCTCAAAT GTCCAAAGAA

GTAAATGCTA GAATTGAACC TTTTTTGAAA ACAACACCAC GACCACTTAG ACTTCCGAAT

GGGCCTCCCT GTTCTCAGCG GTCCAAATTC CTGCTGATGG ATGCCTTAAA ATTAAGCATT

GAGGACCCAA GTCATGAAGG AGAGGGAATA CCGCTATATG ATGCAATCAA ATGCATGAGA

ACATTCTTTG GATGGAAGGA ACCCAATGTT GTTAAACCAC ACGAAAAGGG AATAAATCCA

AATTATCTTC TGTCATGGAA GCAAGTACTG GCAGAACTGC AGGACATTGA GAATGAGGAG

AAAATTCCAA AGACTAAAAA TATGAAGAAA ACAAGTCAGC TAAAGTGGGC ACTTGGTGAG

AACATGGCAC CAGAAAAGGT AGACTTTGAC GACTGTAAAG ATGTAGGTGA TTTGAAGCAA

TATGATAGTG ATGAACCAGA ATTGAGGTCG CTTGCAAGTT GGATTCAGAA TGAGTTTAAC

AAGGCATGCG AACTGACAGA TTCAAGCTGG ATAGAGCTCG ATGAGATTGG AGAAGATGTG

GCTCCAATTG AACACATTGC AAGCATGAGA AGGAATTATT TCACATCAGA GGTGTCTCAC

TGCAGAGCCA CAGAATACAT AATGAAGGGA GTGTACATCA ATACTGCCTT GCTTAATGCA

TCTTGTGCAG CAATGGATGA TTTCCAATTA ATTCCAATGA TAAGCAAGTG TAGAACTAAG

GAGGGAAGGC GAAAGACCAA CTTGTATGGT TTCATCATAA AAGGAAGATC CCACTTAAGG

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GCCATAGGCC AGGTTTCAAG GCCCATGTTC TTGTATGTGA GAACAAATGG AACCTCAAAA

ATTAAAATGA AATGGGGAAT GGAGATGAGG CGTTGCCTCC TCCAGTCACT TCAACAAATT

GAGAGTATGA TTGAAGCTGA GTCCTCTGTC AAAGAGAAAG ACATGACCAA AGAGTTCTTT

GAGAACAAAT CAGAAACATG GCCCATTGGA GAGTCCCCCA AAGGAGTGGA GGAAAGTTCC

ATTGGGAAGG TCTGCAGGAC TTTATTAGCA AAGTCGGTAT TCAACAGCTT GTATGCATCT

CCACAACTAG AAGGATTTTC AGCTGAATCA AGAAAACTGC TTCTTATCGT TCAGGCTCTT

AGGGACAACC TGGAACCTGG GACCTTTGAT CTTGGGGGGC TATATGAAGC AATTGAGGAG

TGCCTGATTA ATGATCCCTG GGTTTTGCTT AATGCTTCTT GGTTCAACTC CTTCCTTACA

CATGCATTGA GTTAGTTGTG GCAGTGCTAC TATTTGCTAT CCATACTGTC CAAAAAAGTA

CCTTGTTTCT ACT

PB1

(SEO ID NO: 2)

AGCGAAAGCA GGCAAACCAT TTGAATGGAT GTCAATCCGA CCTTACTTTT CTTAAAAGTG

CCAGCACAAA ATGCTATAAG CACAACTTTC CCTTATACTG GAGACCCTCC TTACAGCCAT

GGGACAGGAA CAGGATACAC CATGGATACT GTCAACAGGA CACATCAGTA CTCAGAAAAG

GGAAGATGGA CAACAAACAC CGAAACTGGA GCACCGCAAC TCAACCCGAT TGATGGGCCA

CTGCCAGAAG ACAATGAACC AAGTGGTTAT GCCCAAACAG ATTGTGTATT GGAGGCGATG

GCTTTCCTTG AGGAATCCCA TCCTGGTATT TTTGAAAACT CGTGTATTGA AACGATGGAG

GTTGTTCAGC AAACACGAGT AGACAAGCTG ACACAAGGCC GACAGACCTA TGACTGGACT

CTAAATAGAA ACCAACCTGC TGCAACAGCA TTGGCCAACA CAATAGAAGT GTTCAGATCA

AATGGCCTCA CGGCCAATGA GTCTGGAAGG CTCATAGACT TCCTTAAGGA TGTAATGGAG

TCAATGAACA AAGAAGAAAT GGGGATCACA ACTCATTTTC AGAGAAAGAG ACGGGTGAGA

GACAATATGA CTAAGAAAAT GATAACACAG AGAACAATGG GTAAAAAGAA GCAGAGATTG

AACAAAAGGA GTTATCTAAT TAGAGCATTG ACCCTGAACA CAATGACCAA AGATGCTGAG

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CTAGCAAGCA TCGATTTGAA ATATTTCAAT GATTCAACAA GAAAGAAGAT TGAAAAAATC

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GCACCCAATC ATGAAGGGAT TCAAGCCGGA GTCGACAGGT TTTATCGAAC CTGTAAGCTA

CTTGGAATCA ATATGAGCAA GAAAAAGTCT TACATAAACA GAACAGGTAC ATTTGAATTC

ACAAGTTTTT TCTATCGTTA TGGGTTTGTT GCCAATTTCA GCATGGAGCT TCCCAGTTTT

GGGGTGTCTG GGATCAACGA GTCAGCGGAC ATGAGTATTG GAGTTACTGT CATCAAAAAC

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CTCAGACGGC AAAAATAGTG AATTTAGCTT GTCCTTCATG AAAAAATGCC TTGTTTCTAC T

PB2

(SEQ ID NO: 3)

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GAGCAAGGAC AAACTTTATG GAGTAAAATG AATGATGCCG GATCAGACCG AGTGATGGTA

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CAGTCGATTG CCGAAGCAAT AATTGTGGCC ATGGTATTTT CACAAGAGGA TTGTATGATA

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CATCAACTTT TAAGACATTT TCAGAAGGAT GCGAAAGTGC TTTTTCAAAA TTGGGGAGTT

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GTGTTGGTAA TGAAACGGAA ACGGGACTCT AGCATACTTA CTGACAGCCA GACAGCGACC

AAAAGAATTC GGATGGCCAT CAATTAGTGT CGAATAGTTT AAAAACGACC TTGTTTCTAC T

NP

(SEQ ID NO: 4)

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GGTGACGATG CAACGGCTGG TCTGACTCAC ATGATGATCT GGCATTCCAA TTTGAATGAT

GCAACTTATC AGAGGACAAG AGCTCTTGTT CGCACCGGAA TGGATCCCAG GATGTGCTCT

CTGATGCAAG GTTCAACTCT CCCTAGGAGG TCTGGAGCCG CAGGTGCTGC AGTCAAAGGA

GTTGGAACAA TGGTGATGGA ATTGGTCAGA ATGATCAAAC GTGGGATCAA TGATCGGAAC

TTCTGGAGGG GTGAGAATGG ACGAAAAACA AGAATTGCTT ATGAAAGAAT GTGCAACATT

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CGGAACCCAG GGAATGCTGA GTTCGAAGAT CTCACTTTTC TAGCACGGTC TGCACTCATA

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TTCATCAAAG GGACGAAGGT GCTCCCAAGA GGGAAGCTTT CCACTAGAGG AGTTCAAATT

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ATCAGCATAC AACCTACGTT CTCAGTACAG AGAAATCTCC CTTTTGACAG AACAACCATT

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AGGATGATGG AAAGTGCAAG ACCAGAAGAT GTGTCTTTCC AGGGGCGGGG AGTCTTCGAG

CTCTCGGACG AAAAGGCAGC GAGCCCGATC GTGCCTTCCT TTGACATGAG TAATGAAGGA

TCTTATTTCT TCGGAGACAA TGCAGAGGAG TACGACAATT AAAGAAAAAT ACCCTTGTTT CTACT

M

(SEQ ID NO: 5)

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GTGATCCTCT CACTATTGCC GCAAATATCA TTGGGATCTT GCACTTGACA TTGTGGATTC

TTGATCGTCT TTTTTTCAAA TGCATTTACC GTCGCTTTAA ATACGGACTG AAAGGAGGGC

CTTCTACGGA AGGAGTGCCA AAGTCTATGA GGGAAGAATA TCGAAAGGAA CAGCAGAGTG

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NS

(SEQ ID NO: 6)

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HA

(SEQ ID NO: 7)

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GTTTTGAGTTCTACCACAAGTGTGACAATGAATGCATGGAAAGTGTAAGAAATGGGACTTATGATTATCC

CAAATATTCAGAAGAGTCAAAGTTGAACAGGGAAAAGGTAGATGGAGTGAAATTGGAATCAATGGGGATC

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NA

(SEO ID NO: 8)

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GGACGACCTAAAGAAAAAACAATCTGGACTAGTGCGAGCAGCATTTCTTTTTGTGGCGTGAATAGTGATA

CTGTAGATTGGTCTTGGCCAGACGGTGCTGAGTTGCCATTCAGCATTGACAAGTAGTCTGTTCAAAAAAC

TCCTTGTTTCTACT

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All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 27

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<211> LENGTH: 2233
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

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attgtcgagc ttgcggaaaa aacaatgaaa gagtatgggg aggacctgaa aatcgaaaca 120

aacaaatttg cagcaatatg cactcacttg gaagtatgct tcatgtattc agattttcac 180

ttcatcaatg agcaaggcga gtcaataatc gtagaacttg gtgatccaaa tgcacttttg 240

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aaggagaata gattcatcga aattggagta acaaggagag aagttcacat atactatctg 420

gaaaaggcca ataaaattaa atctgagaaa acacacatcc acattttctc gttcactggg 480

gaagaaatgg ccacaaaggc agactacact ctcgatgaag aaagcagggc taggatcaaa 540

accagactat tcaccataag acaagaaatg gccagcagag gcctctggga ttcctttcgt 600

cagtccgaga gaggagaaga gacaattgaa gaaaggtttg aaatcacagg aacaatgcgc 660

aagcttgccg accaaagtct cccgccgaac ttctccagcc ttgaaaattt tagagcctat 720

gtggatggat tcgaaccgaa cggctacatt gagggcaagc tgtctcaaat gtccaaagaa 780

gtaaatgcta gaattgaacc ttttttgaaa acaacaccac gaccacttag acttccgaat 840

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gaggacccaa gtcatgaagg agagggaata ccgctatatg atgcaatcaa atgcatgaga 960

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tatgatagtg atgaaccaga attgaggtcg cttgcaagtt ggattcagaa tgagtttaac 1260

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gagggaaggc gaaagaccaa cttgtatggt ttcatcataa aaggaagatc ccacttaagg 1560

aatgacaccg acgtggtaaa ctttgtgagc atggagtttt ctctcactga cccaagactt 1620

gaaccacata aatgggagaa gtactgtgtt cttgagatag gagatatgct tataagaagt 1680

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gagagtatga ttgaagctga gtcctctgtc aaagagaaag acatgaccaa agagttcttt 1860

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<211> LENGTH: 2341
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

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gggacaggaa caggatacac catggatact gtcaacagga cacatcagta ctcagaaaag 180

ggaagatgga caacaaacac cgaaactgga gcaccgcaac tcaacccgat tgatgggcca 240

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gctttccttg aggaatccca tcctggtatt tttgaaaact cgtgtattga aacgatggag 360

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aatggcctca cggccaatga gtctggaagg ctcatagact tccttaagga tgtaatggag 540

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gacaatatga ctaagaaaat gataacacag agaacaatgg gtaaaaagaa gcagagattg 660

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t 2341

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<212> TYPE: DNA
<213> ORGANISM: Influenza virus

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aagaagtaca catcaggaag acaggagaag aacccagcac ttaggatgaa atggatgatg 180

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tcacctctgg ctgtgacatg gtggaatagg aatggaccaa taacaaatac agttcattat 360

ccaaaaatct acaaaactta ttttgaaaga gtcgaaaggc taaagcatgg aacctttggc 420

cctgtccatt ttagaaacca agtcaaaata cgtcggagag ttgacataaa tcctggtcat 480

gcagatctca gtgccaagga ggcacaggat gtaatcatgg aagttgtttt ccctaacgaa 540

gtgggagcca ggatactaac atcggaatcg caactaacga taaccaaaga gaagaaagaa 600

gaactccagg attgcaaaat ttctcctttg atggttgcat acatgttgga gagagaactg 660

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gtatcagcag atccactagc atctttattg gagatgtgcc acagcacaca gattggtgga 900

attaggatgg tagacatcct taggcagaac ccaacagaag agcaagccgt ggatatatgc 960

aaggctgcaa tgggactgag aattagctca tccttcagtt ttggtggatt cacatttaag 1020

agaacaagcg gatcatcagt caagagagag gaagaggtgc ttacgggcaa tcttcaaaca 1080

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cagtcgattg ccgaagcaat aattgtggcc atggtatttt cacaagagga ttgtatgata 1260

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t 2341

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<211> LENGTH: 1565
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

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agagcatccg tcggaaaaat gattggtgga attggacgat tctacatcca aatgtgcacc 180

gaactcaaac tcagtgatta tgagggacgg ttgatccaaa acagcttaac aatagagaga 240

atggtgctct ctgcttttga cgaaaggaga aataaatacc ttgaagaaca tcccagtgcg 300

gggaaagatc ctaagaaaac tggaggacct atatacagga gagtaaacgg aaagtggatg 360

agagaactca tcctttatga caaagaagaa ataaggcgaa tctggcgcca agctaataat 420

ggtgacgatg caacggctgg tctgactcac atgatgatct ggcattccaa tttgaatgat 480

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ctgatgcaag gttcaactct ccctaggagg tctggagccg caggtgctgc agtcaaagga 600

gttggaacaa tggtgatgga attggtcaga atgatcaaac gtgggatcaa tgatcggaac 660

ttctggaggg gtgagaatgg acgaaaaaca agaattgctt atgaaagaat gtgcaacatt 720

ctcaaaggga aatttcaaac tgctgcacaa aaagcaatga tggatcaagt gagagagagc 780

cggaacccag ggaatgctga gttcgaagat ctcacttttc tagcacggtc tgcactcata 840

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gccagtgggt acgactttga aagggaggga tactctctag tcggaataga ccctttcaga 960

ctgcttcaaa acagccaagt gtacagccta atcagaccaa atgagaatcc agcacacaag 1020

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tgggccataa ggaccagaag tggaggaaac accaatcaac agagggcatc tgcgggccaa 1260

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<213> ORGANISM: Influenza virus

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ttctact 1027

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<212> TYPE: DNA
<213> ORGANISM: Influenza virus

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tggacatcaa gacagccaca cgtgctggaa agcagatagt ggagcggatt ctgaaagaag 240

aatccgatga ggcacttaaa atgaccatgg cctctgtacc tgcgtcgcgt tacctaactg 300

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cgaacttcag tgtgattttt gaccggctgg agactctaat attgctaagg gctttcaccg 480

aagagggagc aattgttggc gaaatttcac cattgccttc tcttccagga catactgctg 540

aggatgtcaa aaatgcagtt ggagtcctca tcggaggact tgaatggaat gataacacag 600

ttcgagtctc tgaaactcta cagagattcg cttggagaag cagtaatgag aatgggagac 660

ctccactcac tccaaaacag aaacgagaaa tggcgggaac aattaggtca gaagtttgaa 720

gaaataagat ggttgattga agaagtgaga cacaaactga agataacaga gaatagtttt 780

gagcaaataa catttatgca agccttacat ctattgcttg aagtggagca agagataaga 840

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ccgacactgt tgacacagta ctcgagaaga atgtgacagt gacacactct gttaacctgc 180

tcgaagacag ccacaacgga aaactatgta gattaaaagg aatagcccca ctacaattgg 240

ggaaatgtaa catcgccgga tggctcttgg gaaacccaga atgcgaccca ctgcttccag 300

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gagatttcat cgactatgag gagctgaggg agcaattgag ctcagtgtca tcattcgaaa 420

gattcgaaat atttcccaaa gaaagctcat ggcccaacca caacacaaac ggagtaacgg 480

cagcatgctc ccatgagggg aaaagcagtt tttacagaaa tttgctatgg ctgacggaga 540

aggagggctc atacccaaag ctgaaaaatt cttatgtgaa caaaaaaggg aaagaagtcc 600

ttgtactgtg gggtattcat cacccgccta acagtaagga acaacagaat ctctatcaga 660

atgaaaatgc ttatgtctct gtagtgactt caaattataa caggagattt accccggaaa 720

tagcagaaag acccaaagta agagatcaag ctgggaggat gaactattac tggaccttgc 780

taaaacccgg agacacaata atatttgagg caaatggaaa tctaatagca ccaatgtatg 840

ctttcgcact gagtagaggc tttgggtccg gcatcatcac ctcaaacgca tcaatgcatg 900

agtgtaacac gaagtgtcaa acacccctgg gagctataaa cagcagtctc ccttaccaga 960

atatacaccc agtcacaata ggagagtgcc caaaatacgt caggagtgcc aaattgagga 1020

tggttacagg actaaggaac attccgtcca ttcaatccag aggtctattt ggagccattg 1080

ccggttttat tgaaggggga tggactggaa tgatagatgg atggtatggt tatcatcatc 1140

agaatgaaca gggatcaggc tatgcagcgg atcaaaaaag cacacaaaat gccattaacg 1200

ggattacaaa caaggtgaac actgttatcg agaaaatgaa cattcaattc acagctgtgg 1260

gtaaagaatt caacaaatta gaaaaaagga tggaaaattt aaataaaaaa gttgatgatg 1320

gatttctgga catttggaca tataatgcag aattgttagt tctactggaa aatgaaagga 1380

ctctggattt ccatgactca aatgtgaaga atctgtatga gaaagtaaaa agccaattaa 1440

agaataatgc caaagaaatc ggaaatggat gttttgagtt ctaccacaag tgtgacaatg 1500

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aatgcatgga aagtgtaaga aatgggactt atgattatcc caaatattca gaagagtcaa 1560

agttgaacag ggaaaaggta gatggagtga aattggaatc aatggggatc tatcagattc 1620

tggcgatcta ctcaactgtc gccagttcac tggtgctttt ggtctccctg ggggcaatca 1680

gtttctggat gtgttctaat ggatctttgc agtgcagaat atgcatctga gattagaatt 1740

tcagagatat gaggaaaaac acccttgttt ctact 1775

<210> SEQ ID NO 8
<211> LENGTH: 1413
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 8

agcaaaagca ggggtttaaa atgaatccaa atcagaaaat aataaccatt ggatcaatct 60

gtctggtagt cggactaatt agcctaatat tgcaaatagg gaatataatc tcaatatgga 120

ttagccattc aattcaaact ggaagtcaaa accatactgg aatatgcaac caaaacatca 180

ttacctataa aaatagcacc tgggtaaagg acacaacttc agtgatatta accggcaatt 240

catctctttg tcccatccgt gggtgggcta tatacagcaa agacaatagc ataagaattg 300

gttccaaagg agacgttttt gtcataagag agccctttat ttcatgttct cacttggaat 360

gcaggacctt ttttctgacc caaggtgcct tactgaatga caagcattca agtgggactg 420

ttaaggacag aagcccttat agggccttaa tgagctgccc tgtcggtgaa gctccgtccc 480

cgtacaattc aagatttgaa tcggttgctt ggtcagcaag tgcatgtcat gatggcatgg 540

gctggctaac aatcggaatt tcaggtccag ataatggagc agtggctgta ttaaaataca 600

acggcataat aactgaaacc ataaaaagtt ggaggaagaa aatattgagg acacaagagt 660

ctgaatgtgc ctgtgtaaat ggttcatgtt ttactataat gactgatggc ccgagtgatg 720

ggctggcctc gtacaaaatt ttcaagatcg aaaaggggaa ggttactaaa tcaatagagt 780

tgaatgcacc taattctcac tatgaggaat gttcctgtta ccctgatacc ggcaaagtga 840

tgtgtgtgtg cagagacaat tggcatggtt cgaaccggcc atgggtgtct ttcgatcaaa 900

acctggatta tcaaatagga tacatctgca gtggggtttt cggtgacaac ccgcgtcccg 960

aagatggaac aggcagctgt ggtccagtgt atgttgatgg agcaaacgga gtaaagggat 1020

tttcatatag gtatggtaat ggtgtttgga taggaaggac caaaagtcac agttccagac 1080

atgggtttga gatgatttgg gatcctaatg gatggacaga gactgatagt aagttctctg 1140

tgaggcaaga tgttgtggca atgactgatt ggtcagggta tagcggaagt ttcgttcaac 1200

atcctgagct gacagggcta gactgtatga ggccgtgctt ctgggttgaa ttaatcaggg 1260

gacgacctaa agaaaaaaca atctggacta gtgcgagcag catttctttt tgtggcgtga 1320

atagtgatac tgtagattgg tcttggccag acggtgctga gttgccattc agcattgaca 1380

agtagtctgt tcaaaaaact ccttgtttct act 1413

<210> SEQ ID NO 9

<400> SEQUENCE: 9

000

<210> SEQ ID NO 10
<211> LENGTH: 2342
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

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<400> SEQUENCE: 10

agcgaaagca ggcaaaccat ttgaatggat gtcaatccga ccttactttt cttaaaagtg 60

ccagcacaaa atgctataag cacaactttc ccttataccg gagaccctcc ttacagccat 120

gggacaggaa caggatacac catggatact gtcaacagga cacatcagta ctcagaaaag 180

ggaagatgga caacaaacac cgaaactgga gcaccgcaac tcaacccgat tgatgggcca 240

ctgccagaag acaatgaacc aagtggttat gcccaaacag attgtgtatt ggaagcaatg 300

gctttccttg aggaatccca tcctggtatt tttgaaaact cgtgtattga aacgatggag 360

gttgttcagc aaacactgag tagacaagct gacacaaggc cgacagacct atgactggac 420

tttaaataga aaccagcctg ctgcaacagc attggccaac acaatagaag tgttcagatc 480

aaatggcctc acggccaatg agtcaggaag gctcatagac ttccttaagg atgtaatgga 540

gtcaatgaaa aaagaagaaa tggggatcac aactcatttt cagagaaaga gacgggtgag 600

agacaatatg actaagaaaa tgataacaca gagaacaata ggtaaaagga aacagagatt 660

gaacaaaagg ggttatctaa ttagagcatt gaccctgaac acaatgacca aagatgctga 720

gagagggaag ctaaaacgga gagcaattgc aaccccaggg atgcaaataa gggggtttgt 780

atactttgtt gagacactgg caaggagtat atgtgagaaa cttgaacaat cagggttgcc 840

agttggaggc aatgagaaga aagcaaagtt ggcaaatgtt gtaaggaaga tgatgaccaa 900

ttctcaggac accgaacttt ctttcaccat cactggagat aacaccaaat ggaacgaaaa 960

tcagaatcct cggatgtttt tggccatgat cacatatatg accagaaatc agcccgaatg 1020

gttcagaaat gttctaagta ttgctccaat aatgttctca aacaaaatgg cgagactggg 1080

aaaagggtat atgtttgaga gcaagagtat gaaacttaga actcaaatac ctgcagaaat 1140

gctagcaagc attgatttga aatatttcaa tgattcaaca agaaagaaga ttgaaaaaat 1200

ccgaccgctc ttaatagagg ggactgcatc attgagccct ggaatgatga tgggcatgtt 1260

caatatgtta agcactgtat taggcgtctc catcctgaat cttggacaaa agagatacac 1320

caagactact tactggtggg atggtcttca atcctctgac gattttgctc tgattgtgaa 1380

tgcacccaat catgaaggga ttcaagccgg agtcgacagg ttttatcgaa cctgtaagct 1440

acttggaatc aatatgagca agaaaaagtc ttacataaac agaacaggta catttgaatt 1500

cacaagtttt ttctatcgtt atgggtttgt tgccaatttc agcatggagc ttcccagttt 1560

tggggtgtct gggatcaacg agtcagcgga catgagtatt ggagttactg tcatcaaaaa 1620

caatatgata aacaatgatc ttggtccagc aacagctcaa atggcccttc agttgttcat 1680

caaagattac aggtacacgt accgatgcca tagaggtgac acacaaatac aaacccgaag 1740

atcatttgaa ataaagaaac tgtgggagca aacccgttcc aaagctggac tgctggtctc 1800

cgacggaggc ccaaatttat acaacattag aaatctccac attcctgaag tctgcctaaa 1860

atgggaattg atggatgagg attaccaggg gcgtttatgc aacccactga acccatttgt 1920

cagccataaa gaaattgaat caatgaacaa tgcagtgatg atgccagcac atggtccagc 1980

caaaaacatg gagtatgatg ctgttgcaac aacacactcc tggatcccca aaagaaatcg 2040

atccatcttg aatacaagtc aaagaggagt acttgaagat gaacaaatgt accaaaggtg 2100

ctggaattta tttgaaaaat tcttccccag cagttcatac agaagaccag tcgggatatc 2160

cagtatggtg gaggctatgg tttccagagc ccgaattgat gcacggattg atttcgaatc 2220

tggaaggata aagaaagaag agttcactga gatcatgaag atctgttcca ccattgaaga 2280

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gctcagacgg caaaaatagt gaatttagct tgtccttcat gaaaaaatgc cttgtttcta 2340

ct 2342

<210> SEQ ID NO 11
<211> LENGTH: 2341
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 11

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tcgcagtctc gcacccgcga gatactcaca aaaaccaccg tggaccatat ggccataatc 120

aagaagtaca catcaggaag acaggagaag aacccagcac ttaggatgaa atggatgatg 180

gcaatgaaat atccaattac agcagacaag aggataacgg aaatgattcc tgagagaaat 240

gagcaaggac aaactttatg gagtaaaatg aatgatgccg gatcagaccg agtgatggta 300

tcacctctgg ctgtgacatg gtggaatagg aatggaccaa tgacaaatac agttcattat 360

ccaaaaatct acaaaactta ttttgaaaga gtcgaaaggc taaagcatgg aacctttggc 420

cctgtccatt ttagaaacca agtcaaaata cgtcggagag ttgacataaa tcctggtcat 480

gcagatctca gtgccaagga ggcacaggat gtaatcatgg aagttgtttt ccctaacgaa 540

gtgggagcca ggatactaac atcggaatcg caactaacga taaccaaaga gaagaaagaa 600

gaactccagg attgcaaaat ttctccttta atggttgcat acatgttgga gagagaactg 660

gtccgcaaaa cgagattcct cccagtggct ggtggaacaa gcagtgtgta cattgaagtg 720

ttgcatttga ctcaaggaac atgctgggaa cagatgtata ctccaggagg ggaagtgaag 780

aatgatgatg ttgatcaaag cttgattatt gctgctagga acatagtgag aagagctgca 840

gtatcagcag acccactagc atctttattg gagatgtgcc acagcacaca gattggtgga 900

attaggatgg tagacatcct taagcagaac ccaacagaag agcaagccgt ggatatatgc 960

aaggctgcaa tgggactgag aattagctca tccttcagtt ttggtggatt cacatttaag 1020

agaacaagcg gatcatcagt caagagagag gaagaggtgc ttacgggcaa tcttcaaaca 1080

ttgaagataa gagtgcatga gggatctgaa gagttcacaa tggttgggag aagagcaaca 1140

gccatactca gaaaagcaac caggagattg attcagctga tagtgagtgg gagagacgaa 1200

cagtcgattg ccgaagcaat aattgtggcc atggtatttt cacaagagga ttgtatgata 1260

aaagcagtta gaggtgatct gaatttcgtc aatagggcga atcagcgact gaatcctatg 1320

catcaacttt taagacattt tcagaaggat gcgaaagtgc tttttcaaaa ttggggagtt 1380

gaacctatcg acaatgtgat gggaatgatt gggatattgc ccgacatgac tccaagcatc 1440

gagatgtcaa tgagaggagt gagaatcagc aaaatgggtg tagatgagta ctccagcacg 1500

gagagggtag tggtgagcat tgaccggttc ttgagagtca gggaccaacg aggaaatgta 1560

ctactgtctc ccgaggaggt cagtgaaaca cagggaacag agaaactgac aataacttac 1620

tcatcgtcaa tgatgtggga gattaatggt cctgaatcag tgttggtcaa tacctatcaa 1680

tggatcatca gaaactggga aactgttaaa attcagtggt cccagaaccc tacaatgcta 1740

tacaataaaa tggaatttga accatttcag tctttagtac ctaaggccat tagaggccaa 1800

tacagtgggt ttgtaagaac tctgttccaa caaatgaggg atgtgcttgg gacatttgat 1860

accgcacaga taataaaact tcttcccttc gcagccgctc caccaaagca aagtagaatg 1920

cagttctcct catttactgt gaatgtgagg ggatcaggaa tgagaatact tgtaaggggc 1980

aattctcctg tattcaacta caacaaggcc acgaagagac tcacagttct cggaaaggat 2040

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gctggcactt taaccgaaga cccagatgaa ggcacagctg gagtggagtc cgctgttctg 2100

aggggattcc tcattctggg caaagaagac aggagatatg ggccagcatt aagcatcaat 2160

gaactgagca accttgcgaa aggagagaag gctaatgtgc taattgggca aggagacgtg 2220

gtgttggtaa tgaaacgaaa acgggactct agcatactta ctgacagcca gacagcgacc 2280

aaaagaattc ggatggccat caattagtgt cgaatagttt aaaaacgacc ttgtttctac 2340

t 2341

<210> SEQ ID NO 12
<211> LENGTH: 2234
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 12

agcgaaagca ggtactgatt caaaatggaa gattttgtgc gacaatgctt caatccgatg 60

attgtcgagc ttgcggaaaa aacaatgaaa gagtatgggg aggacctgaa aatcgaaaca 120

aacaaatttg cagcaatatg cactcacttg gaagtatgct tcatgtattc agatttccac 180

ttcatcaatg agcaaggcga gtcaataatc gtagaacttg gtgatcctaa tgcacttttg 240

aagcacagat ttgaaataat cgagggaaga gatcgcacaa tggcctggac agtagtaaac 300

agtatttgca acactacagg ggctgagaaa ccaaagtttc taccagattt gtatgattac 360

aaggaaaata gattcatcga aattggagta acaaggagag aagttcacat atactatctg 420

gaaaaggcca ataaaattaa atctgagaaa acacacatcc acattttctc gttcactggg 480

gaagaaatgg ccacaagggc cgactacact ctcgatgaag aaagcagggc taggatcaaa 540

accaggctat tcaccataag acaagaaatg gccagcagag gcctctggga ttcctttcgt 600

cagtccgaga gaggagaaga gacaattgaa gaaaggtttg aaatcacagg aacaatgcgc 660

aagcttgccg accaaagtct cccgccgaac ttctccagcc ttgaaaattt tagagcctat 720

gtggatggat tcgaaccgaa cggctacatt gagggcaagc tgtctcaaat gtccaaagaa 780

gtaaatgcta gaattgaacc ttttttgaaa acaacaccac gaccacttag acttccgaat 840

gggcctccct gttctcagcg gtccaaattc ctgctgatgg atgccttaaa attaagcatt 900

gaggacccaa gtcatgaagg agagggaata ccgctatatg atgcaatcaa atgcatgaga 960

acattctttg gatggaagga acccaatgtt gttaaaccac acgaaaaggg aataaatcca 1020

aattatcttc tgtcatggaa gcaagtactg gcagaactgc aggacattga gaatgaggag 1080

aaaattccaa agactaaaaa tatgaaaaaa acaagtcagc taaagtgggc acttggtgag 1140

aacatggcac cagaaaaggt agactttgac gactgtaaag atgtaggtga tttgaagcaa 1200

tatgatagtg atgaaccaga attgaggtcg cttgcaagtt ggattcagaa tgagttcaac 1260

aaggcatgcg aactgacaga ttcaagctgg atagagcttg atgagattgg agaagatgtg 1320

gctccaattg aacacattgc aagcatgaga aggaattatt tcacatcaga ggtgtctcac 1380

tgcagagcca cagaatacat aatgaagggg gtgtacatca atactgcctt acttaatgca 1440

tcttgtgcag caatggatga tttccaatta attccaatga taagcaagtg tagaactaag 1500

gagggaaggc gaaagaccaa cttgtatggt ttcatcataa aaggaagatc ccacttaagg 1560

aatgacaccg acgtggtaaa ctttgtgagc atggagtttt ctctcactga cccaagactt 1620

gaaccacaca aatgggagaa gtactgtgtt cttgagatag gagatatgct tctaagaagt 1680

gccataggcc aggtttcaag gcccatgttc ttgtatgtga ggacaaatgg aacctcaaaa 1740

attaaaatga aatggggaat ggagatgagg cgttgtctcc tccagtcact tcaacaaatt 1800

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gagagtatga ttgaagctga gtcctctgtc aaagagaaag acatgaccaa agagttcttt 1860

gagaacaaat cagaaacatg gcccattgga gagtctccca aaggagtgga ggaaagttcc 1920

attggggaag gtctgcagga ctttattagc aaagtcggta tttaacagct tgtatgcatc 1980

tccacaacta gaaggatttt cagctgaatc aagaaaactg cttcttatcg ttcaggctct 2040

tagggacaat ctggaacctg ggacctttga tcttgggggg ctatatgaag caattgagga 2100

gtgcctaatt aatgatccct gggttttgct taatgcttct tggttcaact ccttccttac 2160

acatgcattg agttagttgt ggcagtgcta ctatttgcta tccatactgt ccaaaaaagt 2220

accttgtttc tact 2234

<210> SEQ ID NO 13
<211> LENGTH: 1565
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 13

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accaaacggt cttacgaaca gatggagact gatggagaac gccagaatgc cactgaaatc 120

agagcatccg tcggaaaaat gattggtgga attggacgat tctacatcca aatgtgcaca 180

gaacttaaac tcagtgatta tgagggacgg ttgatccaaa acagcttaac aatagagaga 240

atggtgctct ctgcttttga cgaaaggaga aataaatacc tggaagaaca tcccagtgcg 300

gggaaagatc ctaagaaaac tggaggacct atatacagaa gagtaaacgg aaagtggatg 360

agagaactca tcctttatga caaagaagaa ataaggcgaa tctggcgcca agctaataat 420

ggtgacgatg caacggctgg tctgactcac atgatgatct ggcattccaa tttgaatgat 480

gcaacttatc agaggacaag ggctcttgtt cgcaccggaa tggatcccag gatgtgctct 540

ctgatgcaag gttcaactct ccctaggagg tctggagccg caggtgctgc agtcaaagga 600

gttggaacaa tggtgatgga attggtcagg atgatcaaac gtgggatcaa tgatcggaac 660

ttctggaggg gtgagaatgg acgaaaaaca agaattgctt atgaaagaat gtgcaacatt 720

ctcaaaggga aatttcaaac tgctgcacaa aaagcaatga tggatcaagt gagagagagc 780

cggaacccag ggaatgctga gttcgaagat ctcacttttc tagcacggtc tgcactcata 840

ttgagagggt cggttgctca caagtcctgc ctgcctgcct gtgtgtatgg acctgccgta 900

gccagtgggt acgactttga aagagaggga tactctctag tcggaataga ccctttcaga 960

ctgcttcaaa acagccaagt gtacagccta atcagaccaa atgagaatcc agcacacaag 1020

agtcaactgg tgtggatggc atgccattct gccgcatttg aagatctaag agtattgagc 1080

ttcatcaaag ggacgaaggt ggtcccaaga gggaagcttt ccactagagg agttcaaatt 1140

gcttccaatg aaaatatgga gactatggaa tcaagtacac ttgaactgag aagcaggtac 1200

tgggccataa ggaccagaag tggaggaaac accaatcaac agagggcatc tgcgggccaa 1260

atcagcatac aacctacgtt ctcagtacag agaaatctcc cttttgacag aacaaccgtt 1320

atggcagcat tcactgggaa tacagagggg agaacatctg acatgaggac cgaaatcata 1380

aggatgatgg aaagtgcaag accagaagat gtgtctttcc aggggcgggg agtcttcgag 1440

ctctcggacg aaaaggcagc gagcccgatc gtgccttcct ttgacatgag taatgaagga 1500

tcttatttct tcggagacaa tgcagaggag tacgacaatt aaagaaaaat acccttgttt 1560

ctact 1565

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<210> SEQ ID NO 14
<211> LENGTH: 1027
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 14

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ctctatcatc ccgtcaggcc ccctcaaagc cgagatcgca cagagacttg aagatgtctt 120

tgcagggaag aacaccgatc ttgaggttct catggaatgg ctaaagacaa gaccaatcct 180

gtcacctctg actaagggga ttttaggatt tgtgttcacg ctcaccgtgc ccagtgagcg 240

aggactgcag cgtagacgct ttgtccaaaa tgcccttaat gggaacgggg atccaaataa 300

catggacaaa gcagttaaac tgtataggaa gctcaagagg gagataacat tccatggggc 360

caaagaaatc tcactcagtt attctgctgg tgcacttgcc agttgtatgg gcctcatata 420

caacaggatg ggggctgtga ccactgaagt ggcatttggc ctggtatgtg caacctgtga 480

acagattgct gactcccagc atcggtctca taggcaaatg gtgacaacaa ccaacccact 540

aatcagacat gagaacagaa tggttttagc cagcactaca gctaaggcta tggagcaaat 600

ggctggatcg agtgagcaag cagcagaggc catggaggtt gctagtcagg ctaggcaaat 660

ggtgcaagcg atgagaacca ttgggactca tcctagctcc agtgctggtc tgaaaaatga 720

tcttcttgaa aatttgcagg cctatcagaa acgaatgggg gtgcagatgc aacggttcaa 780

gtgatcctct cgctattgcc gcaaatatca ttgggatctt gcacttgata ttgtggattc 840

ttgatcgtct ttttttcaaa tgcatttacc gtcgctttaa atacggactg aaaggagggc 900

cttctacgga aggagtgcca aagtctatga gggaagaata tcgaaaggaa cagcagagtg 960

ctgtggatgc tgacgatggt cattttgtca gcatagagct ggagtaaaaa actaccttgt 1020

ttctact 1027

<210> SEQ ID NO 15
<211> LENGTH: 890
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 15

agcaaaagca gggtgacaaa gacataatgg atccaaacac tgtgtcaagc tttcaggtag 60

attgctttct ttggcatgtc cgcaaacgag ttgcagacca agaactaggt gatgccccat 120

tccttgatcg gcttcgccga gatcagaaat ccctaagagg aaggggcagc actcttggtc 180

tggacatcga gacagccaca cgtgctggaa agcagatagt ggagcggatt ctgaaagaag 240

aatccgatga ggcacttaaa atgaccatgg cctctgtacc tgcgtcgcgt tacctaaccg 300

acatgactct tgaggaaatg tcaagggaat ggtccatgct catacccaag cagaaagtgg 360

caggccctct ttgtatcaga atggaccagg cgatcatgga taaaaacatc atactgaaag 420

cgaacttcag tgtgatttttt gaccggctgg agactctaat attgctaagg gctttcaccg 480

aagagggagc aattgttggc gaaatttcac cattgccttc tcttccagga catactgctg 540

aggatgtcaa aaatgcagtt ggagtcctca tcggaggact tgaatggaat gataacacag 600

ttcgagtctc tgaaactcta cagagattcg cttggagaag cagtaatgag aatgggagac 660

ctccactcac tccaaaacag aaacgagaaa tggcgggaac aattaggtca gaagtttgaa 720

gaaataagat ggttgattga agaagtgaga cacaaactga aggtaacaga gaatagtttt 780

-continued

gagcaaataa catttatgca agccttacat ctattgcttg aagtggagca agagataaga 840

actttctcat ttcagcttat ttaataataa aaaacaccct tgtttctact 890

<210> SEQ ID NO 16
<211> LENGTH: 1027
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 16

agcaaaagca ggtagatatt gaaagatgag tcttctaacc gaggtcgaaa cgtacgtact 60

ctctatcatc ccgtcaggcc ccctcaaagc cgagatcgca cagagacttg aagatgtctt 120

tgcagggaag aacaccgatc ttgaggttct catggaatgg ctaaagacaa gaccaatcct 180

gtcacctctg actaagggga ttttaggatt tgtgttcacg ctcaccgtgc ccagtgagcg 240

aggactgcag cgtagacgct ttgtccaaaa tgcccttaat gggaacgggg atccaaataa 300

catggacaaa gcagttaaac tgtataggaa gctcaagagg gagataacat tccatggggc 360

caaagaaatc tcactcagtt attctgctgg tgcacttgcc agttgtatgg gcctcatata 420

caacaggatg ggggctgtga ccactgaagt ggcatttggc ctggtatgtg caacctgtga 480

acagattgct gactcccagc atcggtctca taggcaaatg gtgacaacaa ccaatccact 540

aatcagacat gagaacagaa tggttttagc cagcactaca gctaaggcta tggagcaaat 600

ggctggatcg agtgagcaag cagcagaggc catggaggtt gctagtcagg ctagacaaat 660

ggtgcaagcg atgagaacca ttgggactca tcctagctcc agtgctggtc tgaaaaatga 720

tcttcttgaa aatttgcagg cctatcagaa acgaatgggg gtgcagatgc aacggttcaa 780

gtgatcctct cactattgcc gcaaatatca ttgggatctt gcacttgaca ttgtggattc 840

ttgatcgtct ttttttcaaa tgcatttacc gtcgctttaa atacggactg aaaggagggc 900

cttctacgga aggagtgcca aagtctatga gggaagaata tcgaaaggaa cagcagagtg 960

ctgtggatgc tgacgatggt cattttgtca gcatagagct ggagtaaaaa actaccttgt 1020

ttctact 1027

<210> SEQ ID NO 17
<211> LENGTH: 1027
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 17

agcaaaagca ggtagatatt gaaagatgag tcttctaacc gaggtcgaaa cgtacgttct 60

ctctatcatc ccgtcaggcc ccctcaaagc cgagatcgca cagagacttg aagatgtctt 120

tgcagggaag aacaccgatc ttgaggttct catggaatgg ctaaagacaa gaccaatcct 180

gtcacctctg actaagggga ttttaggatt tgtgttcacg ctcaccgtgc ccagtgagcg 240

aggactgcag cgtagacgct ttgtccaaaa tgcccttaat gggaacgggg atccaaataa 300

catggacaaa gcagttaaac tgtataggaa gctcaagagg gagataacat tccatggggc 360

caaagaaatc tcactcagtt attctgctgg tgcacttgcc agttgtatgg gcctcatata 420

caacaggatg ggggctgtga ccactgaagt ggcatttggc ctggtatgtg caacctgtga 480

acagattgct gactcccagc atcggtctca taggcaaatg gtgacaacaa ccaacccact 540

aatcagacat gagaacagaa tggttttagc cagcactaca gctaaggcta tggagcaaat 600

ggctggatcg agtgagcaag cagcagaggc catggaggtt gctagtcagg ctaggcaaat 660

ggtgcaagcg atgagaacca ttgggactca tcctagctcc agtgctggtc tgaaaaatga 720

-continued

tcttcttgaa aatttgcagg cctatcagaa acgaatgggg gtgcagatgc aacggttcaa 780

gtgatcctct cgctattgcc gcaaatatca ttgggatctt gcacttgata ttgtggattc 840

ttgatcgtct ttttttcaaa tgcatttacc gtcgctttaa atacggactg aaaggagggc 900

cttctacgga aggagtgcca aagtctatga gggaagaata tcgaaaggaa cagcagagtg 960

ctgtggatgc tgacgatggt cattttgtca gcatagagct ggagtaaaaa actaccttgt 1020

ttctact 1027

<210> SEQ ID NO 18
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 18

gctggagtaa aaaactacct tg 22

<210> SEQ ID NO 19
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 19

ugagguagua gguuguaugg uu 22

<210> SEQ ID NO 20
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 20

atactgccaa aaaagtacct t 21

<210> SEQ ID NO 21
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 21

cgaatagttt aaaaacgacc ttgt 24

<210> SEQ ID NO 22
<211> LENGTH: 2233
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 22

agcgaaagca ggtactgatc caaaatggaa gattttgtgc gacaatgctt caatccgatg 60

attgtcgagc ttgcggaaaa aacaatgaaa gagtatgggg aggacctgaa aatcgaaaca 120

aacaaatttg cagcaatatg cactcacttg gaagtatgct tcatgtattc agattttcac 180

ttcatcaatg agcaaggcga gtcaataatc gtagaacttg gtgatccaaa tgcacttttg 240

aagcacagat ttgaaataat cgagggaaga gatcgcacaa tggcctggac agtagtaaac 300

agtatttgca acactacagg ggctgagaaa ccaaagtttc taccagattt gtatgattac 360

aaggagaata gattcatcga aattggagta acaaggagag aagttcacat atactatctg 420

gaaaaggcca ataaaattaa atctgagaaa acacacatcc acattttctc gttcactggg 480

gaagaaatgg ccacaaaggc agactacact ctcgatgaag aaagcagggc taggatcaaa 540

accagactat tcaccataag acaagaaatg gccagcagag gcctctggga ttcctttcgt 600

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cagtccgaga gaggagaaga gacaattgaa gaaaggtttg aaatcacagg aacaatgcgc 660

aagcttgccg accaaagtct cccgccgaac ttctccagcc ttgaaaattt tagagcctat 720

gtggatggat tcgaaccgaa cggctacatt gagggcaagc tgtctcaaat gtccaaagaa 780

gtaaatgcta gaattgaacc ttttttgaaa acaacaccac gaccacttag acttccgaat 840

gggcctccct gttctcagcg gtccaaattc ctgctgatgg atgccttaaa attaagcatt 900

gaggacccaa gtcatgaagg agagggaata ccgctatatg atgcaatcaa atgcatgaga 960

acattctttg gatggaagga acccaatgtt gttaaaccac acgaaaaggg aataaatcca 1020

aattatcttc tgtcatggaa gcaagtactg gcagaactgc aggacattga gaatgaggag 1080

aaaattccaa agactaaaaa tatgaagaaa acaagtcagc taaagtgggc acttggtgag 1140

aacatggcac cagaaaaggt agactttgac gactgtaaag atgtaggtga tttgaagcaa 1200

tatgatagtg atgaaccaga attgaggtcg cttgcaagtt ggattcagaa tgagtttaac 1260

aaggcatgcg aactgacaga ttcaagctgg atagagctcg atgagattgg agaagatgtg 1320

gctccaattg aacacattgc aagcatgaga aggaattatt tcacatcaga ggtgtctcac 1380

tgcagagcca cagaatacat aatgaaggga gtgtacatca atactgcctt gcttaatgca 1440

tcttgtgcag caatggatga tttccaatta attccaatga taagcaagtg tagaactaag 1500

gagggaaggc gaaagaccaa cttgtatggt ttcatcataa aaggaagatc ccacttaagg 1560

aatgacaccg acgtggtaaa ctttgtgagc atggagtttt ctctcactga cccaagactt 1620

gaaccacata aatgggagaa gtactgtgtt cttgagatag gagatatgct tataagaagt 1680

gccataggcc aggtttcaag gcccatgttc ttgtatgtga gaacaaatgg aacctcaaaa 1740

attaaaatga aatggggaat ggagatgagg cgttgcctcc tccagtcact tcaacaaatt 1800

gagagtatga ttgaagctga gtcctctgtc aaagagaaag acatgaccaa agagttcttt 1860

gagaacaaat cagaaacatg gcccattgga gagtccccca aaggagtgga ggaaagttcc 1920

attgggaagg tctgcaggac tttattagca aagtcggtat tcaacagctt gtatgcatct 1980

ccacaactag aaggattttc agctgaatca agaaaactgc ttcttatcgt tcaggctctt 2040

agggacaacc tggaacctgg gacctttgat cttgggggggc tatatgaagc aattgaggag 2100

tgcctgatta atgatccctg ggttttgctt aatgcttctt ggttcaactc cttccttaca 2160

catgcattga gttagttgtg gcagtgctac tatttgctat ccatactgtc caaaaaagta 2220

ccttgtttct act 2233

<210> SEQ ID NO 23
<211> LENGTH: 2341
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 23

agcgaaagca ggtcaattat attcaatatg gaaagaataa aagaactacg aaatctaatg 60

tcgcagtctc gcacccgcga gatactcaca aaaaccaccg tggaccatat ggccataatc 120

aagaagtaca catcaggaag acaggagaag aacccagcac ttaggatgaa atggatgatg 180

gcaatgaaat atccaattac agcagacaag aggataacgg aaatgattcc tgagagaaat 240

gagcaaggac aaactttatg gagtaaaatg aatgatgccg gatcagaccg agtgatggta 300

tcacctctgg ctgtgacatg gtggaatagg aatggaccaa taacaaatac agttcattat 360

ccaaaaatct acaaaactta ttttgaaaga gtcgaaaggc taaagcatgg aacctttggc 420

cctgtccatt ttagaaacca agtcaaaata cgtcggagag ttgacataaa tcctggtcat 480

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gcagatctca gtgccaagga ggcacaggat gtaatcatgg aagttgtttt ccctaacgaa 540

gtgggagcca ggatactaac atcggaatcg caactaacga taaccaaaga gaagaaagaa 600

gaactccagg attgcaaaat ttctcctttg atggttgcat acatgttgga gagagaactg 660

gtccgcaaaa cgagattcct cccagtggct ggtggaacaa gcagtgtgta cattgaagtg 720

ttgcatttga ctcaaggaac atgctgggaa cagatgtata ctccaggagg ggaagtgagg 780

aatgatgatg ttgatcaaag cttgattatt gctgctagga acatagtgag aagagctgca 840

gtatcagcag atccactagc atctttattg gagatgtgcc acagcacaca gattggtgga 900

attaggatgg tagacatcct taggcagaac ccaacagaag agcaagccgt ggatatatgc 960

aaggctgcaa tgggactgag aattagctca tccttcagtt ttggtggatt cacatttaag 1020

agaacaagcg gatcatcagt caagagagag gaagaggtgc ttacgggcaa tcttcaaaca 1080

ttgaagataa gagtgcatga gggatatgaa gagttcacaa tggttgggag aagagcaaca 1140

gccatactca gaaaagcaac caggagattg attcagctga tagtgagtgg gagagacgaa 1200

cagtcgattg ccgaagcaat aattgtggcc atggtatttt cacaagagga ttgtatgata 1260

aaagcagtca gaggtgatct gaatttcgtc aatagggcga atcaacgatt gaatcctatg 1320

catcaacttt taagacattt tcagaaggat gcgaaagtgc tttttcaaaa ttggggagtt 1380

gaacctatcg acaatgtgat gggaatgatt gggatattgc ccgacatgac tccaagcatc 1440

gagatgtcaa tgagaggagt gagaatcagc aaaatgggtg tagatgagta ctccagcacg 1500

gagagggtag tggtgagcat tgaccgtttt ttgagaatcc gggaccaacg aggaaatgta 1560

ctactgtctc ccgaggaggt cagtgaaaca cagggaacag agaaactgac aataacttac 1620

tcatcgtcaa tgatgtggga gattaatggt cctgaatcag tgttggtcaa tacctatcaa 1680

tggatcatca gaaactggga aactgttaaa attcagtggt cccagaaccc tacaatgcta 1740

tacaataaaa tggaatttga accatttcag tctttagtac ctaaggccat tagaggccaa 1800

tacagtgggt ttgtaagaac tctgttccaa caaatgaggg atgtgcttgg gacatttgat 1860

accgcacaga taataaaact tcttcccttc gcagccgctc caccaaagca aagtagaatg 1920

cagttctcct catttactgt gaatgtgagg ggatcaggaa tgagaatact tgtaaggggc 1980

aattctcctg tattcaacta taacaaggcc acgaagagac tcacagttct cggaaaggat 2040

gctggcactt taactgaaga cccagatgaa ggcacagctg gagtggagtc cgctgttctg 2100

aggggattcc tcattctggg caaagaagac aagagatatg ggccagcact aagcatcaat 2160

gaactgagca accttgcgaa aggagagaag gctaatgtgc taattgggca aggagacgtg 2220

gtgttggtaa tgaaacggaa acgggactct agcatactta ctgacagcca gacagcgacc 2280

aaaagaattc ggatggccat caattagtgt cgaatagttt aaaaacgacc ttgtttctac 2340

t 2341

<210> SEQ ID NO 24
<211> LENGTH: 1076
<212> TYPE: DNA
<213> ORGANISM: Influenza B

<400> SEQUENCE: 24

atgtcgctgt ttggagacac aattgcctac ctgctttcat tgacagaaga tggagaaggc 60

aaagcagaac tagcagaaaa attacactgc tggttcggtg ggaaagaatt tgacctagac 120

tctgccttgg aatggataaa aaacaaaaga tgcttaactg atatacaaaa agcactaatt 180

ggtgcctcta tctgcttttt aaaacccaaa gaccaggaaa gaaaaagaag attcatcaca 240

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gagcccctat caggaatggg aacaacagca acaaaaaaga aaggcctgat tctagctgag 300

agaaagatga gaagatgtgt gagctttcat gaagcatttg aaatagcaga aggccatgaa 360

agctcagcgc tactatattg tctcatggtc atgtacctga atcctggaaa ttattcaatg 420

caagtaaaac taggaacgct ctgtgctttg tgcgagaaac aagcatcaca ttcacacagg 480

gctcacagca gagcagcgag atcttcagtg cccggagtga gacgagaaat gcagatggtc 540

tcagctatga acacagcaaa aacaatgaat ggaatgggaa aaggagaaga cgtccaaaaa 600

ctggcagaag agctgcaaag caacattgga gtcttgagat ctcttggggc aagtcaaaag 660

aatggggaag gaattgcaaa ggatgtaatg gaagtgctaa agcagagctc tatgggaaat 720

tcagctcttg tgaagaaata cctataatgc tcgaaccatt tcagattctt tcaatttgtt 780

cttttatctt atcagctctc catttcatgg cttggacaat agggcatttg aatcaaataa 840

aaagaggtgt aaacatgaag atacgaataa aaggtccaaa taaagagaca ataaacagag 900

aggtatcaat tttgagacac agttaccaaa aagaaatcca ggccaaagaa acaatgaagg 960

aagtactctc tgacaacatg gaagtattga gtgaccacat agtaattgag gggctttctg 1020

ccgaagagat aataaaaatg ggtgaaacag ttttggaagt agaagaattg cattaa 1076

<210> SEQ ID NO 25
<211> LENGTH: 2313
<212> TYPE: DNA
<213> ORGANISM: Influenza B

<400> SEQUENCE: 25

atgacattgg ccaaaattga attgttaaaa caactgctaa gggacaatga agccaaaaca 60

gttttgaagc aaacaacggt agaccaatat aacataataa gaaaattcaa tacatcaagg 120

attgaaaaga atccttcact aaggatgaag tgggccatgt gttctaattt tcccttggct 180

ctaaccaagg gcgatatggc aaatagaatc cccttggaat acaaaggaat acaacttaaa 240

acaaatgctg aagacatagg aaccaaaggc caaatgtgct caatagcagc agttacttgg 300

tggaatacat atggaccaat aggagatact gaaggtttcg aaagggtcta cgaaagcttt 360

tttctcagaa aaatgagact tgacaacgcc acttggggcc gaataacttt tggcccagtt 420

gaaagagtga gaaaaagggt actgctaaac cctctcacca aggaaatgcc tccggatgag 480

gcgagcaatg tgataatgga aatattgttc cctaaagaag caggaatacc aagagaatcc 540

acttggatac atagggaact gataaaagaa aaaagagaaa aattgaaagg aacaatgata 600

actccaatcg tactggcata catgcttgaa agagaactgg ttgctcgaag aagattcttg 660

ccagtggcag gagcaacatc agctgagttc atagaaatgc tacactgctt acaaggtgaa 720

aattggagac aaatatatca cccaggaggg aataaattaa ctgagtctag gtctcaatca 780

atgatagtag cttgtagaaa aataatcaga agatcaatag tcgcttcaaa cccactggag 840

ctagctgtag aaattgcaaa caagactgtg atagatactg aacctttaaa gtcatgtctg 900

gcagccatag acggaggtga tgtagcttgt gacataataa gagctgcatt aggactaaag 960

atcagacaaa gacaaagatt tggacggctt gagctaaaaa gaatatcagg aagaggattc 1020

aaaaatgatg aagaaatatt aatagggaac ggaacaatac agaagattgg aatatgggac 1080

ggggaagagg agttccatgt aagatgtggt gaatgcaggg gaatattaaa aaagagtaaa 1140

atgaaactgg aaaaactact gataaattca gccaaaaagg aggatatgag agatttaata 1200

atcttatgca tggtattttc tcaagacact aggatgttcc aaggggtgag aggagaaata 1260

aattttctta atcgagcagg ccaactttta tctccaatgt accaactcca acgatatttt 1320

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ttgaatagaa gcaacgacct ttttgatcaa tgggggtatg aggaatcacc caaagcaagt 1380

gaactacatg ggataaatga atcaatgaat gcatctgact atacattgaa aggggttgta 1440

gtgacaagaa atgtaattga cgactttagc tctactgaaa cagaaaaagt atccataaca 1500

aaaaatctta gtttaataaa aaggactggg gaagtcataa tgggagctaa tgacgtgagt 1560

gaattagaat cacaagcaca gctgatgata acatatgata cacctaaaat gtgggaaatg 1620

ggaacaacca aagaactggt gcaaaacact tatcaatggg tgctaaaaaa cttggtaaca 1680

ctgaaggctc agtttcttct aggaaaagag gacatgtttc aatgggatgc atttgaagca 1740

tttgagagca taattcctca gaagatggct ggtcagtaca gtggatttgc aagagcagtg 1800

ctcaaacaaa tgagagacca ggaggttatg aaaactgacc agttcataaa gttgttgcct 1860

ttttgtttct caccaccaaa attaaggagc aatggggagc cttatcaatt cttaaaactt 1920

gtgttgaaag gaggagggga aaatttcatc gaagtaagga aagggtcccc tctattttcc 1980

tataatccac aaacagaagt cctaactata tgcggcagaa tgatgtcatt aaaagggaaa 2040

attgaagatg aagaaaggaa tagatcaatg gggaatgcag tattagcagg ctttctcgtt 2100

agtggcaagt atgacccaga tcttggagat ttcaaaacta ttgaagaact tgaaaagctg 2160

aaaccggggg aaaaggcaaa catcttactt tatcaaggaa agccagttaa agtagttaaa 2220

aggaaaaggt atagtgcttt gtccaatgac atttcacaag gaataaagag acaaagaatg 2280

acagttgagt ccatggggtg ggccttgagc taa 2313

<210> SEQ ID NO 26
<211> LENGTH: 2313
<212> TYPE: DNA
<213> ORGANISM: Influenza B

<400> SEQUENCE: 26

atgacattgg ccaaaattga attgttaaaa caactgctaa gggacaatga agccaaaaca 60

gttttgaagc aaacaacggt agaccaatat aacataataa gaaaattcaa tacatcaagg 120

attgaaaaga atccttcact aaggatgaag tgggccatgt gttctaattt tcccttggct 180

ctaaccaagg gcgatatggc aaatagaatc cccttggaat acaaaggaat acaacttaaa 240

acaaatgctg aagacatagg aaccaaaggc caaatgtgct caatagcagc agttacttgg 300

tggaatacat atggaccaat aggagatact gaaggtttcg aaagggtcta cgaaagcttt 360

tttctcagaa aaatgagact tgacaacgcc acttggggcc gaataacttt tggcccagtt 420

gaaagagtga gaaaaagggt actgctaaac cctctcacca aggaaatgcc tccggatgag 480

gcgagcaatg tgataatgga aatattgttc cctaaagaag caggaatacc aagagaatcc 540

acttggatac atagggaact gataaaagaa aaaagagaaa aattgaaagg aacaatgata 600

actccaatcg tactggcata catgcttgaa agagaactgg ttgctcgaag aagattcttg 660

ccagtggcag gagcaacatc agctgagttc atagaaatgc tacactgctt acaaggtgaa 720

aattggagac aaatatatca cccaggaggg aataaattaa ctgagtctag gtctcaatca 780

atgatagtag cttgtagaaa aataatcaga agatcaatag tcgcttcaaa cccactggag 840

ctagctgtag aaattgcaaa caagactgtg atagatactg aacctttaaa gtcatgtctg 900

gcagccatag acggaggtga tgtagcttgt gacataataa gagctgcatt aggactaaag 960

atcagacaaa gacaaagatt tggacggctt gagctaaaaa gaatatcagg aagaggattc 1020

aaaaatgatg aagaaatatt aatagggaac ggaacaatac agaagattgg aatatgggac 1080

ggggaagagg agttccatgt aagatgtggt gaatgcaggg gaatattaaa aaagagtaaa 1140

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atgaaactgg aaaaactact gataaattca gccaaaaagg aggatatgag agatttaata 1200

atcttatgca tggtattttc tcaagacact aggatgttcc aaggggtgag aggagaaata 1260

aattttctta atcgagcagg ccaactttta tctccaatgt accaactcca acgatatttt 1320

ttgaatagaa gcaacgacct ttttgatcaa tgggggtatg aggaatcacc caaagcaagt 1380

gaactacatg ggataaatga atcaatgaat gcatctgact atacattgaa aggggttgta 1440

gtgacaagaa atgtaattga cgactttagc tctactgaaa cagaaaaagt atccataaca 1500

aaaaatctta gtttaataaa aaggactggg gaagtcataa tgggagctaa tgacgtgagt 1560

gaattagaat cacaagcaca gctgatgata acatatgata cacctaaaat gtgggaaatg 1620

ggaacaacca aagaactggt gcaaaacact tatcaatggg tgctaaaaaa cttggtaaca 1680

ctgaaggctc agtttcttct aggaaaagag gacatgtttc aatgggatgc atttgaagca 1740

tttgagagca taattcctca gaagatggct ggtcagtaca gtggatttgc aagagcagtg 1800

ctcaaacaaa tgagagacca ggaggttatg aaaactgacc agttcataaa gttgttgcct 1860

ttttgtttct caccaccaaa attaaggagc aatggggagc cttatcaatt cttaaaactt 1920

gtgttgaaag gaggaggga aaatttcatc gaagtaagga aagggtcccc tctattttcc 1980

tataatccac aaacagaagt cctaactata tgcggcagaa tgatgtcatt aaaagggaaa 2040

attgaagatg aagaaaggaa tagatcaatg gggaatgcag tattagcagg ctttctcgtt 2100

agtggcaagt atgacccaga tcttggagat ttcaaaacta ttgaagaact tgaaaagctg 2160

aaaccggggg aaaaggcaaa catcttactt tatcaaggaa agccagttaa agtagttaaa 2220

aggaaaaggt atagtgcttt gtccaatgac atttcacaag gaataaagag acaaagaatg 2280

acagttgagt ccatggggtg ggccttgagc taa 2313

<210> SEQ ID NO 27
<211> LENGTH: 1124
<212> TYPE: DNA
<213> ORGANISM: Influenza C

<400> SEQUENCE: 27

caatggcaca tgaaatactg attgctgaaa cagaggcatt tctaaaaaat gttgctcctg 60

agaccaggac agcaataatt tcagcaataa caggtggaaa atcagcctgc aaatcagcag 120

ctaaactgat taagaatgaa catcttccct taatgtctgg agaagctacc acaatgcaca 180

ttgttatgag gtgcttatat cctgaaataa aaccatggaa gaaggcaagc gacatgctga 240

ataaagcaac ttctagtttg aaaaaatcag aaggaagaga catcagaaag caaatgaaag 300

cagctggaga cttcttggga gtggagtcaa tgatgaaaat gagggccttc agagatgacc 360

aaataatgga aatggttgaa gaagtatatg atcacccaga cgactacaca ccagacatcc 420

gaataggaac aatcacagct tggttgagat gcaaaaacaa gaaaagtgaa agatacagga 480

gtaatgtctc agaaagtgga cgaacagctt taaaaattca tgaagtaaga aaagccagca 540

cagcaatgaa cgaaattgct ggtattactg gccttggaga agaagcacta tctctccaaa 600

gacaaacaga aagtttggcc atattatgca atcacacttt tggaagtaat ataatgagac 660

cccacttgga aaaagcaata aaaggagttg aaggcagagt tggagagatg ggacgaatgg 720

caatgaaatg gttagttgtt ataatatatt tctctataac aagtcaacct gcttctgctt 780

gcaatctaaa aacctgtcta aacctattta acaatactga tgcagtaact gttcattgtt 840

ttaatgaaaa ccaaggatac atgctaacat tagcctcttt gggattagga ataattacta 900

tgttgtattt attagtaaaa atcataattg aacttgtcaa tggttttgtg ctcggcagat 960

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gggagagatg gtgtggagat ataaagacca caattatgcc tgaaattgac tcgatggaaa   1020

aagatattgc cctctctagg gagagacttg acctgggaga ggatgctcct gacgaaaccg   1080

acaactcacc aattcctttt tccaatgatg gtatttttga aatt                    1124
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What is claimed is:

1. A recombinant vector comprising an influenza M viral segment having a nucleotide other than U/T at position 1011, a nucleotide other than G at position 1012, a nucleotide other than A at position 1013, a nucleotide other than U/T at position 1014, a nucleotide other than G at position 1015, a nucleotide other than G at position 1016, a nucleotide other than A at position 1017, or any combination thereof, wherein the numbering refers to $cRNA^+$ corresponding to a sequence having SEQ ID NO:16.

2. The vector of claim **1** wherein the viral segment has a nucleotide other than G at position 1012, a nucleotide other than A at position 1013, and a nucleotide other than U at position 1014.

3. The vector of claim **1** wherein the viral segment has at least two of a nucleotide other than G at position 1012, a nucleotide other than A at position 1013, or a nucleotide other than U at position 1014.

4. A method to prepare influenza virus, comprising: contacting a cell with at least one of:

a vector comprising a promoter operably linked to an influenza virus PA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB1 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB2 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus HA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NP cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus M cDNA linked to a transcription termination sequence, and a vector comprising a promoter operably linked to an influenza virus NS cDNA linked to a transcription termination sequence, wherein the mutant M cDNA has a nucleotide other than U/T at position loll, a nucleotide other than G at position 1012, a nucleotide other than A at position 1013, a nucleotide other than U/T at position 1014, a nucleotide other than G at position 1015, a nucleotide other than 0 at position 1016, a nucleotide other than A at position 1017, or any combination thereof, wherein the numbering refers to $cRNA^+$ corresponding to a sequence having SEQ ID NO:16 and optionally wherein the cDNA for HA and/or NA has sequences for a heterologous HA or NA; and optionally,

a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB1, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB2, and a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NP, and optionally a vector comprising a promoter operably linked to a DNA segment encoding influenza virus HA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus M1, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus M2, or a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NS2.

5. The method of claim **4** further comprising isolating the virus.

6. Virus obtained by the method of claim **5**.

7. An isolated recombinant influenza virus comprising a viral segment for PB1, PB2, PA, and NP, a viral segment for M having a nucleotide other than U/T at position 1011, a nucleotide other than G at position 1012, a nucleotide other than A at position 1013, a nucleotide other than U/T at position 1014, a nucleotide other than G at position 1015, a nucleotide other than G at position 1016, a nucleotide other than A at position 1017, or any combination thereof, wherein the numbering refers to $cRNA^+$ corresponding to a sequence having SEQ ID NO:16, a viral segment for NS, a viral segment, for a heterologous NA, and a viral segment for a heterologous HA.

8. The isolated recombinant influenza virus of claim **7** wherein the viral segment for PB1, PB2, PA, NS, M, and NP encode viral proteins having at least 90% amino acid sequence identity to proteins encoded by SEQ ID Nos. 1-6.

9. The isolated recombinant influenza virus of claim **7** wherein the viral segment for HA is for H1, H3, H5 or H7.

10. The isolated recombinant influenza virus of claim **7** wherein the viral segment for PB2 encodes a PB2 with a serine at position 360 and has at least 90% amino acid sequence identity to a polypeptide encoded by II) NO:3 but which viral segment does not encode a PB2 with SEQ ID NO:3.

11. The isolated recombinant virus of claim **7** which has one or more but less than 25 substitutions in PB2 relative to PB2 encoded by SEQ ID NO:3.

12. The isolated recombinant virus of claim **11** wherein the one or more substitutions include conservative substitutions.

13. The isolated recombinant influenza virus of claim **7** further comprising one or more of the following: 142N, 225C, 356R, or 550L in PA relative to numbering of positions in a PA encoded by SEQ ID NO:1; 112G, 247H, 507V, or 644A in PB1 relative to numbering in positions in a PB1 encoded by SEQ ID NO:2; 202L, 323L or 504V in PB2 relative to numbering of positions in a PB2 encoded by SEQ ID NO:3; 74K, 112L, 116L, 417D, or 442A in NP relative to numbering of positions in a NP encoded by SEQ ID NO:4; 97A and/or 100H in M1 relative to numbering of positions in a M1 encoded by SEQ ID NO:5; and/or 55E and/or 140Q in NS1 relative to numbering of positions in a NS1 encoded by SEQ ID NO:6, or combinations thereof, and optionally at least one of 142N in PA1, 55K in NS1 or 97A and/or 100H in M1; or further comprising one or more of the following:

40I, 40L, 112G, 180W, 247H, 507V, or 644A in PB1; 202L and/or 323L in PB2; 74K, 112L, 116L, 377N, 417D, or 422L in NP; 30P, 55K, 118K, 161T or 140Q in NS1; 142N, 225C, 356R, 401K, or 550L in PA; or one or more of 247H in PB1; 202L and/or 323L in PB2; 74K in NP; 55E in NS1; or 142N in PA.

14. The isolated recombinant virus of claim **7** which has a U at position 4 in the viral segment for any one of PB1, PB2 or PA.

15. The isolated recombinant influenza virus of claim **7** wherein at least one of the PA, PB1, PB2, NP, NS, and M viral segments has a C to U promoter mutation.

16. The isolated recombinant influenza virus of claim **8** wherein the PA, PB1, PB2, NP, NS, and M viral segments comprise sequences for at least one of the following: a PB1 having the amino acid sequence encoded by SEQ ID NO:2 or PB1 with at least 95% amino acid sequence identity to the PB1 encoded by SEQ ID NO:2; a PB2 having the amino acid sequence encoded by SEQ ID NO:3 or PB2 with at least 95% amino acid sequence identity to the PB2 encoded by SEQ NO:3; a PA having the amino acid sequence encoded by SEQ ID NO:1 or PA with at least 95% amino acid sequence identity to the PA encoded by SEQ ID NO:1; a NP having the amino acid sequence encoded by SEQ ID NO:4 or NP with at least 95% amino acid sequence identity to the NP encoded by SEQ ID NO:4; a M having the amino acid sequence encoded by SEQ ID NO:5 or M with at least 95% amino acid sequence identity to the M encoded by SEQ ID NO:5; or a NS having the amino acid sequence encoded by SEQ ID NO:6 or NS with at least 95% amino acid sequence identity to the NS encoded by SEQ ID NO:6 or wherein the PA, PB1, PB2, NP, NS, and M viral segments comprise sequences for at least one of the following: a PB1 having the amino acid sequence encoded by SEQ ID NO:10 or PB1 with at least 95% amino acid sequence identity to the PB1 encoded by SEQ ID NO:10; a PB2 having the amino acid sequence encoded by SEQ ID NO:11 or PB2 with at least 95% amino acid sequence identity to the PB2 encoded by SEQ ID NO:11; a PA having the amino acid sequence encoded by SEQ ID NO:12 or PA with at least 95% amino acid sequence identity to the PA encoded by SEQ ID NO:12; a NP having the amino acid sequence encoded by SEQ ID NO:13 or NP with at least 95% amino acid sequence identity to the NP encoded by SEQ ID NO:13; a M having the amino acid sequence encoded by SEQ ID NO:14 or M with at least 95% amino acid sequence identity to the M encoded by SEQ ID NO:14; or a NS having the amino acid sequence encoded by SEQ ID NO:15 or NS with at least 95% amino acid sequence identity to the NS encoded by SEQ ID NO:15.

17. A cell infected in vitro with the virus of claim **7**.

18. The cell of claim **17** which is a cell in an embryonated egg.

19. The cell of claim **17** which is a Vero cell.

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